Alzheimer's Research Moves to Mice

The first mouse models for studying amyloid's role in Alzheimer's may help solve one of the most vexing issues in research on the neurodegenerative disease

ALZHEIMER'S RESEARCHERS ARE A LOT LIKE the blind men studying the elephant: each grabbing onto a different part of the disease and coming to a different conclusion about what causes it. And Alzheimer's is a beast with at least as many unusual parts as that elephant. Over the years, neurobiologists have caught hold of and identified numerous biochemical and physiological abnormalities in patients' brains. What's been missing is a good picture of what those abnormalities mean—largely due to the lack of good experimental systems for sorting out which are causes and which are effects.

Now, three research teams have independently developed mouse models that may at last give researchers a better understanding of the role of one particular abnormality in Alzheimer's brains. And should their efforts prove fruitful, then the models would have another important role as a test for potential therapeutic drugs to stave off the devastating symptoms of Alzheimer's—now estimated to afflict about one-quarter of all people who live to age 85.

The new models are all aimed at pinning down the role of a small protein called β–amyloid. Neurobiologists have known for about a decade that the abnormal "plaques" that stud the brains of Alzheimer's patients consist of degenerating nerve terminals surrounding a protein core of β–amyloid fibrils. Ever since researchers discovered that protein in the plaques, they've been of two minds about what it's doing. Some have maintained that amyloid deposition triggers brain neuron degeneration, thereby causing the memory loss and other symptoms of Alzheimer's. Others have held that the deposits are not causative at all, but are merely the end result of the brain neuron breakdown. The mouse models are "very important," says Zaven Khachaturian, associate director of the National Institute on Aging, because they could help resolve that issue.

To pin down β–amyloid's role, the three groups genetically engineered new strains of mice in which abnormal β-amyloid deposits build up in the brain—the first time anyone has accomplished that, although many researchers have been trying.

Until now, most of the biochemical evidence linking β–amyloid deposition to Alzheimer's disease had come from studies on human brains obtained at autopsy. The big disadvantage of that approach has been that researchers mainly see what the pathological lesions look like at the end of the disease, says Donald Price of Johns Hopkins University School of Medicine, an expert on Alzheimer's pathology. Only rarely do they get to see what early or intermediate-stage lesions look like.

Yet another problem for neuroscientists hunting β–amyloid's role has been the fact that besides the human, the only other species that develop amyloid plaques and the other characteristic symptoms of Alzheimer's are nonhuman primates, which are both rare and expensive to maintain.

The upshot of all this has been, Price says, that researchers "can't study the evolution of the abnormalities at a cellular and molecular level." Indeed, the participants at a Dahlem Conference on neurodegenerative diseases, held in Berlin last August, agreed that the lack of a good lab animal model was one of the biggest obstacles holding up their efforts to understand what causes Alzheimer's.

Enter the three groups that are now reporting the first successes toward developing those long-sought models. Dana Wirak of Miles Research Center in West Haven, Connecticut, Axel Unterbeck of Bayer's Pharma Research Center Aprath in Wuppertal, Germany, Bruce Trapp of Johns Hopkins University School of Medicine, and their colleagues describe their results on p. 323 of this issue of *Science*; Barbara Cordell and her colleagues at California Biotechnology Inc. in Mountain View, California, describe theirs in the 18 July issue of *Nature*; and the results of Anja Kammesheidt, Rachel Neve, and their colleagues at the University of California, Irvine, are not yet published.

All three groups used similar genetic engineering approaches. They injected genes encoding human β–amyloid into newly fertilized mouse eggs, which were then implanted into foster mothers to develop. Within this approach, however, there were significant differences between the groups' models.

Which of the models will provide the most information about human Alzheimer's is anyone's guess at this early stage. Most likely, no one model will provide the whole story. Instead each could provide illumination about a different aspect of the amyloid problem, leaving the complete puzzle to be pieced together—much as the blind men could have assembled a picture of their elephant had they managed to pool their information. As Miles lab's Wirak puts it, "It's unrealistic to expect any animal model to mimic truly a human disease. We're trying to divide a very complex disease into small packets and understand the various aspects separately."

Complex, it is. β–Amyloid, which contains about 40 amino acids, is synthesized as part of a larger protein, called the amyloid precursor protein (APP). Although Wirak and his colleagues have produced several lines of transgenic mice, each carrying a different APP gene construct, so far they have found brain amyloid deposits only in those animals that received the simplest construct, which encodes just the β–amyloid segment of APP. But the good news is that these deposits have the same appearance as the β–amyloid fibrils in the cores of typical Alzheimer's plaques, says Johns Hopkins' Trapp, who has been doing the pathological studies on the mouse brains. Also encouraging is the finding that the artificially induced β–amyloid deposition occurs mainly in the hippocampus, a part of the brain important for memory and learning—and one of the principal areas affected by Alzheimer's.

MODELS AT A GLANCE

Laboratory	Gene Construct	Main Locations of Amyloid Deposition	Cellular Location
Cordell	APP with inhibitor insert	Cortex and hippocampus	Extracellular
Neve	C-terminal 104 amino acids	Cortex and hippocampus	Intracellular (lysosomes)
Wirak	β–amyloid	Hippocampus	Intracellular

But there were also some significant differences between β–amyloid deposition in the transgenic mice and that in human Alzheimer's. At least so far, Wirak's group has seen few of the deposits in the mouse cortex, a part of the brain that is severely affected in Alzheimer's disease, even though the gene construct used to make the mice contains regulatory sequences that allow β–amyloid to be made in all types of brain neurons. The concentration of the deposits in the hippocampus suggests, says Trapp, that their formation requires something, as yet unknown, in addition to β–amyloid synthesis.

And where the deposits do occur, they are inside nerve cells, rather than outside as they are in typical Alzheimer's plaques. Currently, too, the nerve terminals don't show signs of degeneration like those in plaques.

But Wirak argues that the β–amyloid deposition inside cells might not be as atypical of the human condition as it seems. In fact, he says, it might throw light on a major problem plaguing researchers who favor the idea that β–amyloid deposition causes Alzheimer's. They've been unable to come up with a good explanation of how β–amyloid might find its way to the cell exterior where the Alzheimer's plaques are located.

The intact APP molecule is normally embedded in nerve cell membranes in such a way that nearly half of the β–amyloid segment is buried in the membrane with the remainder extending outside the cell. "When you see the chemistry of that protein, it's very difficult to understand how β–amyloid gets out of the [nerve] cell," Wirak says.

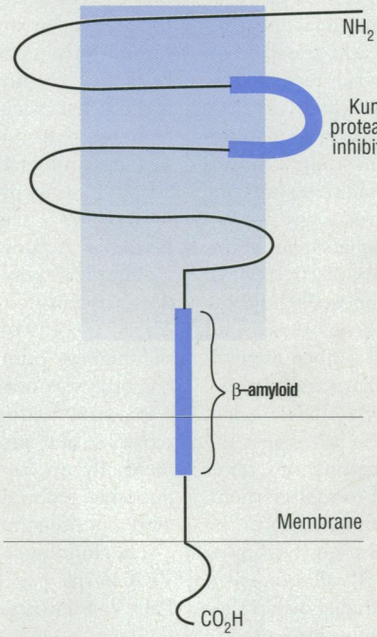
One possibility is that improper handling of APP inside nerve cells leads to the intracellular accumulation of β–amyloid or other APP fragments. That accumulation in turn could cause neuronal degeneration, releasing β–amyloid into the area outside the cells, with the resulting formation of plaques. Indeed, most Alzheimer's researchers think that β–amyloid deposits in the brain originate in brain neurons, but a few have proposed that they came in from the blood stream.

Until now, it has not been possible to test the hypothesis that β–amyloid accumulation in neurons can cause them to degenerate, says Wirak, but that can be done with his group's transgenic mice. They are currently only about 1 year old—about middle-aged for mice—and they can be followed as they grow older to see whether more typical plaques form. In addition, Wirak and his colleagues will begin testing the transgenic animals in a month or so to determine whether they display any behavioral or cognitive deficiencies.

While the Wirak group has tested several APP gene constructs, Cordell and her colleagues have so far focused on one in particular—because they believe that the protein it encodes is important for the development of Alzheimer's plaques. This is what might be called the long form of APP, which includes an insert resembling a substance known as the "Kunitz protease inhibitor." Nerve cells make APP both with and without the insert, and there's some evidence, although this, like much else in Alzheimer's research, is still controversial, that plaques form when the balance between the two is shifted in favor of the form containing the insert.

Cordell and her group decided to test that hypothesis, she says, by "genetically programming the mice to make more of the form with inhibitor." The result: The animals show amyloid deposits, primarily in the cortex and hippocampus. And in this case, they are extracellular. "That could potentially be very exciting," notes Wirak, since one of the big questions in Alzheimer's research concerns how β–amyloid gets out of cells.

Cordell's transgenic mice currently range in age from about 4 months to about 17 months. The amyloid deposits in the animals are not plaques, she says, "at least not yet. What we see in these brains is not full-blown Alzheimer's disease, but we have the first step toward building a good model." The Cal Bio researchers will follow the animals to see whether the amyloid deposits progress to full-fledged plaques.



Conundrum. *How does β–amyloid, which is partially embedded in the nerve cell membrane, get out of the precursor protein and into Alzheimer's plaques?*

The Neve group's choice of a genetic construct to put into their mice was also influenced by a view of how amyloid might contribute to neuronal degeneration, but since that view is different from Cordell's, the construct was also different. About 2 years ago, Neve and her colleagues showed that a peptide consisting of the 104 amino acids on the carboxyl terminal of APP (this peptide contains the β–amyloid segment) is toxic to nerve cells growing in lab cultures. "This may be the killer fragment," she says. So Neve and Kammesheidt transferred the portion of the APP gene that encodes the fragment into their transgenic mice.

And these animals also develop brain amyloid deposits, mostly concentrated in the hippocampus and cortex, Neve says. The deposits are intracellular, but the Irvine workers can already detect signs of degeneration in the neurons with the deposits, even though the transgenic animals are not quite 5 months old. That may reflect the known neurotoxicity of that particular APP fragment.

One aspect of Neve's work may relate to the idea that improper intracellular handling of APP is what leads to neuronal degeneration and plaque formation. In her group's transgenic mice, the amyloid peptide accumulates inside the membranous particles called lysosomes. "I think that's the part we're most excited about," Neve says. Her group has previously shown that the peptide also accumulates in neuronal lysosomes in Alzheimer's brains, possibly because APP is being broken down abnormally by the neurons.

While the three models described here are the first to see the light of day, they may not have long to enjoy their preeminence. Wirak notes, for example, that his group hasn't completed the work on the transgenic mice made with other gene constructs. Researchers will also want to test mutants of the APP gene, especially one discovered earlier this year by John Hardy and his colleagues of St. Mary's Hospital Medical School in London. They found that a mutation in the APP gene is linked to Alzeimer's in two families with a hereditary form of the disease (*Science*, 22 February, p. 876).

That gave new credence to the idea that APP is involved in Alzheimer's etiology, although the particular mutation identified by Hardy can't be a major cause of the disease, since it doesn't occur in most Alzheimer's families. But APP with that mutation is still a prime candidate for transfer into mice to see if that will induce Alzheimer's pathology in the animals.

And then the researchers also want to model other aspects of Alzheimer's pathology, such as the neurofibrillary tangles that are another distinctive feature of the diseased brains. In the end, it's likely that contributions from many "blind men" will be needed to identify the curious beast that lurks in the brains of Alzheimer's patients. Only then will researchers have a chance to protect what the blind men's elephant is so well known for. ■ JEAN MARX