A Reductionist View of Alzheimer's Disease

PETER T. LANSBURY, JR.†

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received October 16, 1995

Introduction

Alois Alzheimer's first description, almost 90 years ago, of the disease which now bears his name was based on his recognition of a correlation between abnormal brain pathology and cognitive dysfunction.[1] The brains of his patients were characterized by the colocalization of neuronal degeneration and insoluble plaques in regions involved in memory formation. Alzheimer raised the question of whether the amyloid plaques, which he believed to be carbohydrate (hence the name "amyloid") but have since been shown to be primarily proteinaceous, were a cause or a result of the neurodegeneration. This issue has still not been unequivocally resolved, since the Alzheimer's disease (AD) brain is only analyzed at a single postmortem time point in the pathogenic pathway. Until the formation of amyloid plaques can be followed *in vivo,* before the onset of symptoms and during the slow progression of the disease, it will not be possible to completely elucidate the cause and effect relationship between pathology and symptoms. In the meantime, a fierce debate has raged over this issue.[2−4]

The fact that the number of amyloid plaques does not correlate to the severity of symptoms at the time of death has been used to argue that amyloid formation is a result of AD neurodegeneration. This argument seems to hinge on the assumption that the severity of symptoms is directly correlated to the progression of the pathogenic pathway. However, symptoms are difficult to quantify and can be disguised by certain individuals (education level and age of AD onset are correlated). In contrast, the argument that amyloid formation causes neurodegeneration is supported by a growing amount of convergent genetic and biochemical evidence. This evidence, some of which is discussed below, strongly suggests that at least some forms of AD result from the abnormally rapid accumulation of amyloid plaques (the brain of an 85-year-old asymptomatic individual will contain some amyloid plaque, yet significantly less than an 85-year-old AD patient).[2−5]

The AD amyloid plaque is a complex mixture of damaged neurons, proteins, and other macromolecules, presumably debris from lysed neurons. On treatment with a chemical denaturant, most of this debris is removed, leaving an insoluble, fibrous plaque core. The plaque core amyloid fibril is an ordered aggregate comprising primarily[6] variants of a 4 kDa protein, collectively designated the β amyloid protein.[3] The β protein is produced by proteolysis of the amyloid precursor protein (APP), a transmembrane precursor protein of unknown function that is encoded on chromosome 21 (Figure 1).[3] Chromosome 21 is trisomic in Down's syndrome (DS): DS patients consequently express unusually high levels of APP. They also invariably develop AD in their thirties or forties.[3] Furthermore, the postmortem brains of infants with DS who have died of other complications are characterized by extensive brain amyloid, supporting the notion that amyloid formation precedes symptoms. The DS−AD linkage motivated the construction of a transgenic mouse which overexpresses human APP. This mouse deposits AD-like amyloid plaques but has not yet been reported to exhibit symptoms reminiscent of AD.[7] There are several forms of inherited, early-onset AD caused by point mutations in APP. Individuals bearing these mutations become symptomatic in their fourth or fifth decade, but their disease is

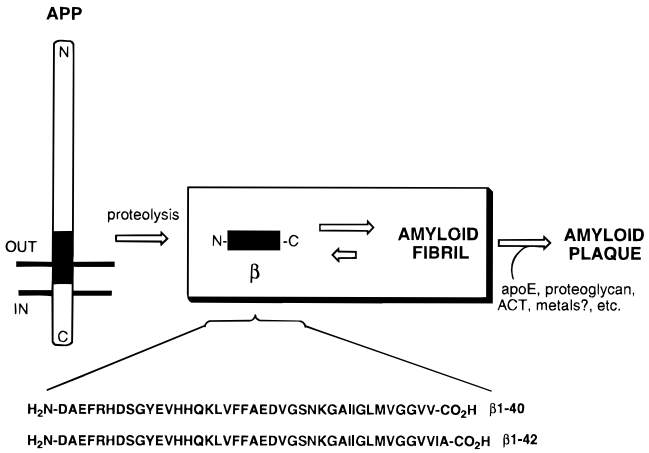

Figure 1. A schematic view of the genesis of AD amyloid plaque. The boxed reaction, especially in the forward direction, is the focus of this Account. Acceleration of this reaction may cause some forms of Alzheimer's disease. The sequences of the two major forms of the β protein are also shown. β1−40 is the major circulating variant, whereas β1−42 is the predominant variant in the amyloid plaque. The sequence of the plaque-associated peptide NAC is shown at the bottom.[43] A similarity among a seven amino acid sequence within NAC (underlined), the hydrophobic C-terminus of the β protein (residues 36−42), and a sequence in the prion protein may be significant.[36]

Peter T. Lansbury, Jr., is currently Associate Professor of Neurology at the Harvard Medical School and a principal investigator at the Center for Neurologic Diseases at Brigham and Women's Hospital. Peter grew up in Buffalo, NY, and received his undergraduate education at Princeton University. He obtained his Ph.D. in chemistry from Harvard University in 1985, working in the laboratory of Professor E. J. Corey. He subsequently spent a postdoctoral fellowship in the laboratory of the late Professor E. T. Kaiser at the Rockefeller University. The Lansbury research group has focused on developing chemical methods and applying chemical ideas and approaches to the understanding of Alzheimer's disease and scrapie.

† Current address: Department of Neurology, Harvard Medical School and Center for Neurologic Diseases, Brigham and Women's Hospital, 221 Longwood Ave., Boston, MA 02115.

(1) Alzheimer, A. *Alg. Z. Psychiatry* **1907**, *64*, 146−8.
(2) Schellenberg, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8552−59.
(3) Selkoe, D. J. *J. NIH Res.* **1995**, *7*, 57−64.
(4) Kosik, K. S. *J. Cell. Biol.* **1994**, *127*, 1501−4.
(5) Selkoe, D. J. *Neuron* **1991**, *6*, 487−498.
(6) A minor component protein of the insoluble fibrillar material, NAC,[36,43] has recently been identified and will be discussed later in the text.

S0001-4842(95)00159-2 CCC: $12.00

otherwise indistinguishable from the much more common late-onset form of AD. The responsible mutations flank the β protein sequence within APP (Figure 1) and may affect its proteolytic excision (*vide infra*). Finally, it is important to note that the β protein is not only produced in conjunction with AD; it circulates at comparable concentrations in the blood and cerebrospinal fluid of AD patients and unaffected individuals.[8]

We have concentrated our attention on a single step in the formation of the amyloid plaque: the conversion of the β protein into an amyloid fibril (box in Figure 1). Our reductionist approach is based on the premise that the rate of this reaction is accelerated in AD.[9−11] This Account will focus on our efforts to elucidate the mechanism of amyloid fibril formation and to illustrate how differences between the normal and AD brains could influence this reaction.

The β Protein Is Multiconformational in Solution

The amphiphilic β protein sequence (Figure 1) reflects the fact that the protein is derived from a sequence in APP which partially overlaps its putative transmembrane sequence. The β protein variants β1−39 and β1−42 exist in aqueous solution as a mixture of rapidly equilibrating conformers,[12−14] and have detergent properties.[15] The insolubility of β1−39 and β1−42 precludes their analysis by solution NMR methods. Our studies of a synthetic peptide based on the C-terminal sequence of β1−42, β34−42, led to the proposal that intermolecular hydrophobic interactions involving the C-terminal portion of the β protein are the driving force in amyloid formation.[16] This proposal has been supported by our kinetic studies of *in vitro* amyloid formation (*vide infra*),[9,17] and by subsequent studies of AD tissue.[18,19]

The Amyloid Fibril Is an Ordered, Noncrystalline Protein Aggregate

The X-ray diffraction pattern of naturally-derived AD amyloid fibrils[20] resembles those observed by Pauling in his pioneering studies of *B. mori* silk and polyalanine.[21] Thus, the Pauling cross-β silk fibril, with its constituent antiparallel β sheet, has been adopted as a model for the amyloid fibril.[22] Repeated attempts to obtain single crystals of an amyloid protein which are suitable for analysis by high-resolution crystallographic methods have been unsuccessful.

We have developed a method to obtain high-resolution structural information about the amyloid fibril. Incorporation of ^{13}C atoms into the peptide backbone at specific sites transformed two underutilized methods for protein structure determination, Fourier-transform infrared spectroscopy (FTIR) and solid-state nuclear magnetic resonance spectroscopy (SSNMR), into powerful methods for amyloid structure elucidation. Replacing a backbone amide carbonyl carbon with ^{13}C lowers the infrared vibrational frequency of that specific amide I mode.[23−25] Since the amide I frequency is known to depend on secondary structure,[26] this process, which we call isotope-editing, allows one to obtain *local* structural information which cannot be extracted from the natural abundance FTIR spectrum due to coupled and unresolved bands.[23,27]

Rotational resonance SSNMR, developed by Robert Griffin and co-workers at the Francis Bitter Magnet laboratory at the Massachusetts Institute of Technology (MIT),[28] allowed us to measure distances of up to 6 Å between 20 pairs of ^{13}C-labeled carbon atoms in the insoluble, noncrystalline β34−42 amyloid fibril.[29−31] A simulated annealing protocol developed by Bruce Tidor of MIT was then used to describe a library of potential β34−42 monomer structures which were consistent with the measured intramolecular ^{13}C−^{13}C distances, the ^{13}C chemical shifts, and the individual amide I frequencies.[30] The resultant extended monomers were aligned on the basis of intermolecular rotational resonance effects which uniquely describe a single highly pleated antiparallel β sheet.[30] The specificity of the intermolecular interactions found in the β34−42 amyloid fibril suggests that amyloid formation may have an enormous entropic cost.

(7) Games, D.; Adams, D.; Alessandrini, R.; Barbour, R.; Berthelette, P.; Blackwell, C.; Carr, T.; Clemens, J.; Donaldson, T.; Zhao, J.; *et al. Nature* **1995**, *373*, 523−527. Other recent efforts to produce mouse models: LaFerla, F. M.; Tinkle, B. T.; Bieberich, C. J.; Haudenschild, C. C.; Jay, G. *Nature Genet.* **1995**, *9*, 21−30. Hsiao, K. K.; *et al. Neuron* **1995**, *15*, 1203−18. These animals show neurological abnormalities, but no animal demonstrates an AD-like condition. It should be emphasized that the mouse version of AD has not been reported, despite the fact that mice express APP.

(8) Seubert, P.; Vigo-Pelfrey, C.; Esch, F.; Lee, M.; Dovey, H.; Davis, D.; Sinha, S.; Schlossmacher, M.; Whaley, J.; Swindlehurst, C.; McCormack, R.; Wolfert, R.; Selkoe, D.; Lieberburg, I.; Schenk, D. *Nature* **1992**, *359*, 325−327.

(9) Jarrett, J. T.; Berger, E. P.; Lansbury, P. T., Jr. *Biochemistry* **1993**, *32*, 4693−97.

(10) Jarrett, J. T.; Lansbury, P. T., Jr. *Cell* **1993**, *73*, 1055−58.

(11) It is also possible that the reverse reaction may be inhibited, by modification of the amyloid fibril, either covalently or noncovalently.[61,62]

(12) Barrow, C. J.; Zagorski, M. G. *Science* **1991**, *253*, 179−182.

(13) Barrow, C. J.; Yasuda, A.; Kenny, P. T. M.; Zagorski, M. G. *J. Mol. Biol.* **1992**, *225*, 1075−1093.

(14) Zagorski, M. G.; Barrow, C. J. *Biochemistry* **1992**, *31*, 5621−5631.

(15) Soreghan, B.; Kosmoski, J.; Glabe, C. *J. Biol. Chem.* **1994**, *269*, 28551−28554.

(16) Halverson, K.; Fraser, P. E.; Kirschner, D. A.; Lansbury, P. T., Jr. *Biochemistry* **1990**, *29*, 2639−2644.

(17) Jarrett, J. T.; Costa, P. R.; Griffin, R. G.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 9741−9742. This paper reports that C-terminal truncation of peptide models of the β protein slightly increases the solubility of the resultant amyloid fibrils in part, by slowing the disaggregation rate.

(18) Younkin, S.; *et al.* Unpublished results. We thank Dr. Younkin for sharing this information with us prior to its publication.

(19) Iwatsubo, T.; Odaka, A.; Suzuki, N.; Mizusawa, H.; Nukina, N.; Ihara, Y. *Neuron* **1994**, *13*, 45−53.

(20) Kirschner, D. A.; Abraham, C.; Selkoe, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 503−507.

(21) Marsh, R. E.; Corey, R. B.; Pauling, L. *Acta. Crystallogr.* **1955**, *8*, 710−715.

(22) Lansbury, P. T., Jr. *Biochemistry* **1992**, *31*, 6865−6870.

(23) Halverson, K. H.; Sucholeiki, I.; Ashburn, T. T.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 6701−6703.

(24) Bandekar, J.; Krimm, S. *Int. J. Peptide Protein Res.* **1985**, *26*, 407−415.

(25) The magnitude of the isotope-induced shift depends on whether the mode is coupled to neighboring amides.[23]

(26) Krimm, S.; Bandekar, J. In *Advances in Protein Chemistry*; Anfinsen, C., Ed.; 1986; Vol. 38, pp 183−364.

(27) Ashburn, T. T.; Auger, M.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1992**, *114*, 790.

(28) Raleigh, D. P.; Creuzet, F.; Das Gupta, S. K.; Levitt, M. H.; Griffin, R. G. *J. Am. Chem. Soc.* **1989**, *111*, 4502−4503.

(29) Griffiths, J. M.; Ashburn, T. T.; Auger, M.; Costa, P. R.; Griffin, R. G.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1995**, *117*, 3539−3546.

(30) Lansbury, P. T., Jr.; Costa, P. R.; Griffiths, J. M.; Simon, E. J.; Auger, M.; Halverson, K. J.; Kocisko, D. A.; Hendsch, Z. S.; Ashburn, T. T.; Spencer, R. G. S.; Tidor, B.; Griffin, R. G. *Nature Struct. Biol.* **1995**, *2*, 990−8.

(31) Spencer, R. G. S.; Halverson, K. J.; Auger, M.; McDermott, A. E.; Griffin, R. G.; Lansbury, P. T., Jr. *Biochemistry* **1991**, *30*, 10382.

(32) Haass, C.; Schlossmacher, M. G.; Hung, A. Y.; Vigo-Pelfrey, C.; Mellon, A.; Ostaszewski, B. L.; Lieberburg, I.; Koo, E. H.; Schenk, D.; Teplow, D. B.; Selkoe, D. J. *Nature* **1992**, *359*, 322−325.

(33) Shoji, M.; Golde, T. E.; Ghiso, J.; Cheung, T. T.; Estus, S.; Shaffer, L. M.; Cai, X.-D.; McKay, D. M.; Tintner, R.; Frangione, B.; Younkin, S. G. *Science* **1992**, *258*, 126−129.

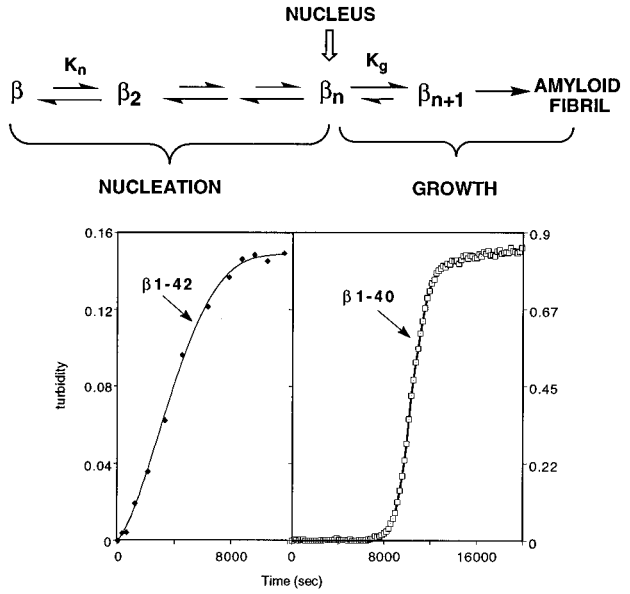

Figure 2. (Top) A simple model depicting a nucleation-dependent polymerization of the β protein. A series of entropically unfavorable association reactions are required in order to produce an ordered nucleus containing *n* monomers. Nucleus formation is rate-limiting and is very sensitive to protein concentration.[10] The rate of nucleation of β1−42 is much faster than that of the C-terminally truncated variant β1−40.[9] (Bottom) A typical experiment showing the time-dependent formation of insoluble amyloid fibrils.[9] In the case of β1−40 (at 80 μM), a striking lag time is seen which corresponds to the time required to form a nucleus as shown in the mechanistic model above. β1−42 nucleates so rapidly at 20 μM that no lag time is observed.

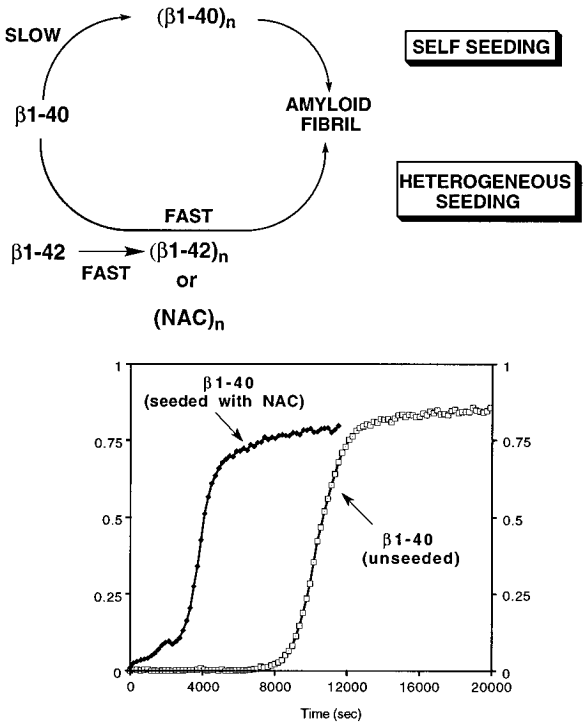

Figure 3. (Top) A schematic depiction of two *in vitro* heterogeneous seeding events which may be important in AD. Nucleation of β1−40 amyloid is slow. However, amyloid fibrils comprising β1−42[9] or NAC[36] are capable of seeding amyloid fibril formation by β1−40. Evidence for the importance of the β1−42 seed *in vivo* has been presented. (Bottom) Kinetic experiments showing heterogeneous seeding of β1−40 (80 μM) by NAC amyloid fibrils (20 μM).[36]

Nucleation Is the Rate-Determining Step of *in Vitro* Amyloid Formation: The β Protein C-Terminus Is Critical in Determining Its Rate

In 1992, Dennis Selkoe of Harvard Medical School and Steven Younkin, then of Case Western Reserve Medical School, reported that the β protein is produced in cell culture, suggesting that β protein production may not be specific to AD, in contrast to the prevailing wisdom at the time.[32,33] Scientists at Athena Neurosciences simultaneously reported that the β protein is found in circulating form in AD patients and unaffected individuals (at similar concentrations), but that the major circulating form of the β protein (β1−40) differs from the primary plaque variant (β1−42) by truncation at its C-terminus.[8] Until that time, no great significance was ascribed to the existence of C-terminal β protein variants; in fact, β proteins in pathological samples were routinely quantified by an immunochemical method which did not distinguish β1−40 and β1−42. However, given the unusual structural properties of our model peptide β34−42, we decided to investigate the kinetic effects of truncation. Studies of synthetic model peptides, including β26−40 and β26−42, and synthetic full-length β protein variants β1−39, β1−40, and β1−42 demonstrated that the rate of amyloid formation is greatly decreased by C-terminal truncation, whereas the morphology and stability of the fibrils is not significantly affected (Figure 2).[9,17]

Amyloid formation by the truncated β protein variants showed a protracted lag time, followed by a sigmoidal growth curve (Figure 2), suggesting the existence of a kinetic barrier to amyloid formation. The barrier may reflect the entropic cost of the ordered aggregation process that is suggested by our structural studies.[9,10,34] This type of process is reminiscent of a crystallization. Like a crystallization, amyloid formation is rate-limited by nucleation, which gives rise to a characteristic lag time. In addition, amyloid formation can also be seeded by a small amount of preformed fibrils, resulting in the elimination of the lag time (Figure 3). Similar studies of other amyloidogenic peptides are consistent with the nucleation-dependent mechanism and demonstrate the specificity of amyloid seeding.[34−38]

Heterogeneous Seeding May Accelerate AD Amyloidogenesis: β1−42 and NAC Are Candidates for *in Vivo* Seeds

Although seeding of amyloid formation was optimal in the case where the seed fibrils comprised the identical peptide as the supersaturated solution, it was also observed to occur, albeit less effectively, in some cases where the sequence of the seed fibril differed slightly from that of the soluble peptide (heterogeneous seeding).[9] For example, β1−42 fibrils are effective seeds for the *in vitro* polymerization of β1−40. Thus, the *in vivo* production of a small amount of

(34) Jarrett, J. T.; Lansbury, P. T., Jr. *Biochemistry* **1992**, *31*, 12345.
(35) Ashburn, T. T.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 11012.
(36) Han, H.; Weinreb, P. H.; Lansbury, P. T., Jr. *Chem. Biol.* **1995**, *2*, 163−9.
(37) Come, J. H.; Fraser, P. E.; Lansbury, P. T., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5959−5963.
(38) Come, J. H.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 4109−4110.

β1−42 could seed amyloid formation by β1−40, the predominant circulating form (Figure 3). On the basis of our kinetic studies, we proposed that the *ratio* of β1−42 to β1−40 would be more important than the total β protein concentration in determining the rate of *in vivo* amyloid formation.[9] A similar proposal was made simultaneously by Younkin, based on studies of β protein production in cell culture.[39] Younkin found that cells expressing an APP mutant that causes early-onset AD produced similar amounts of total β protein, but significantly greater relative amounts of β1−42, than cells expressing wild-type APP.[40] Subsequent neuropathological studies demonstrated the predominance of β1−42 in amyloid plaques derived from patients with early-onset AD as compared to plaques derived from patients with late-onset AD.[19] Recent studies of plasma β protein levels in AD patients and age-matched controls indicate that an increased β1−42/β1−40 ratio results from early-onset AD mutations in APP *as well as* point mutations in the S182 protein encoded on chromosome 14.[18,41] Acceleration of amyloid formation *via* heterogeneous seeding by β1−42 may be responsible for early-onset AD in these individuals. In contrast, individuals who are known to be predisposed to *late*-onset AD (apoE4 homozygotes; see below)[42] had normal circulating levels of both β protein variants,[18] suggesting that amyloid formation in late-onset AD patients may be accelerated by a different mechanism.

We have recently discovered another case of *in vitro* heterogeneous amyloid seeding; its relevance to AD has yet to be determined. In 1993, Tsunao Saitoh's group at the University of California, San Diego, reported that the peptide NAC is present in the insoluble, fibrillar core of the AD amyloid plaque.[43] The sequence of NAC bears some similarilty to the C-terminal sequence of the β protein (Figure 1) and an internal sequence of the prion protein, suggesting that it may also be amyloidogenic.[36] We synthesized NAC and found that the synthetic peptide forms typical amyloid fibrils *via* a nucleation-dependent mechanism.[36] Interestingly, NAC fibrils are competent seeds for the polymerization of β1−40 (Figure 3), and *vice versa*. This heterogeneous seeding event may be important *in vivo*, where NAC fibrillogenesis may trigger amyloid formation by the β protein (or *vice versa*).[36] It may be significant that NAC is derived from a precursor protein (NACP) which is expressed primarily in those neuronal populations which are affected by AD neurodegeneration.[44,45]

Inhibition of Nucleation May Suppress AD Amyloidogenesis: A Possible Explanation for the ApoE Genotype Susceptibility Factor

In 1993, a group at Duke University led by Allen Roses reported that apolipoprotein E genotype is a "susceptibility factor" for AD.[42] Apolipoprotein E, which plays a role in cholesterol transport, occurs as three variants (apoE3, apoE4, and apoE2, in order of decreasing prevalence) in the human population. The apoE4 gene is overrepresented in a typical population of AD patients, and AD patients carrying one (apoE4 heterozygotes) or two (apoE4 homozygotes) copies of the apoE4 gene are diagnosed at an early age relative to apoE3 homozygotes.[42] In contrast, the apoE2 gene seems to be underrepresented and to postpone onset of symptoms. However, despite the significant variation in susceptibility, some apoE2 homozygotes develop AD and some apoE4 homozygotes escape disease.

The apoE susceptibility factor may be related to complex factors which determine the ability of an individual to compensate for neuronal losses and delay the appearance of symptoms.[46] Alternatively, the effect may reflect a direct interaction of apoE with the AD pathogenic pathway, possibly influencing the rate of amyloid formation. Thus, amyloid depostion may be faster in individuals bearing the apoE4 gene and slower in those bearing the apoE2 gene.[46] Consistent with this hypothesis is the finding that possession of the apoE4 gene is correlated with increased amyloid load; specifically, there are more plaques of size comparable to that of the plaques in apoE3 homozygotes.[47] This phenotype could arise in two different ways: apoE4 could more efficiently promote amyloid formation, or apoE3 could more efficiently inhibit the process. Despite suggestions that the former scenario is operative,[48−50] the experimental data from our[51] and other[52] labs strongly support the latter possibility.

We have shown that both apoE3 and apoE4 are effective inhibitors of amyloid nucleation.[51] In the presence of 1% of either variant, significant increases in the lag time are observed (Figure 4). The apoE3 disulfide dimer is a more efficient *in vitro* nucleation inhibitor than either monomer (apoE4 cannot form a covalent dimer), suggesting that apoE3 may be a more efficient *in vivo* amyloid inhibitor than apoE4 due to the presence of the disulfide dimer (apoE2 could form oligomers, which may be excellent inhibitors).[51] The stoichiometry of nucleation inhibition suggests that apoE binds to a prenucleus oligomer of the β protein

(39) Cai, X.-D.; Golde, T. E.; Younkin, S. G. *Science* **1993**, *259*, 514−516.
(40) Suzuki, N.; Cheung, T. T.; Cai, X.-D.; Odaka, A.; Otvos, L., Jr.; Eckman, C.; Golde, T. E.; Younkin, S. G. *Science* **1994**, *264*, 1336−1340.
(41) Sherrington, R.; Rogaev, E. I.; Liang, Y.; Rogaeva, E. A.; St. George-Hyslop, P. H. *Nature* **1995**, *375*, 754−760.
(42) Corder, E. H.; Saunders, A. M.; Strittmatter, W. J.; Schmechel, D. E.; Gaskell, P. C.; Small, G. W.; Roses, A. D.; Haines, J. L.; Pericak-Vance, M. A. *Science* **1993**, *261*, 921−923.
(43) Ueda, K.; Fukushima, H.; Masliah, E.; Xia, Y.; Iwai, A.; Yoshimoto, M.; Otero, D. A. C.; Kondo, J.; Ihara, Y.; Saitoh, T. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11282−11286.
(44) Iwai, A.; Masliah, E.; Yoshimoto, M.; Ge, N.; Flanagan, L.; Rohan de Silva, H. A.; Kittel, A.; Saitoh, T. *Neuron* **1995**, *14*, 467−475.
(45) George, J. M.; Jin, H.; Woods, W. S.; Clayton, D. F. *Neuron* **1995**, *15*, 361−72.
(46) Any arguments based on age of AD onset should be viewed with suspicion; it would be more accurate to refer to the age of AD detection. The clinical heterogeneity of AD, and the lack of any biochemical markers for diagnosis during the life of the patient, makes it difficult to determine the time course of AD pathogenesis.
(47) Hyman, B. T.; West, H. L.; Rebeck, G. W.; Buldyrev, S. V.; Mantegna, R. N.; Ukleja, M.; Havlin, S.; Stanley, H. E. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3586−90.
(48) Ma, J.; Yee, A.; Brewer, H. B.; Das, S.; Potter, H. *Nature* **1994**, *372*, 92−4.
(49) Sanan, D. A.; Weisgraber, K. H.; Russell, S. J.; Mahley, R. W.; Huang, D.; Saunders, A.; Schmechel, D.; Wisniewski, T.; Frangione, B.; Roses, A. D.; Strittmatter, W. J. *J. Clin. Invest.* **1994**, *94*, 860−869.
(50) References 48 and 49 report that fibril morphology, as assessed by electron microscopy, is changed when the fibrils are formed in the presence of apoE4. Changed morphology should not be confused with the promotion of fibril formation.[51] However, the morphology of the amyloid fibrils may be critical for the understanding of the pathogenesis.
(51) Evans, K. C.; Berger, E. P.; Cho, C.-G.; Weisgraber, K. H.; Lansbury, P. T., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 763−767.
(52) Chan, W.; Wood, S. J.; Wetzel, R.; Fornwald, J.; Brawner, M. Unpublished results. We thank Dr. Wetzel for sharing his results with us prior to publication.

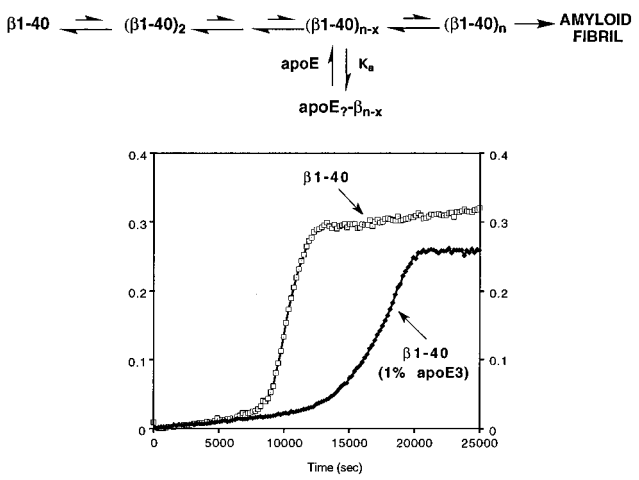

Figure 4. Nucleation inhibition by apoE. (Top) A schematic depiction of our working model of the action of apoE.[51] The relative efficacies of the apoE variants may depend on their relative affinity (K_a) for a β protein oligomer. (Bottom) Kinetic curves showing the effect of 1% apoE (800 nM) on the lag time of β1−40 (80 μM).[51]

which is only sparsely populated (Figure 4). Ron Wetzel and co-workers at SmithKline Beecham have isolated high-molecular-weight complexes of apoE3 and the β protein which may be the inhibitory complex.[52] The ultimate test for this model of apoE action would be to knock out the apoE gene in the transgenic mouse which overexpresses APP.[7] If apoE is an *in vivo* amyloid supressor, then this animal should develop amyloid much more rapidly than an otherwise identical animal which expresses apoE. However, if apoE is an amyloid promoter, then the "knockout" animal should not develop amyloid plaques.

Three Chemical Questions about Amyloid and AD

The structural and mechanistic studies summarized above raise many questions, three of which are detailed below. The first question involves an interesting structural issue which may be relevant to AD. The second and third questions represent alternative approaches to the major issue of whether amyloid causes AD.

(1) Are AD Amyloid Fibrils Homogeneous at the Ultrastructural Level? SSNMR studies indicate that peptide amyloids are homogeneous at the local level,[30,53] but do not address the ultrastructure of the amyloid fibril. It is possible that alternative fibrillar forms exist, analogous to crystal isomorphs[54] or the strains of the scrapie prion.[55] As is the case with prion strains, each isoform could produce slightly different pathology and symptoms, explaining the clinical heterogeneity of AD. Resolution of this issue will require the increased application of techniques such as electron microscopy and atomic force microscopy. If amyloid isomorphs are shown to exist *in vivo*, it will be critical to determine if the isomorphs differ with respect ot their biological activity.

(2) Can New AD Susceptibility Factors be Identified By "Mechanism-Based" Screens? We expect that many AD susceptibility factors analogous to apoE exist. Protein risk factors can be identified by genetic screening of AD patients. Candidate genes for such a screening effort are typically suggested by neuropathological studies, as was the case with apoE[42] and antichymotrypsin.[56−58] Pathological studies of AD brain have also implicated nonprotein risk factors such as zinc and aluminum.[59,60] We propose that new protein and nonprotein risk factors could be identified by mechanism-based screens. Examples are presented above of two types of potential risk factor molecules, the heterogeneous seed (its presence would accelerate amyloid formation) and the nucleation inhibitor (its absence would accelerate amyloid formation). We also envision a third class of molecules which could bind to the amyloid fibril and inhibit its clearance ("amyloid stabilizers"). Two candidates for this class are heparan sulfate proteoglycan[61] and serum amyloid P,[62] which have both been reported to inhibit the *in vitro* proteolysis of amyloid.

(3) Are *in Vivo* Amyloid Inhibitors Potential AD Therapeutics? If amyloid formation is a neccessary step in the pathogenesis of AD, then its inhibition would delay the onset of the disease. This hypothesis can be tested. Combinatorial synthetic methods and high-throughput screening will allow the identification of compounds which inhibit *in vitro* amyloid formation and cross the blood−brain barrier. Inhibition of *in vivo* amyloid formation can be tested in the transgenic mouse model. However, testing for the effect of amyloid inhibition on the progression of the disease requires that these animals become symptomatic, which remains to be seen.[7]

I thank the past and present members of my research group at MIT for their contributions to the planning and execution of the experiments discussed herein. I also acknowledge Steven Younkin of Mayo-Jacksonville for his contributions to the ideas presented here.

AR950159U

(53) Griffiths, J. M.; Ashburn, T. T.; Auger, M.; Costa, P. R.; Griffin, R. G.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1995**, *117*, 3539−46.
(54) Dunitz, J. D.; Bernstein, J. *Acc. Chem. Res.* **1995**, *28*, 193−200.
(55) Bessen, R. A.; Kocisko, D. A.; Raymond, G. J.; Nandan, S.; Lansbury, P. T., Jr.; Caughey, B. *Nature* **1995**, *375*, 698−700.
(56) Kamboh, M. I.; Sanghera, D. K.; Ferrell, R. E.; DeKosky, S. T. *Nature Genet.* **1995**, *10*,
(57) Fraser, P. E.; Nguyen, J. T.; McLachlan, D. R.; Abraham, C. R.; Kirschner, D. A. *J. Neurochem.* **1993**, *61*, 298−305.
(58) Ericksson, S.; Janciauskiene, S.; Lannfelt, L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2313−17.
(59) Bush, A. I.; Pettingell, W. H.; Multhap, G.; Paradis, M. D.; Vonsattel, J.-P.; Gusella, J. F.; Beyreuther, K.; Masters, C. L.; Tanzi, R. E. *Science* **1994**, *265*, 1464−7.
(60) Manyth, P. W.; Ghilardi, J. R.; Rogers, S.; DeMaster, E.; Allen, C. J.; Stimson, E. R.; Maggio, J. E. *J. Neurochem.* **1993**, *61*, 1171−74.
(61) Gupta-Bansal, R.: Frederickson, R. C. A.; Brunden, K. R. *J. Biol. Chem.* **1995**, *270*, 18666−671.
(62) Tennent, G. A.; Lovat, L. B.; Pepys, M. B. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4299−4303.