Alzheimer's Pathology Explored

Current discussions about the causes of Alzheimer's disease focus on the possible role of the amyloid protein. Some researchers think it's important; others don't

A COUPLE OF YEARS AGO, a meeting on Alzheimer's disease would probably have focused on the specific neuronal systems that degenerate in that syndrome. Knowing which types of nerve cells malfunction and die would help researchers understand the patients' symptoms and might even provide a guide for devising therapies. But at a Dahlem conference on neurological diseases held earlier this month, the topic was all but ignored.

And it wasn't because the participants, succumbing to Alzheimer's themselves, had forgotten about it. It was because a new topic has taken center stage in Alzheimer's research in the last few years. "An enormous amount has been learned," says neuropathologist Donald Price of Johns Hopkins University School of Medicine, who helped to organize the Dahlem Conference. "We have identified the nerve systems at risk. Now it's a matter of trying to understand what causes the pathology."

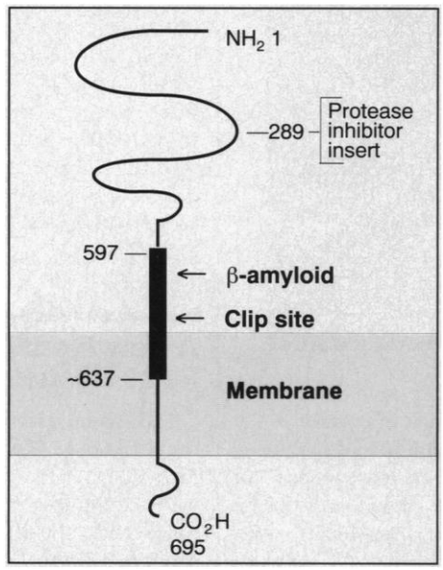
Most of the discussion at the Dahlem conference centered on efforts to understand the possible role of a protein called β-amyloid in the etiology of the disease. Now, β-amyloid hasn't just come on the scene. There has long been reason for thinking that the protein, a major component of the abnormal structures called plaques that stud the diseased portions of Alzheimer's brains, might play a part in the disease.

But researchers have always been of two minds about how important that part is. Some have maintained that β-amyloid deposition in plaques plays a significant role in causing the nerve degeneration seen in Alzheimer's disease, while others argue that it's merely the result of that degeneration.

What's new in the past few years is that the cloning of the gene encoding β-amyloid, in 1987 opened the door for the first time to doing the molecular studies needed for pinning down the role of the protein. Researchers have since learned a great deal about how amyloid is made and what it's normal function might be. Despite that progress, however, they still haven't solved their initial problem since both sides can find some support for their views in the new findings.

What might be called the new era of β-amyloid research began in early 1987 with a brief flurry of optimism when Alzheimer's researchers thought they might have the answer to their conundrum. There appear to be two forms of Alzheimer's, an early onset type that strikes people in their fifties and a late developing type that doesn't become apparent until the seventh or eighth decade of life. Early onset Alzheimer's is thought to be caused by an abnormal gene, and in 1986 genetic studies indicated that the gene was located on human chromosome 21. When the gene encoding β-amyloid was mapped to a nearby site on the same chromosome, hopes soared that it might at least be the gene that causes the early-onset form of Alzheimer's. Those hopes were dashed, however, when further studies indicated that the amyloid gene is some distance away from the Alzheimer's locus.

But all was not lost for β-amyloid, as it happened. Shortly thereafter, Konrad Beyreuther and Axel Unterbeck, who were then at the University of Cologne, Colin Masters of the University of Western Australia in Perth, Melbourne, and their colleagues cloned and sequenced the gene. That revealed that the amyloid protein has interesting properties that pointed to a potential role in Alzheimer's, even if it was not the primary cause of the disease.



Alzheimer's culprit? *Release of β-amyloid from the APP molecule may contribute to Alzheimer's pathology—then again, it may not.*

Their work showed, for example, that β-amyloid, which contains some 40 amino acids, is synthesized as part of a larger molecule, called the amyloid precursor protein, or APP. Researchers soon learned that the structure of APP has changed little during evolution, a finding that suggests that APP has an essential function—and that an interruption of that function might be implicated in the disease. Also encouraging was the discovery that APP is widely made in neurons and in many other cells.

But most intriguing was the finding that cells make two forms of APP, a short form containing 695 amino acids and a long one containing an insert of either 56 or 75 amino acids that goes in at amino acid 289. What caught everyone's attention was the insert. Its structure resembles that of a protein that inhibits certain proteases (protein-cutting enzymes). Could that have anything to do with the normal function of APP or with its involvement in plaque formation?

Well, the answer to the first part of that question is now in, and it's yes. About 2 years ago, work by Beyreuther, who is now at the University of Heidelberg, and Masters, who has moved to the University of Melbourne, showed that APP is embedded in cell membranes, with a short segment on the carboxyl end projecting into the cell interior and the bulk of the protein, including the protease inhibitor insert, outside the cell.

Furthermore, the outer portion of APP can be clipped off and secreted from the cell surface. And last year, two groups, one including Tilman Oltersdorf and his colleagues at Athena Neurosciences, Inc., in South San Francisco and the other including William Van Nostrand and Dennis Cunningham of the University of California, Irvine, recognized that the secreted APP molecule containing the protease inhibitor segment is identical to a known protease inhibitor called protein nexin II.

Van Nostrand and Cunningham also found that human blood platelets contain relatively large amounts of APP, releasing it when activated. This normally happens at injury sites as part of the body's blood-clotting and wound-healing mechanisms,

The Nematode as a Guide to Human Brain Disease

Can a lowly worm help neurobiologists untangle the pathology of Alzheimer's, Huntington's, Parkinson's, and other human brain diseases? That surprising question kept cropping up at a recent Dahlem conference on degenerative brain disorders. Although progress has been made toward understanding those disorders, conference participants had to conclude that they don't yet know nearly enough about how brain cells die (see p. 984). And that's where the lowly worm may help.

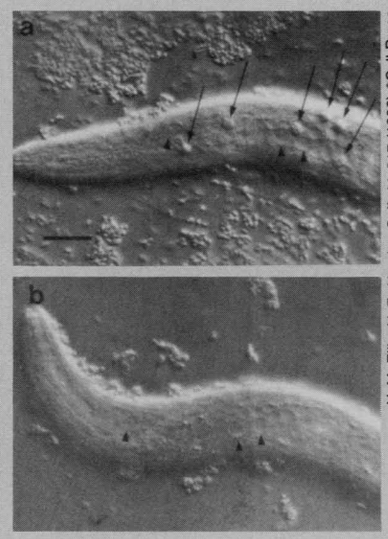
The idea is not as farfetched as it might seem because the worm is not just any worm—it's the nematode *Caenorhabditis elegans*, which has become a favorite laboratory pet of developmental and molecular biologists (*Science*, 15 June, p. 1310). The specific finding that may make the worm relevant to the human neurodegenerative diseases is the isolation of genes that cause nerve cells to die in the nematode, an achievement that opens the door to identifying the biochemical events underlying the cell deaths. The hope is that those same mechanisms will ultimately prove to operate in human beings as well.

How likely is this to be the case? Well, one encouraging sign is the consensus reached by the Dahlem participants that there appear to be few biochemical mechanisms underlying nerve cell death, whether in humans and other mammals or in *C. elegans*. "There are obviously a lot of ways to trigger cell injury," says Dennis Choi, who is studying mammalian nerve cell death at Stanford Medical Center in Palo Alto, "but those of us who are optimistic think there is a convergence [of the mechanisms causing cell death]." And that has so far turned out to be the case in *C. elegans*. "My first thought was that there must be a million ways to make the nerve cells die. But in the worm only a few mechanisms seem to be used," says Robert Horvitz of the Massachusetts Institute of Technology.

Horvitz and his colleagues have been studying the programmed cell deaths that occur normally during *C. elegans* development. About one-fourth of the 407 immature nerve cells that the animal starts with perish before the animal matures, which takes about 3 days. The Horvitz group has identified several genes that are involved in those programmed deaths, but two, called *ced-3* and *ced-4* (because the genes regulate *c*ell *d*eath), are particularly interesting because they encode proteins that work together to kill all the cells that die during the developmental period.

Since programmed cell death is also a feature of mammalian nervous system development, Horvitz suggests that mammals may have genes that act in a similar fashion to those of the *C. elegans* genes. And if the mammalian genes were to be activated abnormally later in the life, he speculates, that might cause the nerve cell deaths occurring in Alzheimer's or the other neurodegenerative diseases. There is a precedent for something like this happening with *ced-3* and *-4* in *C. elegans*.

Martin Chalfie and his colleagues at Columbia University have been studying a different type of neuronal death in *C. elegans*. In contrast to the MIT work in which it's the normal gene products that kill, the Columbia group has identified two genes in which mutations cause the formation of abnormal proteins that wipe out specific nerve cells. "They're poisons, toxic products," Chalfie says. The neurodegeneration caused by the mutations also has a later onset than that produced by the *ced* genes. In one important respect, however, the Columbia results jibe with those of the Horvitz group: they, too, suggest there aren't many ways that nerve cells die. Chalfie notes that he and his colleagues looked long and hard for mutant genes that can kill nerve cells. So far they have found just the two, and preliminary studies indicate that the proteins they encode act in similar ways.



H. M. Ellis, H. R. Horvitz, **Cell** *44*, 817 (1986) ©Cell Press

Nerve death mutant. *Nerve cells that normally die in the* C. elegans *larvum (arrows above) live in* ced-3 *mutants.*

At the moment, neither the Horvitz nor Chalfie group fully understands how the proteins encoded by their genes kill nerve cells, but both are moving toward that goal. Horvitz and Jungying Yuan have recently cloned the *ced-3* and *-4* genes. They find that the *ced-4* sequence suggests that the protein may have calcium-binding sites. "We're working on the assumption that it's a calcium-activated 'deathase,' " Horvitz says. If so, that would be interesting because many of the insults known to kill mammalian nerve cells work by raising calcium ion concentrations inside the cell.

Although the neurodegeneration genes being investigated by the Chalfie group work independently of the *ced* genes, calcium ions may be involved in their mode of action, too. Chalfie and Eve Wolinsky, a postdoc in his lab who has since moved to New York University Medical Center, have recently cloned and sequenced one of the two genes, called *deg-1* (*deg* stands for degeneration).

The gene's sequence indicates that its product is a membrane protein, possibly a receptor for some as yet unidentified agent that regulates nerve cell activity. Its malfunction might lead to an abnormal influx of calcium ions or other ions that could result in the death of nerve cells.

And will any of this work be of benefit to Alzheimer's patients? The answer could well be yes. Horvitz and Chalfie suggest that it may be possible to use their genes to screen for comparable genes that cause nerve cell death in mammals. In addition, it might be possible to use *C. elegans* to screen for drugs that can block neuronal degeneration.

It's only fair to point out that not everyone at the Dahlem conference was buying the proposition that *C. elegans* is a good model for human brain diseases. "It's interesting science, but I don't think that it's relevant to what we do," says Konrad Beyreuther of the University of Heidelberg, who studies Alzheimer's etiology.

He notes, for example, that the nematode life-span is only about 2 weeks, while human beings live more than 70 years. But the worm had its defenders, too. "The mechanism of cell death is unclear," says Yves Barde, a neurobiologist at the Max Planck Institute for Psychiatry in Martinsreid. "That's why the *C. elegans* work is so important." ■ **J.M.**

and Cunningham suggests APP may have a role in wound healing. In addition, Tsunao Saitoh of the University of California, San Diego, David Schubert of the Salk Institute, and their colleagues have shown that the secreted form of APP may have growth factor activity, which could also facilitate wound healing.

Intriguing though these findings may be, there is considerable uncertainty over what they might reveal about the role of APP in plaque deposition in Alzheimer's. According to one interpretation, the secreted form of APP is unlikely to be the source of β-amyloid in the plaques. Roughly a third of the β-amyloid segment of the precursor is buried in the nerve cell membrane. Hence, when the molecule is cleaved before being secreted, this portion remains in the membrane. Indeed, the Athena group has shown that APP secreted by cultured nerve cells contains less than half the β-amyloid molecule.

The discovery that one end of the β-amyloid portion of APP is hidden in the cell membrane presents a problem for researchers who think β-amyloid deposition is an important part of Alzheimer's pathology. Since the buried end is presumably inaccessible to protein-cutting enzymes, it raises the possibility that β-amyloid formation and plaque deposition are secondary events, occurring after something else kills the cells and the membranes degenerate, releasing APP as they deteriorate. That, of course, is what the other camp has been saying all along.

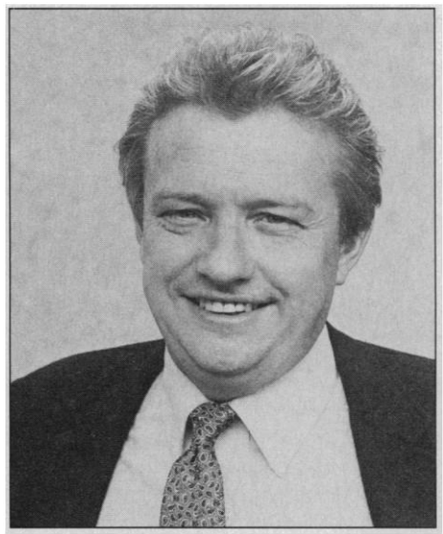
And that camp has been quick to raise other possible causes of the abnormalities seen in Alzheimer's brains. For example, they suggest that the processing of the microtubules that give nerve cells their structure might be defective. One recent indication of the possible importance of microtubules comes from Kenneth Kosik and his colleagues at Harvard Medical School, who have found that inhibiting the synthesis of the tau protein (which helps to stabilize microtubules) produces changes in cultured nerve cells resembling those seen in Alzheimer's disease.

But the defenders of β-amyloid aren't by any means throwing in the towel. One reason is that the findings on how APP is clipped don't come from cells in intact brains, but from cultured nerve cells and platelets. Price points out that no one yet knows whether APP is normally processed the same way by brain neurons. And even if it is, there's always the possibility that Alzheimer's neurons have a defect that results in aberrant APP cleavage and β-amyloid release.

Moreover, recent work has begun to suggest a normal function for APP in the nervous system, and it is compatible with the idea that abnormal APP processing might lead to neuronal degeneration. For example, Edward Koo and Sangram Sisodia in Price's group find that the protein is rapidly transported from the cell body, where it's made, down to the end of the axon. "It seems to be a protein destined for axons and nerve terminals," Price says. "It may play an important role in neuronal connections."

If that is the case, then abnormal APP processing might lead to a severing of the connections, either directly by interfering with the protein's function or indirectly by leading to β-amyloid deposition in the space between neurons, physically blocking their interaction. And because nerve cells apparently need to maintain active connections to survive, the result could be axonal degeneration like that seen in Alzheimer's brains.

In light of those results, the question



Dahlem organizer *Donald Price has seen a major shift in emphasis in Alzheimer's disease research in the past few years.*

becomes: How might abnormal APP processing take place? And while there are no answers yet, the Dahlem participants could at least point to a couple of lines of investigation worth pursuing. They were particularly intrigued, for example, by new results from the groups of Paul Greengard at Rockefeller University and Unterbeck at Molecular Therapeutics, Inc., in West Haven, Connecticut. These researchers have evidence suggesting that addition of a phosphate group to membrane-bound APP by protein kinase C, one of the cell's major regulatory enzymes, stimulates the cellular uptake of the protein and its subsequent degradation.

Two of the fragments that are produced in this way are big enough to contain the entire β-amyloid sequence. If those fragments actually do contain the β-amyloid sequence, they would have the potential of contributing to plaque formation and the protein kinase C system would then be a likely place to look for a defect that could lead to abnormal APP processing and β-amyloid deposition.

Another possibility is that the protease that normally forms secreted APP somehow goes astray and cuts incorrectly, leading to β-amyloid deposition. There are indications that incorrect cleavage of APP can cause this to happen, although how isn't clear. The evidence comes from studies of people with a rare, hereditary form of cerebral hemorrhage that is apparently caused by β-amyloid accumulation around blood vessels in the brain.

Genetic studies by C. Van Broeckhoven of the University of Antwerp and his colleagues strongly point to the APP gene as the one at fault in the disease, and Efrat Levy and Blas Frangione of New York University Medical Center and their colleagues have found that patients who have the condition have a mutation in their APP gene that changes one amino acid in the β-amyloid region. The change occurs near the site where the APP molecule is normally cleaved, and the supposition is that this interferes with its processing and somehow leads to β-amyloid deposition. Although how that might occur still remains to be demonstrated, the Dahlem participants suggested that the cell proteases should be examined to see if they contribute in any way to β-amyloid deposition and Alzheimer's pathology.

Clearly, a number of different lines of research are now needed, along with better animal models for the disease (see box on page 985). But for the moment, the focus of research seems to have come squarely to bear on one molecule: β-amyloid. Sorting out the role of that molecule in the disease would represent a turning point, but it might not unlock the puzzle of the syndrome. As Anita Hardy, a neurogeneticist at London's Institute of Neurology, who summed up the discussion at the end of the meeting, said: "Although major advances have been made, solving Alzheimer's is not around the corner." ■ JEAN MARX

ADDITIONAL READING

J. D. Buxbaum *et al.*, "Processing of Alzheimer β/A4 amyloid precursor protein: Modulation by agents that regulate protein phosphorylation," *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6003 (1990).

A. Caceres and K. S. Kosik, "Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons," *Nature* **343**, 461 (1990).

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