Preface

The field of neurodegenerative diseases is undergoing an unprecedented revolution. The past decade has seen the identification of new mutation mechanisms, such as triplet repeat expansions, and new genes causing familial forms of common neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases. Cellular and animal models based on this genetic information are now available and, importantly, common mechanisms are rapidly emerging among diseases that were once considered unrelated. The field is poised for the development of new therapies based on high throughput screenings and a better understanding of the molecular and cellular mechanisms leading to neurodegeneration.

Molecular Mechanisms of Neurodegenerative Diseases reviews recent progress in this exploding field. By nature, such a book cannot be all inclusive. It focuses on Alzheimer's, Parkinson's, and CAG triplet repeat diseases. In the first chapter, Bill Klein reviews the role of Aβ toxicity in the pathophysiology of Alzheimer's disease. This controversial issue is further examined in the context of transgenic models of Alzheimer's disease by LaFerla and colleagues. Sue Griffin and Robert Mrak, and Caleb Finch and collaborators, then examine the role of glial cells and inflammation in Alzheimer's disease; a review of the role of proteolysis in the generation of abnormal protein fragments by Hook and Mende-Mueller follows. Therapeutic opportunities offered by a better understanding of Alzheimer's disease pathophysiology are examined by Perry Molinoff and his colleagues at Bristol-Myers Squibb.

The chapter on proteolysis by Hook and Mende-Mueller identifies one of the recurring themes that is appearing among neurodegenerative diseases: the formation of abnormal protein fragments, whose misfolding may lead to a cascade of cellular defects, ultimately leading to cell death. Similarities between pathological processes in Parkinson's, Alzheimer's, and related diseases is also the theme of the chapter by Virginia Lee, John Trojanowski, and collaborators, which discusses the role of Tau and synuclein. Despite the identification of mutations in synuclein, and the presence of synuclein in Lewy bodies, the pathophysiology of Parkinson's disease, however, remains poorly understood. Joel Perlmutter and his colleagues review the information we have recently gained on the progression of the disease from brain imaging studies.

BethAnn McLaughlin and Russell Swerdlow then examine the role of dopamine and of mitochondrial dysfunction, respectively, in neurodegeneration.

The last chapters of the book deal with different and complementary aspects of CAG repeat diseases, including SCA1 (Orr and Zoghbi), SCA3 (Opal and Paulson), SBMA (Merry), and Huntington's disease. Chesselet and Levine compare the different mouse models of Huntington's disease, MacDonald and colleagues review the role of proteins interacting with huntingtin, and George Jackson discusses the potential of fly genetics to identify the molecular mechanisms of neurodegenerative diseases.

Despite their differences in focus, many chapters of *Molecular Mechanisms of Neurodegenerative Diseases* overlap, presenting the variety of viewpoints that pervade this dynamic field. Evidently, since new data appear every day, the chapters in a book can only provide the basis for understanding ongoing research. It is hoped that the ideas and concepts presented here will lead, within a few short years, to therapies that prevent, delay the onset, slow the progression, or even cure these devastating neurodegenerative illnesses.

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2

Transgenic Models of Alzheimer's Disease

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2.1. INTRODUCTION

The ability to introduce foreign genes into an animal's genome or to modify or delete existing genes provides a powerful means to study their impact in an intact organism. This technology has been extensively exploited to study the pathogenesis of Alzheimer's disease (AD), a progressive neurodegenerative disease characterized by memory loss and cognitive decline. At the neuropathological level, the AD brain is marked by three principle features: (1) diffuse and neuritic plaques composed primarily of the β-amyloid (Aβ) peptide, (2) intracellular neurofibrillary tangles (NFTs), which consist of hyperphosphorylated tau protein, and (3) neuronal and synaptic loss *(1)*. Thus, to faithfully model AD, an animal model must contain these three principle neuropathological features and the accompanying deficits in memory and cognition. To date, none of the existing models truly fulfills these criteria. Nevertheless, this should not imply that these existing models do not have value, because replicating one or more aspects of the disease provides a valuable experimental system to investigate the underlying pathogenic mechanisms and to evaluate potential therapeutic interventions.

As with virtually any transgenic strategy, approaches to model AD in mice have capitalized on the remarkable advances made in elucidating the genetics of this complex disorder. Therefore, we begin this chapter by presenting a brief overview of the genetics underlying familial AD (FAD). AD can be broadly divided into early-onset or late-onset classifications depending on whether the disease is acquired before or after 60 yr of age. Approximately half of all cases of early-onset autosomal dominant AD can be attributed to missense mutations in three genes encoding transmembrane proteins (Fig. 1): *amyloid precursor protein (APP)* gene on chromosome 21,

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presenilin-1 (PS1) gene on chromosome 14, and *presenilin-2 (PS2)* gene on chromosome 1 *(3)*.

2.2. AUTOSOMAL-DOMINANT FAD GENES

The *APP* gene was the first gene conclusively linked to FAD and to date, seven pathological mutations have been described *(4)*. An important characteristic of all the *APP* mutations identified thus far is that they occur within or in close proximity to the Aβ coding region, consistent with the interpretation that the biological effect of these mutations is an alteration of APP processing, leading to increased Aβ production. The first mutation discovered, a glutamate-to-glutamine substitution at codon 693 (using 770 nomenclature, corresponding to residue 22 of the Aβ sequence), ironically was found not in an AD kindred, but in a family with autosomal-dominant hereditary cerebral hemorrhage with amyloidosis, Dutch type *(5,6)*. The first AD-associated mutation in *APP* was found at codon 717 near the γ-secretase site, and resulted in a valine-to-isoleucine substitution *(7,8)*; since this seminal finding, different missense mutations at this same codon have also been documented in other families, resulting in the substitution of valine with either phenylalanine *(9)* or glycine *(10)*. Another missense mutation at codon 715 near the γ-secretase site has recently been described that results in a valine-to-methionine substitution *(11)*. A double missense mutation near the β-secretase site at codons 670 and 671 was identified in two separate Swedish families and results in a lysine-methionine to asparagine-leucine substitution *(12)*. Another mutation within the Aβ sequence has also been described that results in a glycine-to-alanine substitution at residue 21 *(13)*.

Only 2-3% of early–onset FAD cases are attributable to mutations within *APP*. Genetic linkage analysis revealed another locus implicated in early-onset FAD, which culminated in the identification of the *PS1* gene by positional cloning *(14)*. Shortly thereafter, a homologous gene called *PS2* was identified and cloned *(15–17)*. Nearly half of all FAD cases have been associated with mutations in the presenilin genes, mainly in the *PS1* gene, which accounts for up to 50% of early-onset FAD cases *(3)*. All of the more than 75 mutations in the *PS1* gene described to date are missense mutations, including the "Δexon 9" mutation *(18)*. Mutations in *PS2* are more rare than *PS1* and, thus far, only three missense mutations have been identified: asparagine-to-isoleucine at codon 141 in Volga-Germans, methionine-to-valine at codon 239 in Italian families *(15–17)*, and arginine-to-histidine at codon 62, which is found in a sporadic AD case and may represent a polymorphism with or without biological implications *(19)*.

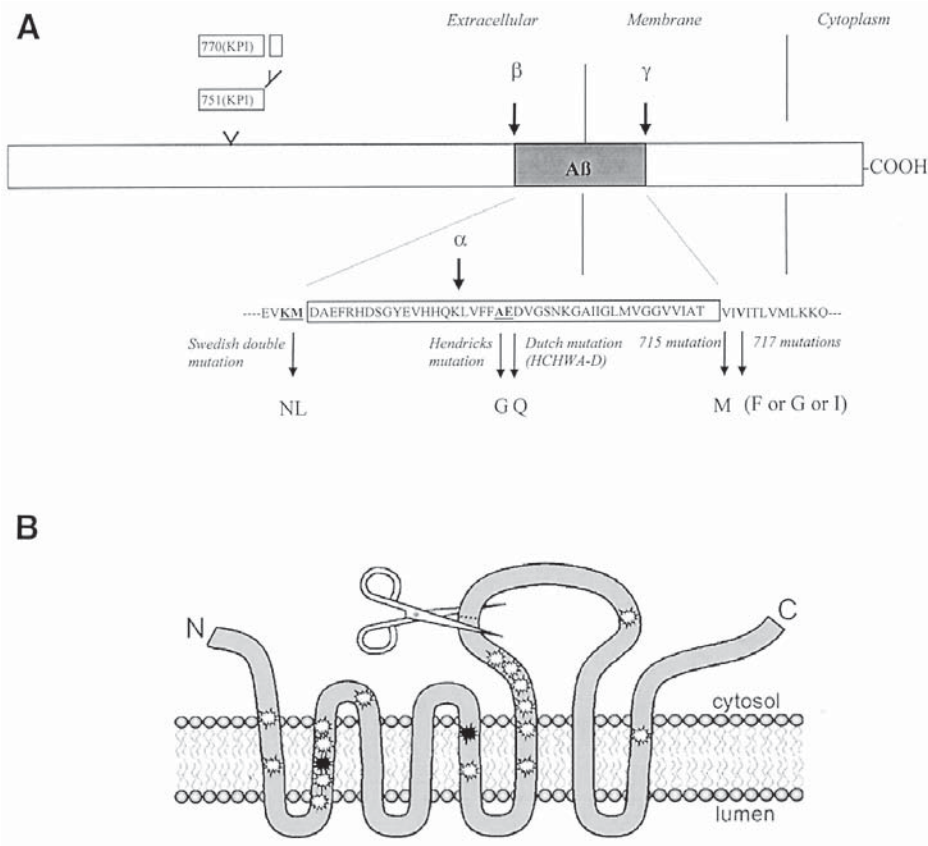

Fig. 1. Schematic diagram of the APP and PS molecules. (A) The position of the Aβ sequence within the APP molecule is indicated by the shaded box, with the 43-amino-acid version of the peptide indicated by single-letter amino acid code within the boxed area. The relative position of the α-, β-, γ- secretase cleavage sites are shown. The amino acid substitutions that have been identified in families with *APP* mutations are shown with the arrow pointing to the substituted amino acids. (Adapted from ref. *2*) (B) Schematic diagram of the presenilin molecule, showing multiple transmembrane domains. Some FAD *PS1* mutations are indicated in white and *PS2* mutations are indicated in black.

2.3. RISK FACTOR GENES

The characterization of a subset of FAD families that developed late-onset AD led to the linkage of a predisposing gene located on chromosome 19, which was identified as *apolipoprotein E* (*ApoE*) *(20–22)*. Three different allelic forms of the *ApoE* gene, referred to as ε2, ε3, and ε4, are present in the population. In 1993, Allen Roses and colleagues recognized that there

was an increased frequency of the ε4 allele among AD patients 65 years of age and older *(21,23)*. In short, possession of the ApoEε4 allele increases the risk of developing AD in a dose-dependent manner, whereas the ε2 allele confers protection from AD *(24)*. Depending on the combination of ApoE alleles inherited, individuals are susceptible to AD to varying degrees. Thus, the presence of each additional ε4 allele leads to an earlier onset of symptoms. In sum, *ApoE* is a genetic risk factor, not a causal agent, because even individuals without an ε4 allele develop AD and vice versa (*see* ref. *25* for review).

The genetics of late-onset AD have not been described as well as the genetics of early-onset FAD. The reason for this may be twofold. First, a significant number of late-onset AD cases may be sporadic (i.e., nongenetic). The second problem is the inherent difficulty associated with studying an aged population. Consequently, if a sizable proportion of late-onset cases are familial, it may be difficult to identify individuals destined to develop late-onset FAD, because they or their kin may die before the disease is manifested. Nevertheless, some candidate genes are emerging, including the identification of a gene on chromosome 12 called α2-macroglobulin, which may be associated with some late-onset FAD cases *(26)*.

2.4. A CENTRAL PATHOLOGIC ROLE FOR Aβ

The Aβ peptide is a heterogenous molecule that can display variability at both the N- and C-termini *(27)*. The significance of the C-terminal heterogenity is particularly notable, as it imparts profound biological consequences of significance to AD pathogenesis. A marked biophysical difference between these two species of Aβ is that the longer form ($A\beta_{42(43)}$) tends to be more amyloidogenic, forming fibrils in vitro more readily than the shorter form ($A\beta_{40}$) *(28)*. In vivo analyses also confirm the more pathogenic nature of the longer form, as immunohistochemical studies of human AD and brains of individuals with Down's Syndrome indicate that the earliest form of Aβ deposits consist of $A\beta_{42(43)}$. Thus far, a consistent theme to emerge is that all mutations within the *APP*, *PS1* and *PS2* genes linked to early-onset FAD eventually lead to enhanced production of either total Aβ levels or the more insoluble $A\beta_{42/43}$ *(29–37)*. The effects of the FAD-linked genes on Aβ has been observed in a variety of experimental systems including cell culture (both transfected cells overexpressing mutant proteins and fibroblasts from FAD patients) and transgenic mice (to be discussed later) and has provided strong evidence for the critical role of Aβ in AD neurodegeneration.

2.5. Aβ TRANSGENIC MICE

Although in vitro experiments had indicated that Aβ was deleterious to a variety of cell types grown in culture *(38)*, the in vivo toxicity of Aβ was less certain and somewhat controversial because direct injection of the peptide into the CNS of various animals yielded conflicting results (*see*, for example, ref. *39*). Therefore, we felt that a transgenic approach might be the most appropriate way to test the neurotoxicity of Aβ in an in vivo context. Rather than express the entire *APP* sequence, we opted to express only the Aβ peptide *(40)*. Moreover, in designing our transgenic model, we also wanted to determine whether Aβ toxicity was mediated as a result of intracellular accumulation or as part of its accumulation in the extracellular milieu. We hypothesized that toxicity might be concentration dependent (i.e., for efficient nucleation of Aβ to occur), and thus we rationalized that the Aβ concentration might reach higher levels inside a contained environment, such as inside the cell. Consequently, two sets of transgenic mice were developed that expressed a cDNA sequence encoding only the mouse Aβ peptide; the only difference between the two sets was that the ''extracellular'' mice contained the neural cell adhesion molecule (NCAM) signal sequence incorporated into the transgene to allow Aβ to be targeted extracellularly.

The neurologic phenotype observed in these mice has been extensively described *(40,41)*. In short, a surprising observation emerged from the study of these Aβ transgenic mice: Only the mice expressing Aβ intracellularly developed pathology. The pathophenotype that developed in the intracellular Aβ transgenic mice consisted of seizures, astrogliosis, neuronal cell death, and extracellular amyloid deposition. Perhaps even more surprising was the lack of neuropathology in the transgenic mice in which the NCAM signal sequence was incorporated; this was true despite the fact that these mice expressed the NCAM–Aβ transgene. These findings indicated that accumulation of Aβ intracellularly can have deleterious neurotoxic effects and highlight a previously underappreciated role of intracellular Aβ in the pathogenesis of AD.

Expressing Aβ intraneuronally initiated a cascade of pathological events that occurred in an age-dependent and region-specific fashion in the "intracellular" Aβ transgenic mice. The earliest phenotypic changes that we observed were changes in neuronal morphology that included neuritic degeneration and cytoplasmic vacuolization. A large percentage (approx 75%) of the transgenic mice also developed profound astrogliosis by 6 mo of age, providing confirmatory evidence of underlying central nervous system (CNS) injury evoked as a result of intracellular Aβ expression. At later time-points, other evidence of neuronal damage were apparent,

including fragmentation of nuclear DNA following TUNEL. Although TUNEL staining, by itself does not discriminate between cells dying by necrosis versus apoptosis *(42)*, we combined terminal deoxynucleotide transferase-mediated dUTP-biotin nick end labeling (TUNEL) with ultrastructural analysis which revealed neurons that were indeed morphologically altered in a manner consistent with cells undergoing apoptosis. Therefore, intracellular Aβ expression triggers neuronal apoptosis. This finding is consistent with apoptotic-inducing properties of Aβ administered in vitro *(43)* and with recent demonstrations that one of the pathologic mechanisms by which certain genes linked to FAD (e.g., presenilins) may induce disease is through enhancement of neuronal apoptosis (*see*, ref. *44* for review).

In addition to the primary neuronal injury, inflammatory or reactive processes were also apparent in the Aβ transgenic mice. Besides the prominent astrogliosis in the transgenic brains, reactive microglia were also present following histochemical staining with the lectin, RCA. Notably, as with the neuronal degeneration, both the astrogliosis and microgliosis occurred in brain regions such as the neocortex and hippocampus, which are major sites of AD pathology. Thus, expression of Aβ intraneuronally is able to trigger many reactive cellular processes within the brain. Finally, the occurrence of these reactive processes in the transgenic brains is relevant because they also represent a significant aspect of AD pathology *(45)*.

The profound extent of neuronal cell loss that occurred in the intracellular Aβ transgenic model is one feature that distinguishes it from other transgenic models. In addition, evidence from light and electron microscopy indicated that the cell death was consistent with an apoptotic pathway. More specifically, we found that neurons were dying by an apoptotic pathway that required p53 expression *(41)*. p53 is a pleiotropic molecule that plays an important role in inducing cellular apoptosis *(46)*. There are now several reports that indicate that p53 expression is elevated in AD brains *(47–49)*; thus, it is quite likely that, as was the case for the Aβ transgenic mice, p53 may play a comparable role in mediating cell death in the AD brain.

Although the occurrence of extracellular Aβ deposits was not as robust in our intracellular Aβ transgenic mice as in some of the mutant APP mice (*see* Subheading 2.6), an important hypothesis regarding the genesis of Aβ plaques has emerged nevertheless. Specifically, we proposed that extracellular deposition of Aβ occurs following neuronal cell death. In part, this hypothesis is supported by our observations that extracellular Aβ deposition occurred in areas in which there was prominent TUNEL labeling in the transgenic brains, indicating that cell death precedes extracellular amyloid deposition in this transgenic model *(41)*.

The finding that expression of intracellular Aβ leads to neuronal cell death via apoptosis in the transgenic brains prompted us to investigate human AD brain postmortem samples to determine if neuronal loss also occurred by apoptosis. We found that indeed there was evidence for neuronal cell death occurring by apoptosis *(50)*, which agrees with numerous other reports *(51,52)*. Curiously, the number of TUNEL-positive cells in the AD brain is substantially higher than one might have predicted *a priori*, given the rapid demise the apoptotic cell undergoes in vitro. The reasons for this are unclear, but many of the cells containing fragmented DNA also paradoxically express bcl-2 *(53)*, which is counterintuitive given that bcl-2 normally represses cell death. Thus, it is plausible that despite the extent of DNA damage, mechanisms exist to retard the loss of postmitotic cells to perhaps allow repair so that the cell can survive.

Histological analysis in combination with the TUNEL procedure allowed us to identify neurons with different degrees of DNA fragmentation, which we interpret to reflect different stages in the cell death pathway. More importantly, from this analysis, we observed that degenerating neurons exhibiting DNA damage also contained *intracellular* Aβ accumulation, despite the absence of any extracellular Aβ. Furthermore, these same cells also contained elevated expression of apoE, which likely stabilizes the hydrophobic Aβ. Therefore, we concluded that, as in the transgenic brains, intracellular accumulation represents a key feature in the pathogenesis of AD that precedes the extracellular accumulation of amyloid plaques *(50)*.

In sum, although expressing Aβ intracellularly in transgenic mice represented an unorthodox approach, these mice did develop some pathological changes that are consistent with those observed in the AD brain. Since then, the suggestion that intracellular Aβ may play an important role in the disease process has been growing. Aβ has traditionally been regarded as manifesting its neurotoxic effects from outside the cell. In part, this is because amyloid plaques are localized extracellularly in the AD brain and because administration of Aβ to cultured cells is known to be toxic *(38)*. However, it is uncertain whether the results of the in vitro studies accurately mimic the means by which Aβ is pathogenic in AD or whether it is the exclusive mechanism by which Aβ can be neurotoxic.

Evidence supporting a role for intracellular Aβ in the pathogenesis of AD includes in vitro work with synthetic Aβ peptide *(54,55)* and with mutant genes linked to FAD *(56,57)*, and findings from transgenic mice and AD brains *(40,41,50,58)*. It is our hypothesis that perturbations of the normal cellular processing of APP or selective reuptake of Aβ would cause the peptide to accumulate intracellularly. As nucleation is concentration

dependent, intracellular Aβ accumulation would facilitate the peptide's formation of neurotoxic aggregates, ultimately causing cell death. The Aβ released following cell death would form an extracellular nidus for neuritic plaque formation, leading to secondary cellular damage by glial activation or other inflammatory responses. Despite evidence supporting this hypothesis, the role of intracellular Aβ in AD remains controversial. However, another age-related disorder called inclusion body myositis (IBM), is characterized by skeletal muscle fiber degeneration associated with accumulation of intracellular Aβ *(59)*.

2.6. *APP* TRANSGENIC AND KNOCKOUT MICE

A frequent approach used to elucidate the function of a protein in an organism is to knock out the encoding gene. Genetically modified mice have been generated that contain a functionally inactive *APP* gene *(60,60a)*; these mice exhibited behavioral deficits, showing slight decreases in motor activity and forelimb grip strength when compared to age-matched controls, which indicates that *APP* is necessary for optimal cellular functioning. Marked reactive gliosis was also observed in the brains of young mice, consistent with absence of *APP* leading to altered neural functioning and the activation of secondary processes. In addition, the *APP* null mice exhibited deficits in spatial learning, impaired long-term potentiation and a reduction in synaptic density, suggesting that *APP* may be necessary for maintenance of synaptic function during aging *(61)*.

Neuroanatomical studies of the brain did not reveal significant differences in the knock out mice as compared to the wild-type controls. Consequently, either *APP* is not essential for mouse embryonic and early neuronal development or the absence of *APP* was compensated by the highly homologous *APP*-like proteins 1 and 2 (*APLP1*, *APLP2*) *(62,63)*. Genetically modified mice have been derived that lack both *APP* and *APLP2 (64)*. The absence of both genes results in early lethality as 80% of double knock out mice die within the first week after birth. Those mice that survive beyond this time-point are reduced in body weight and show several postural and motor abnormalities. Thus, it appears that *APLP2* and *APP* are required for early postnatal development and that *APLP2* and *APP* can compensate for each other functionally. In sum, the results of these single and double knockout mice show that loss of *APP* function does not lead to AD neuropathology in mice, which indicates that *APP* mutations lead to AD by a mechanism other than loss of *APP* function.

To address the pathophysiological role of *APP* in transgenic mice, generally one of two experimental strategies have been utilized: either

expression of the carboxyl fragment of *APP* termed C100 (which consists of 100–104 amino acids from the C' terminus of *APP* including Aβ) or expression of the full-length *APP* molecule, encoded by either genomic or cDNA sequences. Earlier approaches focused on overexpressing wild-type *APP* (APP_{WT}), which resulted in some pathological or behavioral alterations *(65–68)*. Following the identification of *APP* mutations, the consequences of their expression could be measured in the CNS of transgenic mice, particularly in regard to whether they were sufficient to induce AD-like pathology. The most dramatic successes have centered on transgenic models harboring APP molecules that either contained missense mutations near the γ-secretase site *(69)* or the β-secretase site *(70)*. Since then, numerous other laboratories have used comparable approaches. In short, although neither model fully mimics the complete spectrum of AD pathology, the robust Aβ deposits observed in the CNS of these transgenic mice provided confirmatory evidence that mutations within the *APP* gene are, in fact, pathological mutations responsible for some cases of early-onset FAD.

An in depth analysis of all the *APP* transgenic models is beyond the scope of this chapter, therefore we focus on two of the more prominent lines of transgenic mice that developed certain key pathological features, notably extensive extracellular Aβ deposits. Although the transcriptional promoter and *APP* mutant differed in each construct, there were, nevertheless, several histopathological commonalties observed in the brains of both transgenic lines. One common feature shared by the PD-APP *(69)* and prion protein (PrP)–APP_{SW} (PrP-APP_{SW}) *(70)* mice was the age-related increase in Aβ production in the brain. Relative levels of $Aβ_{40}$ and $Aβ_{42(43)}$ were measured using enzyme-linked immunosorbent assay (ELISA). Analysis of Aβ concentrations within the hippocampus of PD–APP mice revealed a 17-fold increase between the ages of 4 and 8 mo and a 500-fold increase from 4 to 18 mo of age *(71)*. Likewise, ELISA analysis of *PrP–APP_{SW}* brain showed 5 and 14 times the amount of $Aβ_{40}$ and $Aβ_{42(43)}$, respectively, in 11- to 13-mo- old transgenic mice when compared to 2- to 5-mo- old transgenic mice. This dramatic age-dependent increase in overall Aβ levels as well as in the $Aβ_{42(43)/40}$ ratio clearly mimics an important characteristic of the human disorder.

Despite the use of different neuronal promoters, both groups observed extracellular deposition of Aβ that was distributed in a region-specific fashion in the brain. This is true, for example, despite the use of the platelet-derived growth factor-beta (PDGF) promoter, which results in widespread neuronal expression throughout the CNS *(72)*. Aβ deposits in the *PD–APP* brain were localized within the hippocampus, corpus callosum, and cerebral cortex but absent in other brain structures, thus paralleling the regional

distribution that occurs in human AD brains. In *PrP–APP*$_{SW}$ mice, Aβ deposits were found in frontal, temporal, and entorhinal cortex, hippocampus, presubiculum, subiculum, and cerebellum. The regional specificity of Aβ accumulation is intriguing because the promoters used in the transgene constructs expressed APP globally in neurons throughout the CNS, suggesting that region-specific factors may influence the accumulation and/or clearance of Aβ.

In addition to widespread Aβ deposition, other pathological markers characteristic of the AD brain such as reactive gliosis and synaptic loss were observed in both mice. Histopathologically, in the *PrP–APP*$_{SW}$ brains, amyloid cores were surrounded by glial fibriallary acidic protein (GFAP) immunoreactive astrocytes and dystrophic neurites. In *PD–APP* brain, the majority of amyloid plaques were also surrounded by extensive GFAP immunoreactive astrocytes and usually associated with dystrophic neurites from surrounding cells *(73)*. In addition, in *PD–APP* transgenic brains, the synaptic density in the molecular layer of the hippocampal dendate gyrus was markedly reduced following visualization with synaptophysin, a presynaptic marker, and MAP2, a dendritic marker *(69)*. This loss of synaptic density within the hippocampus, a structure important for learning and memory, is a major histopathological feature of the AD brain *(74)* and may be a causal factor in the memory loss associated with the disease.

Because an important clinical manifestation of AD is the loss of short-term memory, a comprehensive transgenic model would not only have to contain the major hallmarks of AD pathology (i.e., plaques and tangles) but would also have to exhibit the accompanying behavioral deficits. Several of the *APP* transgenic models do show deficits in certain behavioral tests designed to evaluate learning and memory performance, such as spatial reference and alternation tasks *(67,70,75,76)*. In addition, these transgenic mice also exhibit changes in synaptic plasticity such as induction of long-term potentiation (LTP) *(61,76,77)*.

In sum, transgenic mice expressing mutant *APP* at high levels were successful at producing amyloid deposits distributed in a region-specific manner similar to that observed in the AD brain, along with varying degrees of learning and memory deficits. One of the key features that distinguishes the APP models mentioned earlier from the other models is the high level of expression that is required for plaque formation (*see*, for example, ref. *78*). *PD–APP* transgenic mice expressed human APP to over 10 times the level of endogenous mouse APP at the protein level. The *PrP–APP*$_{SW}$ mice expressed the *APP* transgene mRNA in brain over fivefold the endogenous

APP levels in 14-mo-old transgenic mice. Notably, despite this high level of transgene APP expression and resultant prominent Aβ deposition, there is a relative paucity of neuronal cell death. The implications for the relationship of the genesis of Aβ plaques and neuronal cell death are not clear.

2.7. PRESENILIN TRANSGENIC AND KNOCKOUT MICE

Given the earlier age of disease onset in *PS1* versus *APP* FAD pedigrees (30–50 yr versus 50–60 yr) and the rapid clinical demise that occurs in *PS1* families, one might likely predict that overexpression of *PS1* mutations in transgenic mice would induce more severe pathology than *APP* transgenic mice. Surprisingly, these mice did not develop amyloid plaques. There is no obvious theoretical explanation to account for this observation and it may simply be a reflection of not achieving adequate levels of *PS1* overexpression in the appropriate cell types.

Several groups characterized transgenic mice overexpressing human mutant PS1 molecules *(29,32,33)*. Although the promoter, *PS1* mutation, and background strain of mice differed, two consistent findings emerged. First, overexpression of mutant human *PS1* in transgenic mice results in increased levels of $A\beta_{42(43)}$. Although overexpression of human wild-type PS1 increases $A\beta_{42(43)}$ levels in transgenic mice, these levels are dramatically increased in mice harboring *PS1* missense mutations. Not all *PS1* mutations elevate $A\beta_{42(43)}$ to comparable levels; for example, transgenic mice harboring the methionine-146-leucine (M146V) mutation had a greater increase in $A\beta_{42(43)}$ than did mice overexpressing the leucine-286-valine mutation *(32)*. This disparity may indicate that certain regions of the PS1 protein are more critical, at least with regards to modulating APP processing.

Both the PS1 and PS2 proteins are subject to endoproteolytic processing in vivo; the net effect is that it can be difficult to detect the holoproteins in vivo and that the major detectable species in brain are an approx 27-kDa N-terminal and approx 19-kDa C-terminal fragments *(79)*. The second finding to emerge was that PS1 and PS2 appear to compete for common proteolytic factor(s), as it was observed that saturable levels of the N- and C-terminal fragments accumulate at approximately 1 : 1 stoichiometry in transgenic mice, an effect independent of transgene-derived mRNA levels. These studies reveal that compromised accumulation of murine *PS1* derivatives resulting from overexpression of human *PS1* occurs in a manner independent of endoproteolytic cleavage, consistent with a model in which the abundance of PS1 fragments is regulated coordinately by competition for limiting cellular factors *(79,80)*.

The pathogenic means by which mutations in the presenilin genes lead to AD may involve three mechanisms. One likely mechanism clearly involves elevation of $A\beta_{42(43)}$ levels, as the transgenic models described earlier clearly illustrate; it is still not established whether this effect is primary or whether it lies downstream of other molecular processes. The second mechanism may involve enhanced sensitivity to apoptosis (*see* ref. *44* for review). Recently, *PS1* mutant knock-in mice have been developed that express the human PS1 M146V mutation at normal physiological levels *(81)*; primary hippocampal neurons from these *PS1* mutant knock-in mice exhibit increased vulnerability to Aβ toxicity. The third mechanism may involve disruption of calcium homeostasis *(81,82)*. For example, primary cells from the *PS1* mutant knock-in mice contain elevated calcium stores in the endoplasmic reticulum and deficits in capacitative calcium entry *(82a)*. Thus, although Aβ may be the most obvious readout of mutations in the presenilin genes, it may not necessarily be the primary effect. Recent transgenic data support this hypothesis. Chui et al. *(58)* developed mutant PS1 transgenic mice and found that neurodegeneration was significantly accelerated in mice older than 13 mo, without amyloid plaque formation. However, they reported significantly more neurons containing intracellularly deposited $A\beta_{42}$ in aged mutant transgenic mice, indicating that the pathogenic role of the *PS1* mutation is upstream of the amyloid cascade *(58)*.

To elucidate the physiological role of the PS1 molecule in vivo during development, *PS1*-deficient mice were created by effectively disrupting the murine gene in mouse embryos *(83,84)*. Knocking out the *PS1* gene at this early stage of development has a lethal effect; null mutants have abnormal skeletal deformities and hemorrhages in the central nervous system. These physical characteristics are similar to mice with inactivated Notch 1 *(85,86)*, which is not surprising given the homology between the presenilins and sel-12 *(87)*. These findings may indicate that PS1 expression is required for neuronal survival and normal neurogenesis *(83)*. The PS1 null mice can be "rescued" by being crossed to transgenic mice harboring either wild-type or mutant *PS1 (88,89)*, demonstrating that the PS1 mutation does not lead to a total loss of function during development.

Although overexpression of mutant *PS1* in transgenic mice increases $A\beta_{42(43)}$ levels, Aβ levels are decreased fivefold in the *PS1* knockout embryos *(90)*. The turnover of the membrane-associated fragments of APP was specifically decreased in the null mice. Therefore, it appears that PS1 mediates a proteolytic activity that cleaves the integral membrane domain of APP; simply stated, either PS1 may modulate γ-secretase cleavage of APP or it may even be the γ-secretase molecule *(91)*.

2.8. *APP* AND *PS1* DOUBLY TRANSGENIC MICE

The single transgenic models of AD, defined as carrying one FAD-linked mutation, demonstrate either a limited pathology, as in the case of mice overexpressing *PS1* mutations or a late onset of pathology, as in the case of mice overexpressing *APP* mutations. To create an animal model that develops more severe AD-like neuropathology, including perhaps an earlier age of onset, several laboratories have focused their attention on developing transgenic mice that harbor two or more FAD-linked mutations *(32,33,92)*. Consequently, transgenic mice have been generated that carry a mutant *APP* gene and a mutant *PS1* gene.

Alzheimer's disease-like pathology was accelerated in the double transgenic mice that carried either the A246E or M146L mutation in the *PS1* gene and the APP_{SW} gene *(32,33,92)*. Amyloid deposits were abundant at 6 mo of age and distributed in a region-specific manner in the cerebral cortex and hippocampus. By contrast, similar pathology was not evident in the single APP_{SW} transgenic mice until 9–12 mo of age *(70)*. As mentioned earlier, amyloid deposition was not present in the single *PS1* mutant transgenic mice (see, for example ref *58*). Taken together, these observations suggest that the mutant PS1 acts synergistically with APP_{SW} to accelerate APP processing and amyloid deposition. Because these mice develop plaques at a relatively early age, they may prove to be a more efficient model system to evaluate the usefulness of potential AD therapeutics and, moreover, certainly indicate that overexpression of more than one FAD-linked gene may be essential to develop an animal model that contains all of the hallmark pathological features of AD.

2.9. *ApoE* TRANSGENIC AND KNOCKOUT MICE

ApoE has been identified as a major risk factor that modifies the age of onset for AD *(93)*. Although apoE can bind to and stabilize Aβ *(23)*, its precise physiological role in the CNS or in the pathogenesis of AD remains to be established. To address its role during development, genetically modified mice have been derived in which the gene was effectively knocked out *(94,95)*. In short, no obvious phenotypic alterations were evident in *ApoE* null mice, which appeared to be relatively healthy when compared to wild-type controls; thus, *ApoE* is not essential for development. The *ApoE*-deficient mice, however, had significantly higher levels of serum cholesterol than age-matched controls receiving the same diet, consistent with a known role for apoE in the transport of cholesterol *(96)*.

To elucidate the role of human apoE in brain, the three different protein isoforms (ε2, ε3, or ε4) were individually overexpressed in transgenic mice

on a null murine *ApoE* background *(97–99)*. This approach allowed for the characterization of the effects of human ApoE in mice without the confounding influence of the endogenous *ApoE* gene. Several approaches to express human *ApoE* in null mice have used neuronal specific promoters *(97,99,100)*. *ApoE* is normally expressed to relatively high levels in glial cells, although recent evidence for expression in neurons has also been provided *(101)*. Another approach utilized the GFAP promoter to direct expression to astrocytes, because ApoE in the CNS is primarily found in astrocytes. Immunohistochemical analysis of these transgenic mice at 14 mo of age failed to show any evidence of amyloid deposition or increase in Aβ levels.

To study the effects of human apoE isoforms on Aβ depostion in transgenic mice, *ApoE* transgenic mice (on a mouse null ApoE background) were crossed with *APP* mice containing the valine-717-phenylalanine mutation *(102)*. Aβ deposition was significantly less in ApoE 3 and 4 mice crossed with mutant *APP* mice compared to mutant *APP* mice alone. These findings are somewhat counterintuitive given the strong association between Aβ deposition and apoE isoform *(23)*. If true, these results implicate a potential role for apoE 3 and 4 in increasing clearance and/or decreasing aggregation of Aβ.

2.10. CONCLUDING REMARKS

The goal of these transgenic endeavors is to create an animal model which faithfully mimics the major histopathological and behavioral features of AD. It is expected that such an animal model would be an invaluable tool in the development of treatments to prevent or halt the progression of disease. Currently, none of the transgenic models expressing a single *FAD* gene meets this criterion, but the development of transgenic models that express more than one AD-associated gene may be the key to overcoming this inadequacy. Nevertheless, the single transgenic models have provided novel insights into the pathogenesis of AD. For instance, one consistent theme that has emerged from the genetic studies is that mutations in all of the genes linked to autosomal-dominant AD affect production or accumulation of Aβ. Certainly, transgenic models have been key in providing some of this supporting evidence, which clearly underscores the important pathological role of Aβ in the genesis of AD.

The AD transgenic mice are starting to pave the way for potential and novel therapeutic approaches toward the treatment of this insidious neurodegenerative disorder. One of the most promising of these therapies involves vaccination of transgenic mice with Aβ *(103)*. Schenk et al. showed that immunization of the PD-APP transgenic mice $A\beta_{42}$ at an early age (prior

to onset of AD pathology/plaque formation) essentially prevented the development of Aβ deposition and other neuropathological changes such as neuritic dystrophy and astrogliosis. Likewise, immunization of older mice with well-established neuropathologies also was efficacious in reducing the extent and progression of the pathology. Whether this treatment will be effective (or even safe) in human patients awaits results from clinical trials.

Unexpected advances toward the development of a comprehensive transgenic model of AD occasionally emerge from previously unpredictable avenues of research. One recently described and very exciting model was reported by Capsoni et al. *(104)*. These authors created transgenic mice in which the CMV promoter was used to overexpress a neutralizing antibody directed against nerve growth factor (NGF). Levels of free NGF in the brains of transgenic mice were 53% less than in control mice. Intriguingly, the aged 15- to 17-mo old anti-NGF transgenic mice exhibited AD-type phenotypic changes including β-amyloid plaques, neurofibrillary tangles, tau hyperphosphorylation, neuronal death, and selective behavioral deficits. The mechanism by which neutralization of NGF in the brain leads to the hallmark features of AD neuropathology remains to be determined. Regardless of the mechanism, the bottom line is that this model currently represents the most comprehensive model of AD.

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REFERENCES

1. Yankner, B. A. (1996) Mechanisms of neuronal degeneration in Alzheimer's disease. *Neuron* 16, 921–932.
2. Price, D. L. and Sisosida, S. S. (1998) Mutant genes in familial Alzheimer's disease and transgenic models. *Annu. Rev. Neurosci.* 21, 479–505.
3. Cruts, M., van Duijn, C. M., Backhovens, H., Van den Broeck, M., Wehnert, A., Serneels, S., et al. (1998) Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease. *Hum. Mol. Genet.* 7, 43–51.
4. Goate, A. M. (1998) Monogenetic determinants of Alzheimer's disease: APP mutations. *Cell Mol. Life Sci.* 54, 897–901.
5. Levy, E., Carman, M. D., Fernandez-Madrid, I. J., Power, M. D., Lieberburg, I., van Duinen, S. G., et al. (1990) Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* 248, 1124–1126.
6. Van Broeckhoven, C., Haan, J., Bakker, E., Hardy, J. A., Van Hul, W., Wehnert, A., et al. (1990) Amyloid β protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). *Science* 248, 1120–1122.

7. Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., et al. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349, 704–706.
8. Naruse, S., Igarashi, S., Kobayashi, H., Aoki, K., Inuzuka, T., Kaneko, K., et al. (1991) Mis-sense mutation Val—Ile in exon 17 of amyloid precursor protein gene in Japanese familial Alzheimer's disease. *Lancet* 337, 978–979.
9. Murrell, J., Farlow, M., Ghetti, B., and Benson, M. D. (1991) A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science* 254, 97–99.
10. Chartier-Harlin, M. C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., et al. (1991) Early-onset Alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. *Nature* 353, 844–846.
11. Ancolio, K., Dumanchin, C., Barelli, H., Warter, J. M., Brice, A., Campion, D., et al. (1999) Unusual phenotypic alteration of β amyloid precursor protein (βAPP) maturation by a new val-715 —> met βAPP-770 mutation responsible for probable early-onset Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 96, 4119–4124.
12. Mullan, M., Houlden, H., Windelspecht, M., Fidani, L., Lombardi, C., Diaz, P., et al. (1992) A locus for familial early-onset Alzheimer's disease on the long arm of chromosome 14, proximal to the alpha 1-antichymotrypsin gene. *Nat. Genet.* 2, 340–342.
13. Hendriks, L., van Duijn, C. M., Cras, P., Cruts, M., Van Hul, W., van Harskamp, et al. (1992) Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the β-amyloid precursor protein gene. *Nat. Genet.* 1, 218–221.
14. Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., et al. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 375, 754–760.
15. Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D. M., Oshima, J., Pettingell, W. H., et al. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* 269, 973–977.
16. Li, J., Ma, J., and Potter, H. (1995) Identification and expression analysis of a potential familial Alzheimer disease gene on chromosome 1 related to AD3. *Proc. Natl. Acad. Sci. USA* 92, 12,180–12,184.
17. Rogaev, E. I., Sherrington, R., Rogaeva, E. A., Levesque, G., Ikeda, M., Liang, Y., et al. (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* 376, 775–778.
18. Steiner, H., Romig, H., Grim, M. G., Philipp, U., Pesold, B., Citron, M., et al. (1999) The biological and pathological function of the presenilin-1 Deltaexon 9 mutation is independent of its defect to undergo proteolytic processing. *J. Biol. Chem.* 274, 7615–7618.
19. Lao, J. I., Beyer, K., Fernandez-Novoa, L., and Cacabelos, R. (1998) A novel mutation in the predicted TM2 domain of the presenilin 2 gene in a Spanish patient with late-onset Alzheimer's disease. *Neurogenetics* 1, 293–296.

20. Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., et al. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261, 921–923.
21. Saunders, A. M., and Roses, A. D. (1993) Apolipoprotein E4 allele frequency, ischemic cerebrovascular disease, and Alzheimer's disease. *Stroke* 24, 1416–1417.
22. Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S., et al. (1993) Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 90, 1977–1981.
23. Strittmatter, W. J., Weisgraber, K. H., Huang, D. Y., Dong, L. M., Salvesen, G. S., Pericak-Vance, M., et al. (1993) Binding of human apolipoprotein E to synthetic amyloid β peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 90, 8098–8102.
24. Corder, E. H., Saunders, A. M., Risch, N. J., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Jr., et al. (1994) Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat. Genet.* 7, 180–184.
25. Roses, A. D. (1996) Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu. Rev. Med.* 47, 387–400.
26. Blacker, D., Wilcox, M. A., Laird, N. M., Rodes, L., Horvath, S. M., Go, R. C., et al. (1998) Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nat. Genet.* 19, 357–360.
27. Selkoe, D. J. (1998) The cell biology of β-amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol.* 8, 447–453.
28. Jarrett, J. T., Berger, E. P., and Lansbury, P. T., Jr. (1993. The carboxy terminus of the ß amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* 32, 4693–4697.
29. Borchelt, D. R., Thinakaran, G., Eckman, C. B., Lee, M. K., Davenport, F., Ratovitsky, T., et al. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Aß1-42/1-40 ratio *in vitro* and *in vivo*. *Neuron* 17, 1005–1013.
30. Cai, X. D., Golde, T. E., and Younkin, S. G. (1993) Release of excess amyloid β protein from a mutant amyloid β protein precursor. *Science* 259, 514–516.
31. Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A. Y., Seubert, P., et al. (1992) Mutation of the ß-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. *Nature* 360, 672–674.
32. Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., et al. (1997) Mutant presenilins of Alzheimer's disease increase production of 42- residue amyloid β-protein in both transfected cells and transgenic mice. *Nat. Med.* 3, 67–72.
33. Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C. M., Perez-tur, J., et al. (1996) Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. *Nature* 383, 710–713.
34. Mehta, N. D., Refolo, L. M., Eckman, C., Sanders, S., Yager, D., Perez-Tur, J., et al. (1998) Increased Aβ42(43) from cell lines expressing presenilin 1 mutations. *Ann. Neurol.* 43, 256–258.

35. Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., et al. (1996) Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med.* 2, 864–870.
36. Suzuki, N., Cheung, T. T., Cai, X. D., Odaka, A., Otvos, L., Jr., Eckman, C., et al. (1994) An increased percentage of long amyloid β protein secreted by familial amyloid β protein precursor (βAPP717) mutants. *Science* 264, 1336–1340.
37. Xia, W., Zhang, J., Kholodenko, D., Citron, M., Podlisny, M. B., Teplow, D. B., et al. (1997) Enhanced production and oligomerization of the 42-residue amyloid β-protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J. Biol. Chem.* 272, 7977-7982.
38. Cotman, C. W., Pike, C. J., and Copani, A. (1992) β-Amyloid neurotoxicity: a discussion of *in vitro* findings. *Neurobiol. Aging* 13, 587–590.
39. Podlisny, M. B., Stephenson, D. T., Frosch, M. P., Lieberburg, I., Clemens, J. A., and Selkoe, D. J. (1992) Synthetic amyloid β-protein fails to produce specific neurotoxicity in monkey cerebral cortex. *Neurobiol. Aging* 13, 561–567.
40. LaFerla, F. M., Tinkle, B. T., Bieberich, C. J., Haudenschild, C. C., and Jay, G. (1995) The Alzheimer's Aβ peptide induces neurodegeneration and apoptotic cell death in transgenic mice. *Nat. Genet.* 9, 21–30.
41. LaFerla, F. M., Hall, C. K., Ngo, L., and Jay, G. (1996) Extracellular deposition of β-amyloid upon p53-dependent neuronal cell death in transgenic mice. *J. Clin. Invest.* 98, 1626–1632.
42. Charriaut-Marlangue, C. and Ben-Ari, Y. (1995) A cautionary note on the use of the TUNEL stain to determine apoptosis. *Neuroreport* 7, 61–64.
43. Loo, D. T., Copani, A., Pike, C. J., Whittemore, E. R., Walencewicz, A. J., and Cotman, C. W. (1993) Apoptosis is induced by β-amyloid in cultured central nervous system neurons. *Proc. Natl. Acad. Sci. USA* 90, 7951–7955.
44. Mattson, M. P., Guo, Q., Furukawa, K., and Pedersen, W. A. (1998) Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. *J. Neurochem.* 70, 1–14.
45. Unger, J. W. (1998) Glial reaction in aging and Alzheimer's disease. *Microsc. Res. Tech.* 43, 24–28.
46. Gottlieb, T. M. and Oren, M. (1998) p53 and apoptosis. *Semin. Cancer Biol.* 8, 359–368.
47. de la Monte, S. M., Sohn, Y. K., Ganju, N., and Wands, J. R. (1998) P53- and CD95-associated apoptosis in neurodegenerative diseases. *Lab. Invest.* 78, 401–411.
48. de la Monte, S. M., Sohn, Y. K., and Wands, J. R. (1997) Correlates of p53- and Fas (CD95)-mediated apoptosis in Alzheimer's disease. *J. Neurol. Sci.* 152, 73–83.
49. Kitamura, Y., Shimohama, S., Kamoshima, W., Matsuoka, Y., Nomura, Y., and Taniguchi, T. (1997) Changes of p53 in the brains of patients with Alzheimer's disease. *Biochem. Biophys. Res. Commun.* 232, 418–421.
50. LaFerla, F. M., Troncoso, J. C., Strickland, D. K., Kawas, C. H., and Jay, G. (1997) Neuronal cell death in Alzheimer's disease correlates with apoE uptake and intracellular Aβ stabilization. *J. Clin. Invest.* 100, 310–320.

51. Lassmann, H., Bancher, C., Breitschopf, H., Wegiel, J., Bobinski, M., Jellinger, K., et al. (1995) Cell death in Alzheimer's disease evaluated by DNA fragmentation in situ. *Acta Neuropathol. (Berl.)* 89, 35–41.
52. Su, J. H., Anderson, A. J., Cummings, B. J., and Cotman, C. W. (1994) Immunohistochemical evidence for apoptosis in Alzheimer's disease. *NeuroReport* 5, 2529–2533.
53. Su, J. H., Satou, T., Anderson, A. J., and Cotman, C. W. (1996) Up-regulation of Bcl-2 is associated with neuronal DNA damage in Alzheimer's disease. *NeuroReport* 7, 437–440.
54. Knauer, M. F., Soreghan, B., Burdick, D., Kosmoski, J., and Glabe, C. G. (1992) Intracellular accumulation and resistance to degradation of the Alzheimer amyloid A4/β protein. *Proc. Natl. Acad. Sci. USA* 89, 7437–7441.
55. Yang, A. J., Knauer, M., Burdick, D. A., and Glabe, C. (1995) Intracellular Aβ 1–42 aggregates stimulate the accumulation of stable, insoluble amyloidogenic fragments of the amyloid precursor protein in transfected cells. *J. Biol. Chem.* 270, 14,786–14,792.
56. Martin, B. L., Schrader-Fischer, G., Busciglio, J., Duke, M., Paganetti, P., and Yankner, B. A. (1995) Intracellular accumulation of β-amyloid in cells expressing the Swedish mutant amyloid precursor protein. *J. Biol. Chem.* 270, 26,727–26,730.
57. Wild-Bode, C., Yamazaki, T., Capell, A., Leimer, U., Steiner, H., Ihara, Y., et al. (1997) Intracellular generation and accumulation of amyloid β-peptide terminating at amino acid 42. *J. Biol. Chem.* 272, 16,085–16,088.
58. Chui, D. H., Tanahashi, H., Ozawa, K., Ikeda, S., Checler, F., Ueda, O., et al. (1999) Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation. *Nat. Med.* 5, 560–564.
59. Askanas, V., Engel, W. K., Alvarez, R. B., and Glenner, G. G. (1992) β-Amyloid protein immunoreactivity in muscle of patients with inclusion-body myositis. *Lancet* 339, 560–561.
60. Zheng, H., Jiang, M., Trumbauer, M. E., Sirinathsinghji, D. J., Hopkins, R., Smith, D. W., et al. (1995) β-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell* 81, 525–531.
60a. Muller, U., Cristina, N., Li, Z. W., Wolfer, D. P., Lipp, H. P., Rulicke, T., Brandner, S., Aguzzi, A., and Weissmann, C. (1994) Behavioral and anatomical deficits in mice homozygous for a modified beta-amyloid precursor protein gene. *Cell* 79, 755–765.
61. Dawson, G. R., Seabrook, G. R., Zheng, H., Smith, D. W., Graham, S., O'Dowd, et al. (1999) Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the β-amyloid precursor protein. *Neuroscience* 90, 1–13.
62. Wasco, W., Bupp, K., Magendantz, M., Gusella, J. F., Tanzi, R. E., and Solomon, F. (1992) Identification of a mouse brain cDNA that encodes a protein related to the Alzheimer disease-associated amyloid β protein precursor. *Proc. Natl. Acad. Sci. USA* 89, 10,758–10762.
63. Wasco, W., Gurubhagavatula, S., Paradis, M. D., Romano, D. M., Sisodia, S. S., Hyman, B. T., et al. (1993) Isolation and characterization of APLP2

encoding a homologue of the Alzheimer's associated amyloid β protein precursor. *Nat. Genet.* 5, 95–100.
64. von Koch, C. S., Zheng, H., Chen, H., Trumbauer, M., Thinakaran, G., van der Ploeg, L. H., et al. (1997) Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice. *Neurobiol. Aging* 18, 661–669.
65. Higgins, L. S., Catalano, R., Quon, D., and Cordell, B. (1993) Transgenic mice expressing human β-APP751, but not mice expressing β-APP695, display early Alzheimer's disease-like histopathology. *Ann. NY Acad. Sci.* 695, 224–227.
66. Lamb, B. T., Sisodia, S. S., Lawler, A. M., Slunt, H. H., Kitt, C. A., Kearns, W. G., et al. (1993) Introduction and expression of the 400 kilobase amyloid precursor protein gene in transgenic mice. *Nat. Genet.* 5, 22–30.
67. Moran, P. M., Higgins, L. S., Cordell, B., and Moser, P. C. (1995) Age-related learning deficits in transgenic mice expressing the 751- amino acid isoform of human β-amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* 92, 5341–5345.
68. Quon, D., Wang, Y., Catalano, R., Scardina, J. M., Murakami, K., and Cordell, B. (1991) Formation of β-amyloid protein deposits in brains of transgenic mice. *Nature* 352, 239–241.
69. Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., et al. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature* 373, 523–527.
70. Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., et al. (1996) Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* 274, 99–102.
71. Johnson-Wood, K., Lee, M., Motter, R., Hu, K., Gordon, G., Barbour, R., et al. (1997) Amyloid precursor protein processing and Aβ42 deposition in a transgenic mouse model of Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 94, 1550–1555.
72. Sasahara, M., Fries, J. W., Raines, E. W., Gown, A. M., Westrum, L. E., Frosch, M. P., et al. (1991) PDGF B-chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. *Cell* 64, 217–227.
73. Masliah, E., Sisk, A., Mallory, M., Mucke, L., Schenk, D., and Games, D. (1996) Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F β-amyloid precursor protein and Alzheimer's disease. *J. Neurosci.* 16, 5795–5811.
74. Masliah, E. (1995) Mechanisms of synaptic dysfunction in Alzheimer's disease. *Histol. Histopathol.* 10, 509–519.
75. Moechars, D., Gilis, M., Kuiperi, C., Laenen, I., and Van Leuven, F. (1998) Aggressive behaviour in transgenic mice expressing APP is alleviated by serotonergic drugs. *NeuroReport* 9, 3561–3564.
76. Nalbantoglu, J., Tirado-Santiago, G., Lahsaini, A., Poirier, J., Goncalves, O., Verge, G., et al. (1997) Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. *Nature* 387, 500–505.
77. Chapman, P. F., White, G. L., Jones, M. W., Cooper-Blacketer, D., Marshall, V. J., Irizarry, M., et al. (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat. Neurosci.* 2, 271–276.

78. Malherbe, P., Richards, J. G., Martin, J. R., Bluethmann, H., Maggio, J., and Huber, G. (1996) Lack of β-amyloidosis in transgenic mice expressing low levels of familial Alzheimer's disease missense mutations. *Neurobiol. Aging* 17, 205–214.
79. Thinakaran, G., Borchelt, D. R., Lee, M. K., Slunt, H. H., Spitzer, L., Kim, G., et al. (1996) Endoproteolysis of presenilin 1 and accumulation of processed derivatives *in vivo*. *Neuron* 17, 181–190.
80. Thinakaran, G., Harris, C. L., Ratovitski, T., Davenport, F., Slunt, H. H., Price, D. L., et al. (1997) Evidence that levels of presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors. *J. Biol. Chem.* 272, 28,415–28,422.
81. Guo, Q., Sebastian, L., Sopher, B. L., Miller, M. W., Ware, C. B., Martin, G. M., et al. (1999) Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid β-peptide toxicity: central roles of superoxide production and caspase activation. *J. Neurochem.* 72, 1019–1029.
82. Leissring, M. A., Paul, B. A., Parker, I., Cotman, C. W., and LaFerla, F. M. (1999) Alzheimer's presenilin-1 mutation potentiates inositol 1,4,5-trisphosphate-mediated calcium signaling in Xenopus oocytes. *J. Neurochem.* 72, 1061–1068.
82a. Leissring, M. A., Akbari, Y., Fanger, C. M., Cahalan, M. D., Mattson, M. P., and LaFerla, F. M. (2000) Capacitative calcium entry deficits and elevated luminal calcium content in mutant presenilin-1 knockin mice. *J. Cell Biol.* 149, 793–798.
83. Shen, J., Bronson, R. T., Chen, D. F., Xia, W., Selkoe, D. J., and Tonegawa, S. (1997) Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell* 89, 629–639.
84. Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J., Trumbauer, M. E.,et al. (1997) Presenilin 1 is required for Notch1 and DII1 expression in the paraxial mesoderm. *Nature* 387, 288–292.
85. Conlon, R. A., Reaume, A. G., and Rossant, J. (1995. Notch1 is required for the coordinate segmentation of somites. *Development* 121, 1533–1545.
86. Hrabe de Angelis, M., McIntyre, J., 2nd, and Gossler, A. (1997) Maintenance of somite borders in mice requires the Delta homologue DII1. *Nature* 386, 717–721.
87. Levitan, D. and Greenwald, I. (1995) Facilitation of lin-12-mediated signalling by sel-12, a Caenorhabditis elegans S182 Alzheimer's disease gene. *Nature* 377, 351–354.
88. Davis, J. A., Naruse, S., Chen, H., Eckman, C., Younkin, S., Price, D. L., et al. (1998) An Alzheimer's disease-linked PS1 variant rescues the developmental abnormalities of PS1-deficient embryos. *Neuron* 20, 603–609.
89. Qian, S., Jiang, P., Guan, X. M., Singh, G., Trumbauer, M. E., Yu, H., et al. (1998) Mutant human presenilin 1 protects presenilin 1 null mouse against embryonic lethality and elevates Aβ1-42/43 expression. *Neuron* 20, 611–617.
90. De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., et al. (1998) Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 391, 387–390.

91. Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999) Two transmembrane asparates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature* 398, 513–517.
92. Borchelt, D. R., Ratovitski, T., van Lare, J., Lee, M. K., Gonzales, V., Jenkins, N. A., et al. (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* 19, 939–945.
93. Roses, A. D. (1998) Apolipoprotein E and Alzheimer's disease. The tip of the susceptibility iceberg. *Ann. NY Acad. Sci.* 855, 738–743.
94. Piedrahita, J. A., Zhang, S. H., Hagaman, J. R., Oliver, P. M., and Maeda, N. (1992) Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc. Natl. Acad. Sci. USA* 89, 4471–4475.
95. Plump, A. S., Smith, J. D., Hayek, T., Aalto-Setala, K., Walsh, A., Verstuyft, J. G., et al. (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E- deficient mice created by homologous recombination in ES cells. *Cell* 71, 343–353.
96. Mahley, R. W. (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240, 622–630.
97. Raber, J., Wong, D., Buttini, M., Orth, M., Bellosta, S., Pitas, R. E., et al. (1998) Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: increased susceptibility of females. *Proc. Natl. Acad. Sci. USA* 95, 10,914–10919.
98. Smith, J. D., Sikes, J., and Levin, J. A. (1998) Human apolipoprotein E allele-specific brain expressing transgenic mice. *Neurobiol. Aging* 19, 407–413.
99. Xu, P. T., Schmechel, D., Rothrock-Christian, T., Burkhart, D. S., Qiu, H. L., Popko, B., et al. (1996) Human apolipoprotein E2, E3, and E4 isoform-specific transgenic mice: human-like pattern of glial and neuronal immunoreactivity in central nervous system not observed in wild-type mice. *Neurobiol. Dis.* 3, 229–245.
100. Bowman, B. H., Jansen, L., Yang, F., Adrian, G. S., Zhao, M., Atherton, S. S., et al. (1995) Discovery of a brain promoter from the human transferrin gene and its ulitization for development of transgenic mice that express human apolipoprotein E alleles. *Proc. Natl. Acad. Sci. USA* 92, 12,115–12,119.
101. Xu, P. T., Gilbert, J. R., Qiu, H. L., Ervin, J., Rothrock-Christian, T. R., Hulette, C., et al. (1999) Specific regional transcription of apolipoprotein E in human brain neurons. *Am. J. Pathol.* 154, 601-611.
102. Holtzman, D. M., Bales, K. R., Wu, S., Bhat, P., Parsadanian, M., Fagan, A. M., et al. (1999) Expression of human apolipoprotein E reduces amyloid-β deposition in a mouse model of Alzheimer's disease. *J. Clin. Invest.* 103, R15–R21.
103. Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., et al. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400, 173–177.
104. Capsoni, S., Ugolini, G., Comparini, A., Ruberti, F., Berardi, N., and Cattaneo, A. (2000) Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. *Proc. Natl. Acad. Sci. USA* 97, 6826–6831.