Boring in on β-Amyloid's Role in Alzheimer's

New studies of how the protein β-amyloid is made in cells may help in understanding Alzheimer's brain degeneration

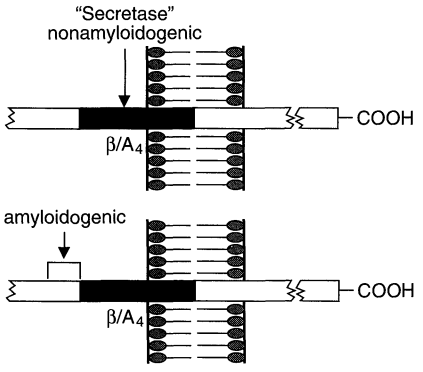
THE U.S. ECONOMY ISN'T THE ONLY THING that goes through boom and bust cycles. Consider what's been happening to the decade-long effort of neurobiologists to prove that the small protein called β-amyloid helps cause Alzheimer's disease. A few years ago, the failure of genetic studies to find a link between the protein's gene and hereditary forms of the disease sent the stock of the "amyloid hypothesis" plummeting. Within the past year, however, in a stunning reversal, further studies have shown that there is indeed a link between the amyloid gene and Alzheimer's—touching off a new bullish phase of β-amyloid research.

But while that work is removing the doubts about the protein's importance, it still has not provided answers to many vexing questions, including one that has long been a thorn in the side of β-amyloid proponents: what actually causes the buildup of β-amyloid deposits in the brains of Alzheimer's patients? Now, several research teams are beginning to answer that question—and the results are surprising.

The expectation had been that β-amyloid would be produced by some aberrant reaction related to the disease. But the picture now emerging suggests that it's formed even in healthy cells by the cell's normal protein-degrading machinery. "This is a big change from what we thought before," says Steven Younkin of Case Western Reserve University School of Medicine in Cleveland, who leads one of the research teams. Although researchers still don't know what causes more β-amyloid deposits to form in the brains of Alzheimer's patients than in those of unaffected people, finding the pathways that make the protein means they can begin a systematic exploration of how β-amyloid production is controlled. And that work might at long last lead to the identification of the genetic and other factors that contribute to Alzheimer's development.

If those factors could be pinned down, it would open the door to improved Alzheimer's therapy—even prevention. "If there's some way that modulation of one or more of the pathways [for making β-amyloid] can slow down neurodegeneration, then we would really have progress," says Alzheimer's expert Carl Banner of the National Institute on Aging, who organized a recent conference dealing with β-amyloid formation.* Since most people develop the disease late in life, usually after age 65, delaying the onset of symptoms by 10 years would be almost as good as a cure, Banner explains.

The original reason for thinking that β-amyloid might be involved in Alzheimer's was its location. The patients' brains contain large numbers of abnormal structures called "senile plaques," which consist of a protein core surrounded by degenerating nerve terminals. In 1984 George Glenner and C. W. Wong of the University of California, San Diego, found that the plaque cores are made of β-amyloid. Neurobiologists have been of two minds about that protein's role ever since, however. Some have argued that the deposition of the protein actually causes the neuronal degeneration of Alzheimer's. Their opponents think that view has it backwards—that the deposition is merely the result of nerve cell breakdown.



A big difference. *By clipping APP within β-amyloid, secretase may prevent plaque formation.*

The "amyloid as cause" faction got a big boost in 1987 after the gene encoding β-amyloid was cloned and mapped to chromosome 21. That was encouraging because people with Down's syndrome, who have an extra copy of chromosome 21, develop an Alzheimer's-like dementia in their twenties and thirties. That suggested that excess β-amyloid production causes the neuronal degeneration underlying their dementia. But even though the amyloid gene may play a role in this form of Alzheimer's, researchers had trouble connecting it to other forms. Especially disappointing was the inability to find a linkage between the amyloid gene and hereditary Alzheimer's, which strikes people in their forties and fifties and is not as common as the late-onset disease.

But it turns out there was a simple explanation for the failure. Traditional genetic linkage studies don't work if two or more different genes can cause what appears clinically to be the same disease. And that turned out to be the case in hereditary Alzheimer's. So there was still a chance that a β-amyloid defect caused the disease in some Alzheimer's families and could be found by looking for amyloid gene mutations within individual families. Then about a year ago, lightning struck.

A large team of researchers led by neurogeneticist John Hardy of St. Mary's Hospital Medical School in London reported in *Nature* (21 February 1991) that they had discovered a mutation in the amyloid protein gene that is associated with the disease in two families with hereditary Alzheimer's (also see *Science*, 22 February 1991, p. 876). Since then, additional Alzheimer's families with a mutation in the same site have been found by Hardy's group and others, although the mutation is very rare. Identification of the "Hardy mutation" showed "that aberrations in the amyloid precursor protein are sufficient, if not necessary, for Alzheimer's. That put the focus on the protein's metabolism more squarely than it was a year ago," Banner says.

In particular, researchers want to find out how β-amyloid is made in cells, an issue that has been problematical for the protein's supporters. The β-amyloid protein, which contains just 42 amino acids, is made as part of a much larger protein, called the amyloid precursor protein (APP). Full-length APP apparently does not harm cells, and is in fact thought to have a normal function that has yet to be identified. It's only when β-amyloid is clipped out of APP by protein-splitting enzymes that problems may start. But clipping out β-amyloid is not so easy, because APP is apparently embedded in the cell membrane with about two-thirds of the β-amyloid segment on the cell exterior and the rest buried in the membrane where it's inaccessible to protein-splitting enzymes, unless the membrane is somehow damaged.

What's more, about 2 years ago, Sam Sisodia of Johns Hopkins University School of Medicine and his colleagues found that the outer APP segment is normally clipped from the membrane and then secreted. Shortly thereafter, a team led by Fred Esch

SOURCE: SAM GANDY AND PAUL GREENGARD ILLUSTRATION: J. CHERRY

*The symposium, "Proteases and Protease Inhibitors: Emerging Roles in the Pathogenesis of Alzheimer's Disease," was held at the National Institutes of Health from 16 to 18 December 1991.

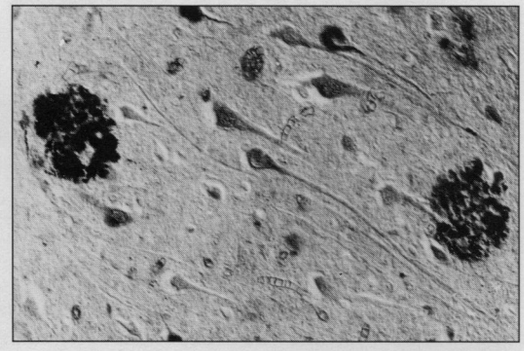
of Athena Neurosciences, Inc. in San Francisco, showed that it occurs either at amino acid 15 or 16 in the β-amyloid segment. Any APP molecule that undergoes this normal "secretase" reaction would thus be unable to produce β-amyloid. Those results left the amyloid faction with a problem: How could APP processing possibly lead to β-amyloid deposition?

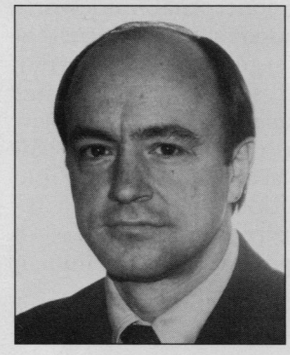
And that's where the new work comes in, starting with two papers in this issue from Younkin and his colleagues (see pages 726 and 728). What the Case Western group and others are now finding is that cells have alternative ways of breaking down APP—and that these routes, unlike the secretase reaction, yield fragments that contain intact β-amyloid and thus have the potential of forming Alzheimer's plaques. Younkin and his colleagues have demonstrated the presence of such "amyloidogenic" fragments in normal human cells, including brain cells. What's more, they show that the fragments are almost certainly formed in the small vesicles known as lysosomes. They find, for example, that compounds that inhibit lysosomal enzymes inhibit the fragment formation.

Researchers already had reason to suspect that APP breakdown might occur in the lysosomes as well as by the secretase reaction. Known to be a site of normal protein breakdown, they are loaded with enzymes "capable of cleaving just about every bond in a normal protein," says Ralph Nixon of Harvard Medical School and McLean Hospital in Belmont, Massachusetts. In fact, other investigators had previously detected APP fragments in lysosomes, but the Younkin group is the first to show directly that the fragments contain intact β-amyloid. "I think these are very strong experiments," says Sisodia. He describes the results as "the best evidence yet" that cells make APP fragments that could lead to the formation of β-amyloid molecules—and therefore that some defect in the processing of amyloid could lead to the plaque formation that characterizes Alzheimer's brains.

Several other researchers also have evidence, much still unpublished, that APP is broken down in the lysosomes to form amyloidogenic fragments. These include Gregory Cole of the University of California, San Diego, who was among those who detected APP fragments in lysosomes in the earlier experiments; Sam Gandy, who works with Paul Greengard at Rockefeller University in New York City; Dennis Selkoe of Harvard Medical School, who is a collaborator on one of the current Younkin papers but is also pursuing the work independently; and Sisodia.

But showing that there's more than one way to cleave APP is only a first step toward a better understanding of amyloid deposition in Alzheimer's, especially since it now seems that amyloidogenic fragments can form during normal APP degradation. As Gandy puts it: "If everyone has these alternatively cleaved molecules, why do some people get amyloid deposits early and some late? I hate to say it's going to be complicated because that's pretty self-evident."





Getting at the problem. *Steve Younkin's research may help explain how senile plaques, such as these, form.*

COURTESY OF DR. GEORGE PERRY, DEPT. OF PATHOLOGY, CASE WESTERN RESERVE UNIVERSITY

Presumably, something shifts the balance away from APP cleavage through the secretase pathway, which should work against amyloid deposition, toward the lysosomal pathway, which may favor it. Selkoe and his poctdoc Christian Haass have shown, for example, that APP, which is on the membrane, can be taken into the lysosomes. So if membrane APP is not cut by the secretase then there is a route for getting it back into the lysosomes.

Of course, the identity of the "something" that shifts the balance between the two degradation pathways remains a big question mark. As Gandy suggested, there are numerous possibilities. One is that some inherent defect in APP—such as the one caused by the Hardy mutation—alters its processing. Indeed, researchers, including Bruce Yankner of Children's Hospital in Boston, have preliminary evidence that the mutant APP may produce more amyloidogenic fragments than the normal protein. "The mutations are important because they give you a handle on the disease," Yankner says.

But the genetic studies have already shown that APP mutations can account for only a small number of Alzheimer's cases, so researchers are going to have to look for additional changes that may lead to amyloid deposition. Other possibilities include a problem with the secretase itself—causing it to become less active or to be produced in smaller amounts as a person ages. Alternatively, the defect could be in reactions that control the action of the secretase. Gandy, Greengard, and their colleagues and also the Younkin group have shown, for example, that the secretase reaction is increased by treatments that stimulate protein phosphorylation. And then the problem might be not with the secretase, but with lysosomal abnormalities that enhance β-amyloid formation.

But no matter how the β-amyloid forms, it looks as if the cell has a tough time getting rid of it. Charles Glabe and his colleagues at the University of California, Irvine, find that the protein forms aggregates inside the lysosomes. "It turns out that β-amyloid is resistant to degradation when it's inside cells," Glabe says, although that raises still another question: How does it get out of the lysosome? As Cole points out: "All the pieces are there, but someone now has to go in and show that you can get amyloid deposition through the lysosomal pathway." One possibility is that it's secreted. Alternatively, an abnormal accumulation of β-amyloid in the lysosomes might lead to cell damage, or even death, with the subsequent release of the lysosomal contents.

Despite the surging interest in the lysosomal pathway as a potential source of the amyloid deposits of Alzheimer's, it's only fair to say that not all the amyloid skeptics have come around. Take for example, Harvard's Nixon. He thinks that the lysosomes are involved all right. Indeed, research by his group shows that amyloid plaques contain lysosomal enzymes, as well as β-amyloid. But he still argues that release of the lysosomes and amyloid formation is the result of nerve cell injury—rather than the other way around. Even the Hardy mutation is consistent with this idea, he says, since it changes an amino acid within the membrane-spanning segment of APP, and that might open the way to membrane damage.

In spite of this kind of skepticism, the market for the amyloid hypothesis was anything but bearish in 1991. The scientific analogue of the Dow-Jones index is *Science Watch*, a publication that tracks research trends by counting the citations papers receive. The last issue of that publication anointed APP research last year's "hottest" field, largely because the Hardy paper got the most citations in biology. Science-watchers should keep their eye on the ticker for the latest amyloid results, because 1992 figures to be even hotter. ■ JEAN MARX