Deciphering Alzheimer's Disease: The Amyloid Precursor Protein Yields New Clues

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WHEN A NEWLY IDENTIFIED MACROMOLECULE APPEARS to play an important and conserved role in biology and is implicated in a major disease, one may expect that the study of its structure and function will advance rapidly. Such is indeed the case for the amyloid β protein precursor (APP). This membrane-spanning glycoprotein is expressed in many mammalian tissues and cell lines and is encoded by a gene that, in humans, is found on chromosome 21. APP has become the subject of scrutiny because an ~40–amino acid fragment of the molecule, referred to as the amyloid β protein (AβP) or A4, is the major proteinaceous component of the myriad amyloid deposits that accumulate extracellularly in the brains of patients with Alzheimer's disease (AD). In recent weeks, seven research reports concerning APP have appeared in *Science*, and their implications for understanding Alzheimer's disease are discussed here.

To put the new work in perspective, one must recall what has been discovered about APP and AβP since identification of the partial sequence in 1984 (*1*). The first full-length cDNA obtained predicted a 695-residue polypeptide (APP_{695}) that spanned the membrane once, with a large, extramembraneous NH_2-terminal portion, and a short tail in the cytoplasm (*2*). The AβP consisted of the 28 amino acids just outside the membrane plus the first 11 to 14 residues of the hydrophobic transmembrane domain. The question thus arose as to how a putatively membrane-inserted portion of APP could be proteolytically cleaved to release a ~40-residue peptide that would accumulate extracellularly.

There are several alternatively spliced APP transcripts. The two most abundantly expressed forms contain exons of 56 and 19 amino acids, one or both of which are inserted at residue 289 of APP_{695}, yielding either 751- or 770-residue proteins (APP_{751}, APP_{770}) (*3*). The 56-residue insert has ~50% homology with the Kunitz serine protease inhibitors (KPI), and APP polypeptides containing it have been shown to inhibit trypsin (*3*). This observation came full circle when a computer search and supporting in vitro experiments revealed that the large NH_2-terminal portion of APP_{751} or APP_{770}, which is secreted by various cultured cells, was identical to a proteinase inhibitor, protease nexin II (PNII) (*4*). It has been proposed that PNII is released from the holoprotein by cleavage near the membrane, may then inactivate serine proteases in the extracellular space, bind to the cell again as a protease-inhibitor complex, and be internalized. Whether such normal processing could somehow be perturbed in AD brain is unknown. A separate line of inquiry has suggested that the secreted forms of APP (particularly those containing the KPI domain) can function as growth-promoting factors (*5*).

While these advances in understanding normal APP structure and function were accruing, progress was also being made in characterizing abnormal deposition in AD. Antibodies to native or synthetic AβP revealed a much greater number and wider distribution of AβP deposits in AD brain than conventional histochemical analyses had suggested. Many of the tissue deposits appeared by light and electron microscopy to be largely nonfilamentous (called "diffuse" or "preamyloid" deposits) (*6, 7*). Such deposits are actually much more abundant than the filamentous amyloid cores of "classical" senile plaques, which are surrounded by altered glial cells and structurally abnormal neurites of varying neurotransmitter specificities. The finding of diffuse AβP deposits in younger subjects with trisomy 21 (Down syndrome) in the absence of surrounding neuronal and glial dystrophy has provided the strongest evidence yet that AβP deposition may precede the cytopathology of AD. Virtually all patients with Down syndrome develop classical senile plaques indistinguishable from those of AD if they survive beyond age 40. The observation of diffuse AβP deposits essentially lacking surrounding cellular changes in clinically unaffected regions of the brain (such as the cerebellum) further supports the hypothesis that deposition of AβP might be an early event and that the local response to its presence might vary among brain regions (*8*).

Because these and other recent observations suggest that AβP deposition may play a seminal role in AD, it is important to delineate the normal and alternative pathways by which APP molecules can be proteolytically processed. In the first of the recent *Science* papers, Sisodia *et al.* (*9*) transfected mammalian cells with cDNA constructs encoding variant APP molecules that lacked some or all of the region of APP immediately extracellular to the transmembrane domain (that is, the NH_2-terminal portion of AβP). By assessing which of these variant molecules could still undergo cleavage and secretion of the extramembranous portion, Sisodia *et al.* deduced that APP cleavage must normally occur within the AβP peptide. Although these recombinant APP experiments indicate the region within which normal cleavage might occur, the actual delineation of the clip site requires direct sequencing of the termini of both the secreted and membrane-retained fragments. This feat has now been accomplished by Esch and co-workers (*10*). Using human kidney cells transfected with either APP_{695} or APP_{751} cDNAs, they purified the large NH_2-terminal fragment from culture medium and showed that it ended at position 15 within AβP (residue 611 of APP_{695}). The ~9-kD COOH-terminal fragment was purified from cell membranes and found to begin at residue 17 of AβP (613 of APP_{695}). The single amino acid separating these termini must be removed from one or the other fragment by an exopeptidase after the original cleavage. The presence of the initial portion of AβP on the secreted APP fragment corroborates an earlier study that described AβP immunoreactivity of such fragments in human cerebrospinal fluid (*11*).

If constitutive processing of APP at this site is confirmed in normal human tissues, then the responsible protease (APP secretase) must not cleave APP molecules that give rise to intact AβP peptides prior to amyloidogenesis. Even during late stages of plaque deposition in AD and Down syndrome, most of the APP molecules expressed in brain and nonneural cells still undergo the constitutive cleavage, since NH_2- or COOH-terminal fragments of the normal size are readily detected in extracellular fluids (cerebrospinal fluid

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and plasma) or tissues, respectively, of such patients. What percentage of APP molecules undergo alternative cleavage to release AβP, and why the APP secretase does not cleave these appropriately remains to be determined. Humans, monkeys, dogs, and certain other mammals have the necessary alternative pathway to generate intact AβP fragments, as in all of these species β-amyloid deposition normally occurs late in life (*12*). In Down syndrome, an increase of transcription of APP because of elevated gene dosage may cause more APP molecules to be diverted to the alternative catabolic pathway, leading to accelerated AβP formation and early β-amyloid deposition. Perhaps a different genetic mechanism alters the regulation of APP biosynthesis in familial (autosomal dominant) forms of AD, resulting in increased use of the alternate pathway and a neuropathological phenotype indistinguishable from that of late Down syndrome.

Now that the primary structure of the secreted form of APP is established, its normal function is being studied. The truncated form of APP containing the KPI domain (alias PNII) is stored in platelets and is released during activation by agents such as thrombin, collagen, or calcium ionophore (*13, 14*). Van Nostrand *et al.* (*13*) show that PNII is localized in platelet α-granules and released with other granule constituents by treatment with physiologic agonists that trigger granule secretion. They point out that PNII, which has both protease inhibitory and growth promoting activities, could be secreted at wound sites where platelets aggregate and thus influence inflammatory responses and tissue repair. Van Nostrand *et al.* speculate that in AD and other disorders (*15*) in which vascular AβP deposition occurs, subtle changes in vessel walls may expose sites that could activate platelets and thus release their PNII. However, this form of APP lacks part of AβP and could not itself serve as the precursor of β-amyloid. The inability of Van Nostrand *et al.* to detect circulating PNII in plasma may relate to experimental design, since chromatography of plasma on Affigel-blue has been shown to yield a fraction enriched in COOH-truncated APP that comigrates and is immunochemically cross-reactive with the PNII in cell media and cerebrospinal fluid (*16*). In the study of platelet APP by Smith *et al.* (*14*), an inhibitor of coagulation factor XIa released from stimulated platelets was shown to be identical to PNII. Thus, another apparent function of the KPI-containing form of APP may be to participate in the regulation of hemostasis (*14*). These reports do not address the question of the function of APP_{695}, the expression of which is largely restricted to neurons.

The remaining three papers under consideration deal with alterations of APP in human disease. Addressing an issue examined by several laboratories in the last 3 years, Johnson *et al.* (*17*) have conducted quantitative in situ hybridization of hippocampal pyramidal neurons to demonstrate a twofold increase in the ratio of APP_{751} to APP_{695} mRNAs in AD versus non-AD subjects. They further show that most hippocampal pyramids contain both transcripts and that the increased ratio is not due to a decrease in APP_{695} mRNA prevalence in AD. The latter result contrasts with earlier RNA blot analyses by these and other investigators which also documented an increase in the ratio of APP_{751} to APP_{695} mRNAs in AD but found it to be due to a decrease in APP_{695} transcripts. The RNA blot technique measures total RNA from all cells, including glia and other nonneuronal cells that express APP. Johnson *et al.* also reported a linear relation between the degree of increase in the ratio of APP_{751} to APP_{695} mRNAs and the density of senile plaques in hippocampus and entorhinal cortex. As Johnson *et al.* emphasized, this finding cannot yet be construed to indicate a cause-and-effect relation between altered APP transcription and amyloid deposition. Indeed, the quantitation of APP mRNAs in human brain remains controversial. A study with ribonuclease H–S1 nuclease protection assays indicated the opposite: that there was no significant change in the differential expression of APP transcripts in AD compared to aged (control) human cortex or in aged monkeys undergoing β-amyloidosis (*18*). Whether local changes in APP transcription underlie AβP deposition in affected brain regions still remains in question.

The final two reports in this group provide a compelling example of a principle of medical research: that the investigation of a very rare disease in a small patient group can provide critical insights into common pathological processes that affect the entire population. It has been known for several years that four families from two coastal villages in the Netherlands have an autosomal dominant form of hereditary cerebral hemorrhage with amyloidosis (HCHWA-D). The subunit protein of the amyloid deposits that affect innumerable meningeal and cerebral blood vessels in this disease is a form of AβP. Moreover, some of the Dutch patients also develop cortical AβP deposits that closely resemble the diffuse plaques of AD and Down syndrome, although no neuritic plaques and neurofibrillary tangles (and no dementia) have been described in HCHWA-D. Now, Van Broeckhoven *et al.* (*19*) have carried out genetic linkage analysis in two of the Dutch families and find that the APP gene is tightly linked to HCHWA-D, suggesting that a mutation responsible for the disease occurred in a common ancestral allele of APP. This idea has essentially been proved by Levy and co-workers (*20*), who detected a point mutation in the APP coding region in two Dutch patients that caused the substitution of a glutamine for glutamic acid at position 22 of AβP (position 618 of APP_{695}). Since no such substitution has previously been observed in DNA encoding the AβP region from normal humans, AD and familial AD patients, or even lower mammals, one can assume that this mutation is not an irrelevant polymorphism but is the primary defect in HCHWA-D. If we consider these data together with the findings of Esch *et al.*, the location of the Dutch mutation just six amino acids downstream from the constitutive APP cleavage site suggests that the mutation could alter the efficiency of binding of the APP secretase, thus allowing some APP molecules to be alternatively processed to liberate intact AβP. Several other ways in which the glutamate to glutamine mutation could augment AβP deposition may also be considered.

Whatever the mechanism, one genetic event that can cause premature and accelerated deposition of AβP in cerebral blood vessels and diffuse plaques is a mutation in AβP itself. The recent discovery (*21*) of AβP-immunoreactive deposits in skin and intestine of AD and some aged non-AD subjects, particularly in and near blood vessels, heightens interest in the possibility that at least some of the AβP that accumulates during AD and aging could be of vascular or circulating origin (*22*). This hypothesis contrasts with the generally held view that neurons are the most likely source for AβP deposition. Very recently, numerous perivascular AβP deposits have been detected deep in the cerebral white matter in AD (*23*); like the meningovascular deposits, these are not in proximity to neurons or neuritic endings. However, no form of APP containing the intact AβP region has yet been demonstrated in plasma. Much study is needed before the question of whether AβP originates from APP in neurons, glia, vascular cells, extracellular fluids (blood and cerebrospinal fluid), or multiple sources can be settled.

Although the detection of an AβP mutation in HCHWA-D is of great interest, subjects with AD have no known defects in the APP coding region. In Down syndrome, duplication of a structurally normal APP gene can apparently result in the AD phenotype. In familial AD, only some cases of which have been linked to chromosome 21 (*24*), there may be more than one genetic mechanism that can augment the deposition of amyloidogenic AβP fragments. The central issue of whether and how AβP (perhaps in concert with certain β-amyloid–associated proteins) can lead to regionally selective cellular pathology remains enigmatic. While we are left with

many questions, the steadily growing evidence that AβP deposition is an early and important pathogenic event in AD recommends the study of β-amyloidosis as a rational route toward developing specific therapies for this still untreatable disease.

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