Alzheimer's Disease: A Cell Biological Perspective

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An almost bewildering number of findings concerning Alzheimer's disease mask the significant recent progress in understanding the molecular basis of some inherited forms of this disease and the proteolytic processing of proteins related to the disease. Alzheimer's disease is an amyloidosis, a condition in which certain proteins or protein fragments precipitate in various tissues as amyloid, fibrillar aggregates with a β-pleated sheet conformation. Alzheimer's is also characterized by neuritic lesions and cell death. Some rare forms of the disease are now known to arise from a mutation in an amyloidogenic protein. Another recent insight is the discovery of an endosomal-lysosomal processing pathway capable of generating protein fragments that can deposit extracellularly as amyloid fibrils. Key future directions for cellular-based research in Alzheimer's disease include the study of membrane trafficking and the passage of intracellular material to the extracellular milieu, molecular signaling among intracellular compartments, the interaction between organelles and the neuronal cytoskeleton, and the nature of cytoskeletal reorganization after neuronal injury.

After decades of debate concerning the cause of Alzheimer's disease (AD), we now have an answer for a small fraction of patients with the disease. There are a handful of families with familial (inherited) AD in which a fundamental genetic defect has been pinpointed. The defect is a mutation on chromosome 21 in the gene encoding a membrane glycoprotein called the amyloid precursor protein (APP), the parent molecule of a considerably smaller peptide that accumulates in the brain and vasculature of patients with AD. This fragment, amyloid β protein (AβP), accumulates at the core of the senile plaque, a neuropathological hallmark of AD. The mutation in the APP gene, discovered by A. Goate, J. Hardy, and their colleagues (*1*), was a single nucleotide change from a C to T at base pair 2149 in exon 17 of APP, which resulted in the substitution of an isoleucine for a valine at codon 717 in APP of a patient with familial AD. This observation triggered the sequencing of the APP from many families with this form of AD. It is clear from this effort that, although a few similar mutations have been identified, these mutations in the APP gene are exceedingly rare. Although AD can be linked to a primary defect in APP in some cases, most cases of familial AD as well as the sporadic cases still do not have a clear etiology (*1a*). The additional mutations found in codon 717 change the valine, which is conserved in APP from all mammalian species that have been sequenced, to either an isoleucine (*2, 3*), a glycine (*4*), or a phenylalanine (*5*). Very recently C. Jones and D. St. Clair of the Edinburgh Alzheimer Research Group have discovered a patient with chronic schizophrenia and a missense alanine to valine mutation at codon 713, a site at the COOH-terminal end of the AβP (*6*).

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APP Expression

APP is a single gene that undergoes alternative splicing to generate several isoforms that are designated by the total number of amino acids in each. Inclusion of various alternatively spliced exons results in isoforms of 770, 751, 714, 695, 563, and 365 amino acids (*7*). The two smallest of these isoforms do not encode the AβP sequence. The amino acid sequence was determined from AβP isolated from the amyloid deposited in meningeal vessels of AD patients (*8*), making it possible for others to clone APP by screening libraries with degenerate oligonucleotide primers. The full-length sequence (*9, 10*) suggested the pivotal nature of APP processing because it revealed that the AβP fragment, consisting of two constitutively expressed exons, was cleaved from APP.

The APP processing event of key interest is the cleavage of the AβP fragment from the precursor. Near the COOH-terminus of APP is a membrane-spanning sequence (Fig. 1). This sequence is inserted into the plasma membrane or the membrane of some subset of organelles. A long NH_2-terminal domain extends extracellularly or intralumenally, depending on the particular membranous element with which APP is associated. A short COOH-terminal tail lies in the cytoplasm. The COOH-terminus of AβP is within the membrane-spanning sequence, raising the question of how proteolytic cleavage could occur within an intramembranous site. The NH_2 terminus of AβP lies at Asp^{672} (numbers refer to APP_{770}).

Codon 717 mutations, which lead to the typical clinical features of AD but with a particularly early onset, lie within the intramembranous region of APP on the COOH-terminal side of AβP. Postmortem examination of patients with AD in which codon 717 was a missense isoleucine, phenylalanine, or glycine reveal the typical senile plaques and neurofibrillary tangles of AD (*2, 3, 5, 11*). [One exception to this is a patient who also had Lewy body inclusions in brainstem and cortical neurons (*2*).] The very conservative nature of some of these mutations makes it unlikely, but not impossible, that an alteration in hydrophobicity alters the relation of APP to the membrane. Alternatively, this mutation may affect APP posttranslational processing.

Trisomy of chromosome 21, which causes Down syndrome, represents another genetic route to the AD phenotype. Individuals with Down syndrome invariably develop the neuropathological features of AD, a process that appears to begin with the deposition of AβP as much as 50 years earlier than occurs in the general population of elderly at risk for AD (*12*). Since APP is encoded on chromosome 21, the gene dosage effect would be predicted to result in high concentrations of APP in these individuals. Gene dosage effects have been reported for eight chromosome 21 gene products in people with Down syndrome in which the increased expression approximates the theoretical value of 1.5 times the disomy levels (*13*). The regulation of APP, however, may be more complex because its expression in trisomic conditions may exceed the expected 50% increase above normal values (*14, 15*). In the trisomy 16 mouse, a model for Down syndrome (many loci on mouse chromosome 16 map to human chromosome 21), APP gene expression varies from tissue to tissue and as a function of development (*15*). Many of the gene products for which gene dosage effects occur are metabolic enzymes, which are constitutively expressed. In contrast, the expression of APP is developmentally regulated and therefore may be affected by additional levels of trans regulation. For example, up-regulation of APP expression occurs in endothelial cells after treatment with interleukin 1 (*16*); complex control elements occur in the region of the APP promoter, including two heptamer sequences that resemble an AP-1 binding

site and a nucleotide sequence that resembles the heat shock control element (*17*).

Related Amyloidoses

The discovery of mutations in APP links AD to several other diseases in which a mutated parent protein or proteolytic fragment of a mutated parent protein precipitates to form amyloid fibrils that have principally a cross β-pleated sheet conformation. Analogous conditions affecting the nervous system (and sometimes also other tissues) include familial amyloidotic polyneuropathy involving transthyretin (*18*); hereditary cerebral hemorrhage with amyloidosis, Icelandic type, involving cystatin C (*19*); hereditary cerebral hemorrhage with amyloidosis, Dutch type, involving APP (*20*); familial amyloidosis, Finnish type, involving gelsolin (*21*); and Gerstmann-Straussler-Scheinker syndrome, involving the prion protein (*22*). Thus amyloidogenic fragments of similar secondary structure can arise from different primary structures.

The mutation site in the APP may have an effect on the disease phenotype. Hereditary cerebral hemorrhage, Dutch type, in which AβP is deposited primarily within the walls of meningeal and cerebral blood vessels, is usually recognized when the patient has a cerebral hemorrhage. Affected patients have a mutation at codon 693, a site within the AβP fragment, which results in a glutamate to glutamine substitution (*20*). The AβP deposited within the blood vessels in the Dutch-type hereditary cerebral hemorrhage contains both the normal and the variant forms of the APP alleles (*23*). Because the gene product of the normal allele contributes to the material deposited as AβP, it is possible that, just as is postulated for the transmission of prion diseases, a nucleating, template-directed conversion of protein conformation (*24*) might also occur in other amyloidoses.

Small differences in a mutation site may affect the phenotype; however, the exact relation between a particular mutation and the disease expression is by no means clear. The anatomical sites at which various amyloid deposits form and the sites of associated lesions, such as the neurofibrillary tangles in AD, appear responsible for the clinical phenotype. Various mutations of transthyretin in familial amyloidotic polyneuropathy result in quite distinct patterns of organ dysfunction (*18*). Various mutations in the prion gene are associated with phenotypes described either as Creutzfeldt-Jakob or as Gerstmann-Straussler-Scheinker diseases (*25*). These historical designations overly simplify the broad range of phenotypes, which includes forms with minimal neuronal loss, minimal gliosis, and minimal or no detectable amyloid plaques or prion amyloid protein. The unsuspected association of the phenotypic entity, fatal familial insomnia, with a prion gene mutation (*26*) may herald additional surprises in inherited neurodegenerative conditions that fail to fit into known phenotypes.

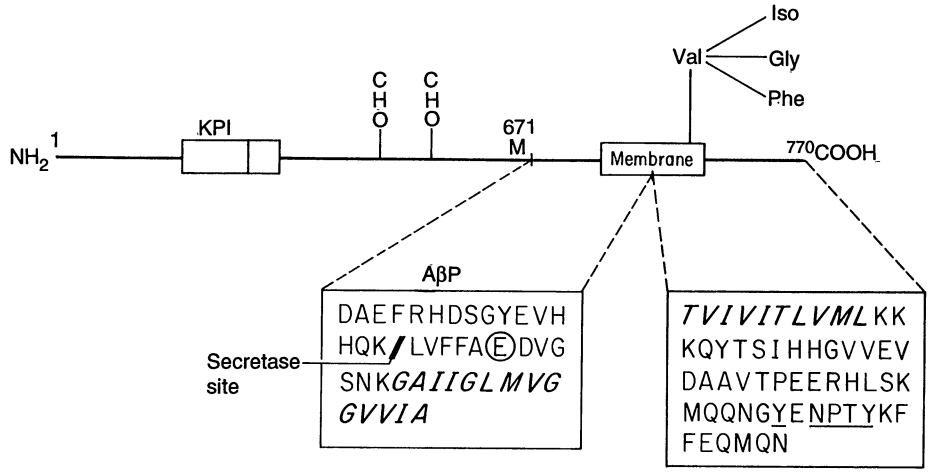

Fig. 1. The amyloid precursor protein. The NH_2-terminus is designated by the number 1, and Met^{671}, the site where the AβP begins, is noted. The AβP box includes 42 amino acids, although it may extend to 43 amino acids in some cases. The circled E in the AβP box indicates the site of the mutation in hereditary cerebral hemorrhage with amyloidosis, Dutch type (*19*). The bold italic amino acids are predicted to be intramembranous. Val^{717} in the intramembranous portion of APP is the site of several missense mutations in familial Alzheimer's disease. A putative endocytotic targeting sequence is underlined. Two glycosylation sites are indicated by CHO and two alternatively spliced exons are boxed. One of these exons is designated KPI because of its homology to Kunitz protease inhibitor sequences.

APP Processing

In order to follow APP processing in cells, full-length isoforms have been transfected into various cell types (*27, 28*). Human embryonic kidney 293 cells stably transfected with full-length APP_{751} and APP_{695} generate a membrane-associated 9-kD COOH-terminal fragment, which is cleaved from APP between Lys^{687} and Leu^{688} within AβP (*28*). This interior cleavage precludes the formation of AβP. The quantitative production of this cleaved form may prevent the deposition of amyloid fibrils. The large NH_2-terminal fragment of approximately 100 kD is secreted, and therefore this enzymatic activity has been referred to as a "secretase." The secreted product of this so-called "constitutive" cleavage has been identified as protease nexin (PN) II (*29*). Although there are no reports that these transfected cells form amyloid fibrils, when the COOH-terminal 100 residues of APP (containing the full AβP region) were transfected into COS-1 cells, 8- to 22-nm fibrils that could be stained with an antibody directed against the COOH-terminus of APP formed near the nuclear membrane (*30*).

How much APP normally is cleaved by the secretase in various cell types is unknown. Because it is difficult to detect APP at the cell surface, other important pathways may generate APP intermediates. The movement of APP down axons in the rapid phase of axonal transport (*31*) suggests that it is associated with organelles that move via a microtubule-based motor. The suspicion that another degradative pathway exists gained credence when APP processing was also reported to occur in an endosomal-lysosomal pathway (*32, 33*). Kidney 293 cells transfected with APP_{695} produced a set of 8- to 12-kD COOH-terminal derivatives, the largest of which are sufficient to include the AβP fragment. Similar fragments were found in normal postmortem human brains and in rat brain (*34*). An even larger COOH-terminal fragment of 22 kD occurs normally in brain microvessels (*35*). Treatment of the 293 cells with ammonium chloride, an inhibitor of endosomal-lysosomal proteolysis, substantially diminished the generation of the derivatives (*32*). In short-term experiments (1 hour), leupeptin had a similar effect. APP is internalized from the cell surface and targeted to late endosome-lysosomes where an array of potentially amyloidogenic COOH-terminal fragments are generated (*36*). Nevertheless, localization of APP to this intracellular membrane-bound compartment, a finding indirectly suggested earlier by antibody labeling (*37*) and by a marked reduction of secreted forms in microglia, astrocytes, and neurons relative to transfected cells (*38*), fails to explain the extracellular deposition of AβP. The sugges-

tion from work in chicken fibroblasts that the lysosome is not a terminal membrane target, but can get re-routed to the plasmalemma, may also apply to APP processing.

Endocytosis is an active process by which up to 50% of the cell surface can be internalized per hour (*40*). The cytoplasmic tail of APP may target the molecule for rapid internalization through coated pits via the use of the NPXY (Asn-Pro-X-Tyr) sequence, one of several similar consensus sequences in which the tyrosine forms a crucial component of the recognition determinant (*41*). In many proteins with this sequence, there is a hydrophobic residue two positions to the NH_2-terminal side of the asparagine, a position occupied by a tyrosine in APP. Other receptors for which the consensus sequence in the cytoplasmic tail is required for rapid endocytosis or coated pit–mediated internalization include the low density lipoprotein receptor (*42*), the mannose-6-phosphate receptor (*43*), and transferrin (*44*). Assuming that the NPXY sequence does target APP to endocytotic vesicles, the concentration of APP in these organelles may occur constitutively or may be regulated by an internalization signal. Fc receptor internalization, another example of coated pit–mediated endocytosis, may depend on sequences that tether the protein to the cytoskeleton, thereby preventing internalization (*45*). Indeed, in neural and glial cell lines large amounts of APP are found in a detergent-insoluble cytoskeletal fraction (*46*). The interaction of APP with the cytoskeleton may be modulated by the phosphorylation state of the cell (*46*), which can also shift the degradation pathway to generate more COOH-terminal fragments (*47*). A phosphorylation-mediated interaction between the cytoskeleton and a receptor protein also occurs in the case of leukocyte function–associated antigen–1 (LFA-1) binding to the actin-associated protein, talin (*48*).

The Amyloid Fibril

The AβP fragment has a molecular mass of approximately 4000 daltons. The exact COOH-terminus of the fragment is not known with certainty, but its maximum length is either 42 or 43 amino acids, ending either at Ala^{713} or Thr^{714} (*9*). However, the fragment can vary in length. The amyloid deposited in the core of a senile plaque can have a blocked NH_2-terminus (*49*), whereas, in cerebral blood vessels, it is more soluble and may terminate at residue 710 (*50*) or 711 (*51*). Amyloid from the AD brain consists of 75 to 100 Å fibrils which, by x-ray diffraction, have principally a cross β-sheet conformation (*52*) with the fiber axis perpendicular to the polypeptide β-strand and parallel to the hydrogen bond direction. Synthetic peptides corresponding to AβP and a number of shorter peptides contained within AβP can spontaneously form amyloid-like fibrils in a β-sheet conformation (*53, 54*). The formation of these fibrils is pH-dependent (*53, 55*), an observation that may explain the generation of potentially amyloidogenic fragments in acidic lysosomes.

Once an amyloidogenic fragment is generated, it undermines the viability of the neuron by a mechanism that is not well understood. APP fragments may be directly toxic to neurons in a way that results in neurofibrillary lesions (*56*). Alternatively, some common insult might result in both the amyloid plaques and the neuritic (neurofibrillary) lesions. This latter scenario seems likely after head trauma or in dementia pugilistica in which APP fragments, as well as amyloid plaques and neurofibrillary tangles, are deposited (*57*). Whether synthetic peptides derived from AβP are directly toxic has stirred considerable controversy. Certain peptides can indeed be toxic to biological membranes. Peptides can be toxic via their interaction with a receptor; however the only evidence that an interaction of the AβP with the plasmalemma occurs via a receptor is the demonstration of its binding to the serpin-enzyme complex receptor (*58*), a finding that has as yet unclear relevance to the pathogenesis of the disease. In contrast, antibacterial peptides are directly toxic to membranes and may exert their cytotoxic effects by forming channels in the membrane (*59*). One class of these peptides, the cecropins, bears a weak homology to a portion of the AβP, including a conserved glutamic acid in position 693 of APP, the site of the mutation in Dutch-type hereditary cerebral hemorrhage. Cecropins act stoichiometrically rather than catalytically to effect their damage in bacteria. However, if they were toxic in humans they would have to act by a somewhat different mechanism because this class of peptides does not harm the eukaryotic plasmalemma; its effects upon the lysosomal membrane are unknown.

The Neuritic Dystrophy

If we are to understand AD, we must understand the formation of the second neuropathological hallmark of the disease—the neuritic lesions. The spectrum of these lesions includes the classical neurofibrillary tangle and the considerably more abundant dystrophic neurites found both around some senile plaques and elsewhere in the neuropil. These lesions invariably accompany the clinical dementia associated with AD; deposition of AβP alone is not sufficient to develop dementia (*60*). The common feature of the various neuritic lesions is their immunoreactivity with antibodies to the microtubule-associated protein tau. The ultrastructural correlates of the neuritic lesions are straight or paired and helical filamentous structures. The principal protein constituent of these filamentous structures is tau (*61–63*), a protein encoded by a single gene, the exon structure of which has been determined in cow and human (*64*). The tau messenger RNA (mRNA) is localized to neurons (*65*), where the protein translation product selectively binds to the population of microtubules in the axon (*66*). The suppression of tau in neuronal cultures with the use of antisense oligonucleotides to tau suggests a role for tau in the selective elongation of the axonal process (*67*). Furthermore, the expression of tau in Sf9 cells by a baculovirus vector induces long, relatively unbranched processes (*68*) that contain microtubules with some organizational features of an axon (*69*).

Tau is a highly heterogeneous molecule, the complexity of which arises both from the expression of alternatively spliced isoforms and from phosphorylation at multiple sites. One or more of these as yet undetermined phosphorylation sites affect binding of tau to microtubules. These sites lie on either side of a series of imperfectly repeated sequences (*70*), which bind to the microtubules. The transition of tau from a microtubule-binding protein to a polymeric filament must first require its dissociation from the microtubule. This transition involves modifications that decrease the mobility of tau and generate a series of phosphorylated tau isoforms referred to as A68 (*62, 63*). These isoforms correlate with a soluble pool of paired helical filaments, and some antibodies to phosphotau epitopes react with AD-associated tau isoforms but not with normal adult tau protein (*63*). The extreme insolubility of some paired helical filaments (*71*) may result from their conjugation to ubiquitin.

As remarkable as the accumulation of tau-reactive filaments in neurons is the complete dissolution of the cytoskeleton in these same neurons (*72*). Although the assembly of tau into filaments may be simply the consequence of its molecular structure, the cause of the massive cytoskeletal loss in affected neurons remains enigmatic. The abnormal phosphorylation state of tau raises the possibility of a more generalized activation of kinases in AD neurons. Direct phosphorylation of neurofilament subunits within the NH_2-terminal head domain could result in their

disassembly (*73*). The hyperphosphorylation of various microtubule-associated proteins could result in their dissociation from microtubules and, consequently, microtubule destabilization.

In addition, the dystrophic neurites may represent sprouts from affected neurons (*74*). Curiously though, these neurites do not have features of growth cones; instead, they have degenerative features including accumulations of filaments and often ubiquitin immunoreactivity. Their ability to elongate may represent a neurite extension not mediated by growth cones that may occur as a result of the polymerization of intracellular filamentous structures. A phenomenon of this nature has been described in the lamprey after axotomy, which results in ectopic axon-like sprouts filled with phosphorylated neurofilaments that emerge from the tips of dendrites (*75*).

The Future

Connection of the diverse facets of AD is one of the challenges for researchers in this field. A focus upon the cell biology of AD promises to be the most rewarding future direction: we must understand what triggers the neuritic lesions of AD, whether other abnormalities precede amyloidogenesis in the majority of AD cases that lack a mutation, and how APP is processed as its associated membrane-bound organelles are routed through cellular compartments. Ultimately the reward of research into Alzheimer's will be great because the complexities of this disease will inevitably shed light on the higher functions of the brain, the target of AD.

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