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β-AMYLOID FORMATION AS A POTENTIAL THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE

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INTRODUCTION

As prolonged life expectancy has increased the proportion of the population that is elderly, Alzheimer's disease has emerged as a major health problem in the United States. Currently, 3 million Americans suffer from Alzheimer's disease. This number is expected to grow to greater than 10 million within the next decade. There is no remission in the progression of the disease, nor is there any truly effective pharmaceutical intervention. Hence, upon onset, the disease progresses inexorably towards increasing mental and physical incapacitation, followed by death. This process commonly lasts from two to twelve years. Presently, the financial burden from institutional care of demented Alzheimer's disease patients is estimated at 40 billion dollars per year. Perhaps the greatest tragedy is the emotional burden to the afflicted individual and his or her family.

Although the cause of Alzheimer's disease is unknown, recent molecular and biological research, in conjunction with classical neuropathological techniques, has contributed detailed insights into the pathogenesis of this disease. Particular attention has been devoted to the β-amyloid plaque, which is the major histopathological hallmark of Alzheimer's disease. Current evidence indicates that β-amyloid deposition may play a central role in the pathogenesis of this disease, and recent findings have prompted

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new concepts in the development of therapies for the treatment of Alzheimer's disease. This review focuses on the current advances in β-amyloid biology and the novel targets for therapeutic intervention that are unfolding in this area of research.

FEATURES OF ALZHEIMER'S DISEASE

Clinically, Alzheimer's disease first presents with impaired short-term memory, which progressively declines along with other cognitive abilities. Ultimately, the individual completely loses his or her presymptomatic persona and becomes incapable of self-care. Postmortem analysis of the victim's brain reveals a somewhat stereotypic histopathological profile of the disease. Two major microscopic hallmarks are evident---extracellular deposits of β-amyloid in senile plaques and intracellular accumulations of filamentous structures referred to as neurofibrillary tangles (Figure 1). Both of these structures were first reported by Alois Alzheimer at the turn of the century (1). These lesions occur at substantially greater frequency in the Alzheimer's disease brain than in neurologically normal aged controls (2–5). Unlike neurofibrillary tangles, which are common to a number of dementing disorders (6–8), β-amyloid plaques are a unique feature of Alzheimer's disease and of the aging process.

Mature β-amyloid plaques are often intimately associated with degenerating neuronal processes. These dystrophic neurites are engorged with an abnormally phosphorylated neuronal cytoskeletal protein, the microtubule-associated protein tau (9, 10). Aberrant tau is also a major constituent of neurofibrillary tangles (11, 12). Additional abnormal tau structures are neuropil threads, which are found in spatial association with neurofibrillary tangles (13–15). Like neurofibrillary tangles, neuropil threads are found in other neurological disorders in which β-amyloid deposition is absent (15). In contrast, dystrophic neurites are only observed where mature plaques are found and are unique to Alzheimer's disease (16). They are referred to as neuritic plaques. Neuritic plaques, neurofibrillary tangles, and neuropil threads are most predominantly found in the neocortex but are seen in other brain areas such as the amygdala and the hippocampus (reviewed in 17).

Neuronal loss, as well as loss of synaptic connections, are additional pathological features of Alzheimer's disease (reviewed in 17 and 18; 19–21). The reduction in neurons and synapses parallels a progressive accumulation of β-amyloid plaques, neurofibrillary tangles, and neuropil threads. The cellular degeneration involves select neuronal populations and appears to be anatomically determined. In general, these vulnerable populations of neurons either project to or are located within brain regions displaying β-amyloid plaques and neurofibrillary tangles. For those populations of neurons pro-

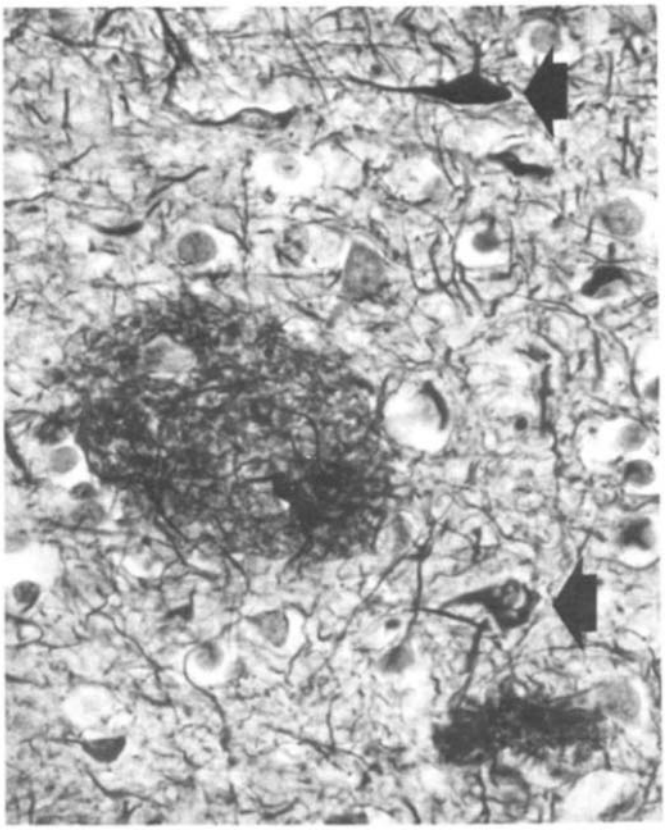

Figure 1 Classical histopathology in Alzheimer's disease brain showing large and small β-amyloid plaques and neurofibrillary tangles (*arrows*) as revealed by impregnation with silver salts. Neuropil threads are evident in the background.

jecting to areas with lesions, a major consequence is deafferentation and loss of synaptic connectivity. Specific subcortical nuclei that send neuronal projections to the cortex exhibit neurofibrillary tangle formation and neuronal degeneration. Other subcortical nuclei that do not project to the neocortex are typically spared and lack neurofibrillary tangles. As a result of the variety of subcortical nuclei and, in turn, the diversity of neurons affected, a number of different neurotransmitter systems and neuropeptide modulators are altered. This broad profile of neurotransmitter alteration has consequently confounded strategies for therapeutic development. Heretofore, much effort has been directed towards developing compounds with short-term benefit

rather than towards preventative or curative therapies. The most extensive drug development activity has been devoted to counteracting the degenerative cholinergic depletions of the basal forebrain. Because cholinergic deafferentation in the disease is severe, and because of the importance of this transmitter system for memory, multiple therapeutic approaches to enhance cholinergic activity have been sought (reviewed in 22). To date, only one compound, tetrahydroaminoacridine (THA or tacrine), an inhibitor of acetylcholin esterase, has been approved for Alzheimer's disease. Unfortunately, the clinical benefit of THA is marginal and treatment is often associated with hepatotoxicity (23–25).

Several issues emerge from observation of the various pathogenic lesions that characterize Alzheimer's disease. What is the earliest pathological event in the process? What is the interrelationship between the observed lesions? What are the operating molecular mechanisms leading to the disease state? These questions are important when considering alternative approaches to therapeutic development.

PATHOGENIC ROLE OF β-AMYLOID

Histological Observations

Understanding the early pathogenesis of Alzheimer's disease is of critical importance in developing a therapeutic strategy for intervention. A picture of the early Alzheimer's disease pathology and its progression to an advanced state is available through analyses of brains from individuals with Down's syndrome. Down's individuals invariably develop histopathology, and often neurological symptoms, that are indistinguishable from Alzheimer's disease (26, 27). Studies of pathological changes in Down's individuals of various ages have demonstrated β-amyloid deposition at early ages, typically in young adults (28–30). These deposits are of diffuse morphology and lack the highly ordered fibrillar structure of β-amyloid present in mature plaques. These diffuse deposits may represent early lesions that precede development of classical mature plaques. Over time, the frequency of mature plaques with neuritic association increases in the Down's brain. Therefore, appearance of dystrophic neurites in association with highly fibrillar plaque amyloid is believed to be a late-stage event and one that invariably accompanies the clinical dementia of Alzheimer's disease.

A relationship between β-amyloid deposition and neurofibrillary tangles appears to exist. From evidence described below, extracellular β-amyloid deposits are likely to promote alterations in the neuronal cytoskeleton, which may ultimately lead to the fibrillar triad of tangles, neuropil threads, and

dystrophic neurites. The studies of brains from young individuals with Down's syndrome also showed that β-amyloid deposition precedes neurofibrillary tangle, neuropil thread, and dystrophic neurite formation (28–30). Presumably, alterations in the neuronal cytoskeletal that form tangle, thread, and dystrophic neuritic structures damage cell function and represent one mechanism by which neurons degenerate and die. Since neurofibrillary tangles are seen in association with a collection of dementing disorders, formation of these filamentous structures may be part of a common neurodegenerative pathway following various initial insults, and β-amyloid deposition may be an example of one such insult. For this scenario, β-amyloid deposition is a causal event indirectly culminating in intellectual impairment through induced neuronal dysfunction.

Formation of β-amyloid also appears to precede and promote alterations in synaptic structure based on both light and electron microscopic studies of Alzheimer's disease brains. Early diffuse deposits are frequently accompanied by presynaptic dilation of synaptic terminals (31–33). However, not all immature deposits are associated with synaptic alterations, which suggests that β-amyloid deposition may occur prior to synaptic damage and loss (31, 32). Presumably, this presynaptic distortion becomes severe and leads to dystrophic neurite formation and synapse loss characteristically associated with neuritic plaques.

Arguments have been presented discounting the histological evidence for the pathogenic role of β-amyloid deposition in Alzheimer's disease. Two observations are frequently cited. The first is that β-amyloid deposition also occurs in brain regions that lack an associated neurodegenerative response. The second argument is that the brains of some aged individuals, who showed no cognitive abnormalities, displayed extensive β-amyloid deposition. Neither of these findings discounts β-amyloid deposition as a fundamental lesion in the pathogenic process. While β-amyloid deposition may be widespread anatomically as a result of general perturbations leading to β-amyloid formation, certain populations of neurons may be more vulnerable than others to the effects of amyloid fibril formation. Local factors in the microenvironment may also influence the putative developmental progression of diffuse deposits to mature plaques. For example, cortical plaques are frequently associated with dystrophic neurites in contrast to cerebellar deposits, which lack neurodegenerative responses (34). With regard to cognitive abnormalities, select individuals may be better able than others to cope functionally with β-amyloid deposition. Time is the greatest risk factor for Alzheimer's disease (35). Perhaps if normal individuals with extensive β-amyloid pathology were to have lived longer they also might have presented with clinical symptoms. In Down's syndrome, β-amyloid deposition precedes clinical symptoms by 20 to 40 years.

Support From Experimental Models

Additional data suggesting that β-amyloid promotes neuronal cytoskeletal alterations comes from two different in vivo models. One model employs a transgenic approach, the other a mechanical method to produce β-amyloid deposits. Transgenic mice, genetically programmed for altered neuronal expression of the human gene encoding β-amyloid, exhibit diffuse immunoreactive β-amyloid deposits in their brains (36). Although only a single genetic alteration was made, these transgenic mice also show intraneuronal cytoskeletal alterations that resemble primordial tangle structures, which are detected by an antibody to aberrant tau (L Higgins & B Cordell, unpublished data). Moreover, some of the transgenic mice that exhibit large β-amyloid deposits have associated structures that are morphologically identical to dystrophic neurites as revealed by immunological and classical silver staining procedures. These Alzheimer's disease-like lesions are never seen in wild-type mice.

The second experimental model makes use of extrinsic application of β-amyloid. When synthetic β-amyloid protein is microinjected into brains of adult rats, aberrant neuronal cytoskeletal alterations are produced (37, 38). In addition, the injection of β-amyloid results in local neuronal degeneration. While the reported in vivo effects of synthetic β-amyloid have been variable, the in vitro neurotoxic effects of this protein are well documented. Chronic incubation of primary cortical and hippocampal cultures with micromolar concentrations of aggregrated β-amyloid results in formation of aberrantly phosphorylated tau protein and progressive neuronal degeneration (39–41). In contrast, soluble β-amyloid causes no neurotoxicity. The β-amyloid aggregates formed in vitro have been shown to adopt a β-pleated sheet conformation similar to fibrils in situ, whereas the soluble monomer assumes a β-helical structure, underscoring the importance of secondary and tertiary structural features of the protein in the pathogenic process (reviewed in 42 and 43). In addition to its direct neurotoxic effects, β-amyloid appears to exert indirect cellular effects. Treatment of cultured cortical neurons with aggregated β-amyloid renders the cells more vulnerable to glutamate excitotoxicity (44, 45) and to injury by glucose deprivation (46).

Genetic Evidence

The strongest evidence demonstrating an etiological role for β-amyloid in Alzheimer's disease is genetic. One compelling correlation between β-amyloid deposition and Alzheimer's disease pathology is found in Down's syndrome. Individuals with Down's syndrome are trisomic for chromosome 21 and invariably develop the pathology of Alzheimer's disease. Therefore,

it is significant that the gene encoding the precursor protein harboring β-amyloid is localized to chromosome 21 and within the obligate Down's region (47–50).

A subset of individuals afflicted with Alzheimer's disease develop the disease in an autosomal dominant pattern of inheritance. Genetic linkage studies of these high-risk families indicated that a rare pathogenic locus resides on the long arm of chromosome 21. Detailed analyses of several of these ethnically diverse families revealed mutations within the gene encoding the β-amyloid precursor protein (reviewed in 51). These mutations segregate in a disease-specific manner; afflicted family members are found to carry the mutation whereas the gene of normal members encodes a wild-type sequence. To date, six different disease-specific coding alterations have been characterized within the β-amyloid gene sequence. The identification of these mutations strongly suggests that β-amyloid does, indeed, play a central role in the pathogenesis of Alzheimer's disease.

Additional genetic loci have been linked to the heritable form of the disease. Most noteworthy is an unidentified locus on chromosome 14 that shows frequent linkage to a number of afflicted families (52–54). A locus on chromosome 19 also segregates with the familial form of Alzheimer's disease (55). While the gene on chromosome 19 is unknown, an interesting disease-related correlation has been recently described; it involves apolipoprotein E, which maps within the chromosome 19 region containing the locus for familial Alzheimer's disease (56). Individuals carrying an allele(s) for the ε4 isoform of the apolipoprotein E gene show a significantly greater risk for the disease. High-avidity binding between β-amyloid and apolipoprotein E ε4 has been described—a physical interaction that may assist in seeding amyloid fibril formation. Therefore, a number of different genetic loci can contribute to the pathogenic state, and it will be of great interest to learn how this genetic heterogeneity relates to the genesis of β-amyloid.

Taken together, the histological, experimental, and genetic evidence implicates β-amyloid formation as a fundamental event in the pathogenesis of Alzheimer's disease. A proposed scheme of the principal features of the disease and the possible interrelationships is illustrated in Figure 2. In this simplified sequence of events, β-amyloid is shown as the central lesion. Both intrinsic and extrinsic factors are known to influence β-amyloid formation. These include intrinsic factors such as genetic mutations on chromosomes 14 and 19, mutations in or an extra copy of the gene encoding β-amyloid, or the presence of the apolipoprotein ε4 allele, as well as extrinsic factors such as age (35), head trauma (57, 58), and the environment (reviewed in 59), all of which have been reported to increase the risk of developing Alzheimer's disease.

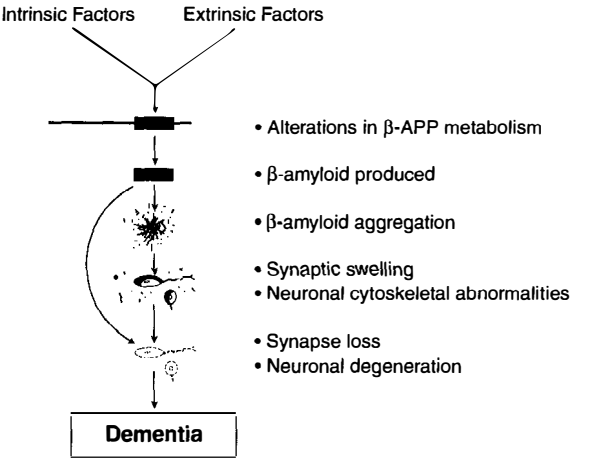

Figure 2 Proposed scheme of Alzheimer's disease pathology.

MOLECULAR BIOLOGY OF β-AMYLOID AND ITS PRECURSOR

Understanding the molecular biology of β-amyloid was made possible by two independent research groups who successfully purified, solubilized, and biochemically characterized the β-amyloid present in the brains of Alzheimer's disease victims (60, 61). Both identified β-amyloid as a 39–43 amino acid protein of novel sequence (Figure 3).

Gene Structure

The identified amino acid sequence of the β-amyloid protein enabled isolation of encoding cDNAs and of the gene. Characterization of cDNAs revealed that β-amyloid is harbored within a larger precursor protein termed β-amyloid precursor protein or β-APP (49). Therefore, proteolytic processing is required in generating the ~4-kDa β-amyloid protein. The precursor displays features of a membrane-associated protein by virtue of its secretory signal sequence and transmembrane-spanning domain. The location of the β-amyloid domain within the precursor is curious in that part is contained within the transmembrane domain and part in the putative extracellular compartment (see Figure 3).

A unique gene spanning 16 exons encodes β-APP; the sequence encoding the ~4-kDa β-amyloid protein is split between two exons (62). The β-APP gene is a member of a multigene family and shows high evolutionary

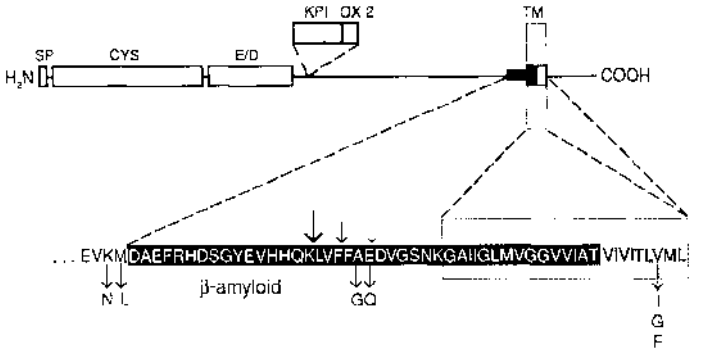

Figure 3 Molecular features of the β-amyloid precursor protein. The β-amyloid domain is shown as a black box within β-APP and as a boxed linear amino acid sequence. Other features of β-APP include a transmembrane-spanning domain (**TM**) (*shaded*), a secretory signal peptide (SP), extracellular domains rich in cysteine residues (CYS), acidic residues (E/D), a Kunitz proteinase inhibitor (KPI), and an immune homologue (OX-2). Both the KPI and OX-2 domains can be either present or absent owing to differential RNA splicing. Disease-specific mutations with amino acid substitutions are indicated; major and minor secretase cleavage sites are depicted with *vertical arrows* on the topology of the β-amyloid sequence.

conservation of amino acid sequence (63–67). The degree of conservation of the β-amyloid domain appears to have important ramifications with respect to the propensity of a species to form pathogenic deposits. To date, β-amyloid deposits have not been observed in rodents for which the β-amyloid sequence differs from the human sequence by three residues. Likewise, deposits have been found in animals with the same primary β-amyloid sequence as in humans, such as nonhuman primates, polar bears, canines, and transgenic mice carrying the human sequence (36, 68–71).

Transcriptional Features

A set of seven different β-APP cDNAs arise by differential splicing of a primary transcript synthesized from the unique copy β-APP gene (49, 72–78). Of these transcripts, three have been shown to be expressed as protein isoforms of 695, 751, and 770 amino acids. The 695 amino acid isoform differs from the larger isoforms in that it lacks a functional proteinase inhibitor domain homologous to the Kunitz family of serine proteinase inhibitors and because it has a restricted expression pattern. In contrast to the isoforms containing Kunitz proteinase inhibitor that are ubiquitously expressed, the 695 amino acid isoform is expressed only in neurons where it is the predominant β-APP isoform (72, 73, 79). The reason for this specific neuronal localization is unknown but presumably reflects the bio-

logical function of the precursor. The normal function of this protein is not yet defined but may involve cell surface remodeling or interactions with juxtaposing cells and/or with the extracellular matrix.

Precursor Metabolism

Based on a variety of studies, a picture of normal β-APP metabolism has emerged. Generally, all isoforms appear to have a similar metabolic profile. Typically, nascent precursor molecules are directed along the constitutive secretory pathway of the cell. As the precursor moves through the secretory pathway, it undergoes extensive posttranslational modification. Once all modifications have occurred, a subset of the molecules are proteolytically cleaved to liberate the large extracellular domain as a soluble entity (80–82). The proteinase(s) responsible for the cleavage, termed secretase, has not been characterized. However, a major cleavage site, as well as secondary sites have been biochemically mapped and shown to occur within the β-amyloid domain (see Figure 3; 83; Z Zhong & B Cordell, in press). Thus, secretase action precludes amyloid formation by disrupting the integrity of the β-amyloid protein. As a result of cleavage, the β-APP exodomain of 100–140 kDa is released from the cell and a carboxyl-terminal fragment of ~9 kDa, which bears a segment of β-amyloid and the cytoplasmic domain of β-APP, is generated. This β-APP remnant of ~9 kDa remains cell-associated because of retention of the transmembrane domain and is ultimately catabolized by cellular degradation pathways. The secretion of β-APP can be positively regulated by a number of agents that act through a common mechanism mediated by protein kinase C (84–86). Secretion of β-APP is not confined to cell culture systems but also occurs in vivo as evidenced by soluble β-APP detected in human plasma (87) and cerebrospinal fluid (88). Not all of the β-APP molecules are secreted. Many remain intact and reside on the cell surface (89, 90) or at the terminals of neurons (91). These uncleaved precursor proteins can be reinternalized, using a consensus "NPXY" endocytotic signal sequence located in the cytoplasmic domain of the protein, and transported back to the cell surface or targeted for degradation (89, 90, 92).

Knowledge of β-APP metabolism raises a number of questions regarding β-amyloid genesis. Where along the intracellular β-APP itinerary is the β-amyloid protein produced? What proteinase(s) and subcellular compartment(s) are involved in releasing the amyloid domain from the precursor backbone? What mechanism(s) is operating to produce β-amyloid? Is aberrant secretion a factor? The answers to many of these questions are not well understood, but a rough picture of β-amyloidogenesis is emerging. In this picture, potential targets for therapeutic intervention can be envisioned.

β-AMYLOID GENESIS

In vitro research using three different experimental approaches has generated a basic understanding of β-amyloid formation. One approach has been to analyze the generation of amyloidogenic carboxyl-terminal fragments of β-APP, the putative processing intermediate to β-amyloid. Additional investigation of β-amyloid processing has been made possible by the recent discovery that normal cultured mammalian cells produce β-amyloid. Finally, synthetic peptide homologues of β-amyloid have permitted definition of the biophysical parameters of amyloid fibril formation.

The position of the β-amyloid domain is a determining factor in the processing of β-APP to β-amyloid. Because the β-amyloid domain spans the extracellular and transmembrane domains of β-APP, it is likely that the primary proteolytic cleavage occurs within the exposed extracellular domain of the precursor rather than within the less accessible membrane-bound domain. Such a cleavage would result in a β-APP carboxyl-terminal fragment carrying an intact β-amyloid domain at its amino terminus.

A number of laboratories have identified carboxyl-terminal fragments harboring an intact β-amyloid domain from cell lysates and from human cerebral cortex (89, 93–95). Amyloidogenic fragments appear to be produced at a number of intracellular sites including the endosomal/lysosomal system (89, 94) and the Golgi complex (J Higaki and B Cordell, unpublished data), but not all may be authentic precursors to β-amyloid. Evidence for a precursor-product relationship between a carboxyl-terminal fragment and β-amyloid stems from in vitro analysis of one Alzheimer's disease-specific mutation (96). In this study, the lysine and methionine codons in the β-APP cDNA immediately amino-terminal to the β-amyloid sequence were replaced by asparagine and leucine codons, respectively, that are identical to the mutations found in some afflicted humans. This mutated cDNA was then used to express β-APP after introduction into cultured mammalian cells. A large and concomitant increase in an amyloidogenic carboxyl-terminal fragment and the β-amyloid protein resulted. The precursor-product relationship between β-amyloid-bearing carboxyl-terminal β-APP fragments and β-amyloid is further demonstrated by the accumulation of such fragments when β-amyloid production is inhibited in cultured cells (J Higaki, N Peet, and B Cordell, unpublished data).

While the primary cleavage event leading to β-amyloid protein appears to involve a carboxyl-terminal intermediate, the exact site(s) of proteolytic cleavage has not been reported. It is possible that the initial cleavage directly generates the amino terminus of β-amyloid. Alternatively, the initial cleavage may be near to the mature β-amyloid sequence, in which case additional cleavages would be required. For this latter scenario, one or multiple

proteinases may be operating. The proteinase(s) responsible for β-amyloid excision constitutes a key target for therapeutic development, and considerable research effort is currently focused on its identification and inhibition. Already a number of putative β-amyloid forming proteinases have been described. Reported candidates include multicatalytic proteinase (97, 98), mast cell chymase (99), metalloendopeptidase 24.15 (100), calcium-activated neutral proteinase (101), a calcium-activated serine proteinase (102), and prolylendopeptidase (103). In general, short peptidic substrates corresponding to the amino and carboxyl termini have been employed to identify and purify each proteinase. Cleavage of native β-APP substrate was evaluated for only one of these proteinases, but unfortunately the specificity of cleavage was not described (101). The physiological relevance of these candidate proteinases has not been demonstrated by parallel inhibition of enzymatic activity with blocked β-amyloid formation.

A major advance in the field was made when workers observed that β-amyloid is produced and released by cultured cells (104–107). This recent finding should greatly facilitate elucidation of amyloid formation. Moreover, it provides cell-based systems with which to identify inhibitors of β-amyloid production. β-Amyloid is secreted from a variety of cell types in vitro (104–106), as well as in vivo (107). That β-amyloid is secreted helps explain the extracellular deposition of this protein. The application of functional inhibitors of different intracellular compartments and/or protein trafficking has shown that the ~4-kDa protein is generated early in the secretory pathway of β-APP biosynthesis. A weakly acidic compartment, probably in the Golgi complex, is the likely site of origin (106, 108). Into what subcellular compartment the β-amyloid protein is directed after the Golgi complex and how it is ultimately released from the cell is not known. Apparently, however, the mechanism of β-amyloid secretion is not used for β-APP secretion, since modulation of secretase activity does not coordinately regulate β-amyloid production (108). Also, the amount of β-amyloid produced per mole of precursor is very low, thus indicating that only a minor subset of β-APP molecules give rise to the ~4-kDa proteolytic product (Z Zhong and B Cordell, unpublished data).

Because β-amyloid is a small protein, synthetic homologues have been employed to understand the physical parameters of fibril and deposit formation. These studies have identified the structural features of β-amyloid that promote seeding, exponential growth, and insolubility of protein aggregates (reviewed in 42 and 43). In fact, synthetic β-amyloid protein can polymerize into fibrils that are morphologically identical to those isolated from the brains of Alzheimer's disease victims. The hydrophobic carboxyl-terminal portion of the β-amyloid molecule is critical in establishing aggregates. Amino acid substitutions in this hydrophobic domain, as well as the

length of the carboxyl-terminus of β-amyloid, greatly influence the rate of aggregation. For example, a β-amyloid protein of 42 residues forms fibrils in hours, compared to a β-amyloid protein of 40 residues that requires days. The β-sheet structure that the protein can adopt under certain extrinsic conditions is also a requirement for aggregation and insolubility. This molecular information has been valuable in understanding the implications of increased expression and concentration of β-amyloid, naturally occurring mutations in its primary sequence, and agents that may serve to assist in seeding fibril formation. The physical studies have important ramifications for potential treatments of the disease. Because small changes in β-amyloid concentration are calculated to have large consequences in the rate of insoluble aggregate formation, therapies that only minimally reduce β-amyloid levels could potentially cause a significant reduction in the number of insoluble deposits and/or their development into mature plaques. Also, compounds that bind β-amyloid and block its ability to seed further molecular addition would be of therapeutic advantage.

An illustration summarizing a number of the aspects of β-amyloid formation is presented in Figure 4. In this sequential process leading to β-amyloid deposition, new targets for therapeutic intervention are highlighted, namely, developing inhibitors of the proteinase(s) that liberate β-amyloid from its precursor and interrupting β-amyloid aggregation.

MECHANISMS OF β-AMYLOID FORMATION

A collection of seemingly different mechanisms appears to be responsible for β-amyloid formation. At the genetic level, mutations in the gene encoding β-APP can produce the disease state (see Figure 3). In vitro expression of one of these mutations (leucine for methionine), generates a 5- to 8-fold increase in β-amyloid production (96, 109). This increased concentration of soluble β-amyloid could dramatically accelerate the rate of fibril formation in individuals carrying the mutation (42). A synthetic peptide homologue of another β-APP genetic mutation (glutamate substituted for glutamine within the β-amyloid domain) was found to have increased stability of a β-sheet conformation that would facilitate fibril formation (110). The pathological consequence of mutations located within the transmembrane domain immediately adjacent to the β-amyloid domain has not been determined, but they may influence the cleavage producing the carboxyl terminus of β-amyloid. Perhaps cleavage is altered with the mutant β-APP such that a β-amyloid protein of 42 residues is generated rather than a less pathogenic β-amyloid protein of 40 residues.

Increased β-APP gene dosage as in Down's syndrome appears to be another mechanism leading to Alzheimer's disease. In addition to an extra

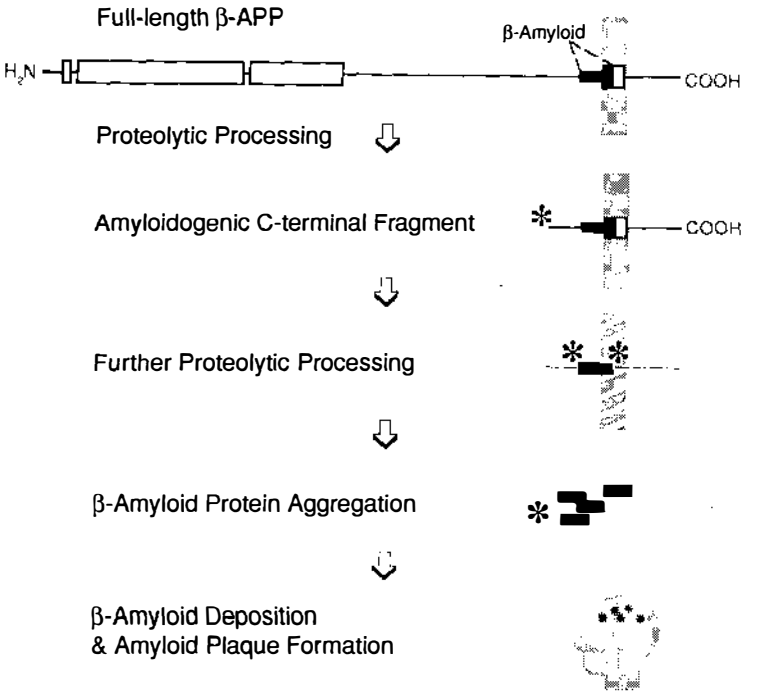

Figure 4 Proteolytic processing of the β-amyloid precursor protein leading to β-amyloid formation and deposition. Possible targets for therapeutic intervention are indicated by asterisks.

allele, Down's individuals have a concomitant 2-fold increase in both β-APP mRNA (79) and protein expression (111), which may generally elevate levels of β-amyloid production. Over-expression of β-APP may also explain the possible association of head trauma and Alzheimer's disease (58). The very rapid appearance of β-amyloid deposition, seen within weeks of severe head trauma (57), may result from increased gene expression because the promoter region controlling β-APP expression has stress-responsive elements (112). General increases in β-APP expression in Alzheimer's disease per se have not been reported. However, alterations in β-APP isoform expression have been found. A number of reports indicate a disease-specific increase in neuronal expression of β-APP isoforms harboring the Kunitz proteinase inhibitor domain (95, 113–115). In one study, a direct linear correlation between increased expression of inhibitor-bearing β-APP and neuritic plaque density was observed (115). In addition, transgenic mice genetically programmed for increased neuronal expression of the 751 amino acid isoform

containing the Kunitz inhibitor domain show β-amyloid deposition in their brains, whereas mice with increased neuronal expression of the isoform normally predominating in this cell type, the 695 amino acid isoform, do not (36).

A unifying mechanism that assimilates and explains the different molecular alterations leading to β-amyloid formation can be put forward. It is proposed that β-amyloid protein derives from aberrant β-APP molecules. These aberrant precursor proteins are discarded via an intracellular degradative pathway exiting at an early point along the biosynthetic process. There are a number of ways a β-APP molecule might qualify as aberrant. Aberrancies could include mutations, excess amounts of wild-type β-APP, expression of the "incorrect" isoform, structural misfolding, and abnormalities in posttranslational modification. For each situation, β-amyloid would be formed as a proteolytic by-product of this degradation process. Similar to other degradative organelles such as lysosomes, the degradative products of aberrant β-APP catabolism would be extruded from the cell into the extracellular compartment. Once liberated, the soluble β-amyloid protein would be able to initiate fibril formation, which would occur at different rates depending on the primary structure and concentration of the protein, as well as conditions in the local microenvironment. This hypothesis has therapeutic implications. It suggests that inhibition of aberrant β-APP degradation should have little or no effect on the biosynthesis and normal function of "correct" β-APP molecules. Potentially, if the proteinase(s) involved in β-amyloidogenesis is inhibited, alternative proteolysis will occur, resulting in non-amyloidogenic degradative fragments. The development of an inhibitor of β-amyloid formation would greatly facilitate testing this hypothesis experimentally.

CONCLUSION

Emerging information on the pathobiology of Alzheimer's disease points to β-amyloid formation as a critical early factor in the process. A central goal in Alzheimer's disease research is now to prevent this early pathologic event. Already, investigators have developed a number of in vitro and in vivo systems that provide insights into the molecular mechanism(s) of the disease process and β-amyloid genesis. Moreover, these experimental systems have revealed novel approaches to therapeutic development. Specifically, compounds that inhibit the proteinase(s) that produce the β-amyloid protein or those that block assembly of this protein into neurotoxic amyloid fibrils are sought. These unique therapeutic targets offer new encouragement for ultimately treating this tragic disorder.

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