NEUROBIOLOGY

Alzheimer's Pathology Begins to Yield Its Secrets

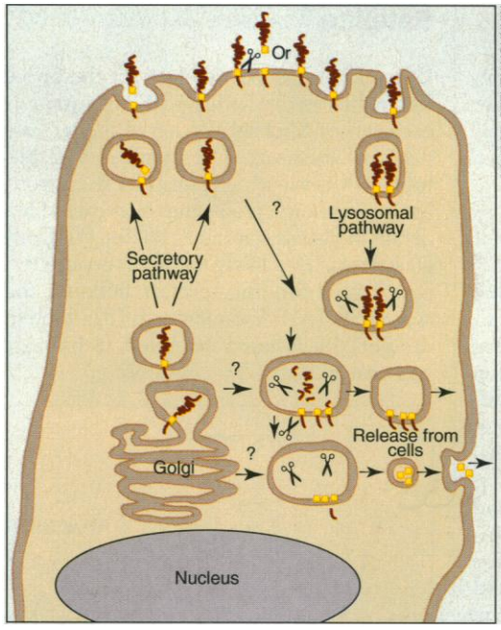
In the past few years there's been a growing—though hardly universal—acceptance of one of the more contentious propositions in neurobiology: that abnormal deposition of the protein known as β-amyloid in the brain results in the nerve cell degeneration underlying some cases of Alzheimer's disease. But even researchers who are fully convinced of this proposition have had a hard time explaining just *how* β-amyloid contributes to the disease. For a start, they have had difficulty understanding even how the protein is made and released by brain cells. But in that area, at least, help is on the way: A flurry of results over the past several months has finally begun to sort out the mechanisms of β-amyloid production.

The most recent findings come from two research teams, one led by Dennis Selkoe of Harvard Medical School and the other by Steven Younkin of Case Western Reserve University. Both groups have strong evidence that people with a particular hereditary form of Alzheimer's develop the disease because they have a mutation in the gene encoding β-amyloid that causes them to make greatly increased amounts of the protein. (The Selkoe group reported their results in the 17 December issue of *Nature* and the Younkin group describes theirs on page 514 of this issue of *Science*.) "The results provide a good explanation of how at least one form of Alzheimer's disease is produced," says Younkin.

The information could be important not only in helping researchers understand why some people get Alzheimer's and others don't, but it may ultimately help in identifying drugs for treating the disease. Still, amid the excitement, researchers in the field are sounding cautionary notes, pointing out in particular that Younkin's and Selkoe's results are based only on studies of cells in culture. "I think these results are interesting," says Alzheimer's researcher Donald Price of Johns Hopkins University School of Medicine. But he adds: "These are in vitro results. One really has to take it further," and show that increased β-amyloid production actually contributes to Alzheimer's pathology in living animals.

The caution reflects the great difficulty that Alzheimer's researchers have had in pinning down the pathogenic mechanisms of the disease. Indeed, the Alzheimer's community has for some time been divided up into pro- and anti-amyloid factions, with the protein's supporters arguing that it contributes to the nerve cell degeneration, and its detractors arguing that its presence in the plaques that characterize Alzheimer's brains is merely the result of that degeneration.

One problem that has faced the pro-amyloid faction is that intact cells appeared to be inherently incapable of producing β-amyloid. The protein, which contains approximately 40 amino acids, is made as part of a much larger molecule, the amyloid precursor protein, or APP. APP is inserted into cell membranes with about two-thirds of the β-amyloid segment sticking out of the cell and the rest buried in the membrane. When researchers first began determining what happens to the APP, they discovered that most of the extracellular segment of the long protein is clipped off by a "secretase" enzyme. And since the secretase cuts within the exposed β-amyloid portion of the molecule, its activity would preclude β-amyloid production. It's possible that other enzymes in the cell could cleave APP at a different place to release intact β-amyloid. But because one end of β-amyloid is buried within the cell membrane, it would appear to be inaccessible to cutting enzymes unless the membrane were already damaged. And that, of course, adds credence to the notion that β-amyloid is simply a byproduct released by dying cells—not a causative agent in Alzheimer's at all.



SOURCE: STEVEN YOUNKIN

Another route. The secretory pathway can't make β-amyloid *(in yellow)*, but an alternate path, possibly in the lysosomes, can.

Beginning about a year ago, however, a series of findings began to offer the amyloid backers a way around this impasse. The first step was the finding, by several groups, including Younkin's and Selkoe's, that the secretase reaction isn't the only way cells can break down APP and that some fragments produced in the alternative pathways contain the complete β-amyloid molecule (*Science*, 7 February 1992, pages 688, 726, and 728). This breakdown appeared to take place in the lysosomes—small intracellular vesicles where protein degradation normally occurs—possibly after β-amyloid has recycled back into the cell from the membrane where the secretase enzyme may act.

In that early work, though, the researchers didn't show that cells further digest the APP fragments so that they actually release the β-amyloid they contain. Then, last fall, Selkoe and his colleagues, in collaboration with a team from the biotech company Athena Neurosciences Inc. of South San Francisco led by Ivan Lieberburg, and also Younkin's group, took the next step forward: They demonstrated that normal nerve cells growing in culture do indeed secrete β-amyloid into the culture fluids.

Since the cultured cells appeared to be healthy, the researchers say, this work suggests that neurons don't have to be damaged to release β-amyloid, boosting the hopes of the amyloid backers. Both the Younkin and Athena groups also found they could identify β-amyloid in samples of human cerebrospinal fluid from both Alzheimer's patients and normal controls—a result which indicates that the cells in brain also release the protein and that it's not just an artifact of their culture systems.

But that just raised the further question of why, if everyone makes β-amyloid, only some people get Alzheimer's disease. And that's where the latest work, representing a third major step, comes in. In that work the Selkoe-Lieberburg and Younkin groups made use of a mutant APP gene present in a Swedish family whose members show an early onset, hereditary form of the disease. The researchers introduced the gene, which was identified by Mike Mullan and his colleagues at the University of South Florida in Tampa, into cultured cells to see if they handle the mutated APP differently from the normal protein.

The answer is yes. "We find that when these cells are transfected with the Swedish mutation, the β peptide is overproduced quite dramatically—by six- to eight-fold," Lieberburg says. The Younkin group found a similar increase in β-amyloid produced from the same gene. Says Younkin: "This result not only offers a good explanation of why these people get [familial] Alzheimer's but also validates that the pathway producing the peptide in

cultured cells is relevant to the disease."

Although the new results suggest that overproduction of β-amyloid causes Alzheimer's disease in the Swedish family, they don't settle the question of β-amyloid's role once and for all. For one thing, Younkin's group actually found lower β-amyloid production when they tested another mutant APP gene that has been associated with familial Alzheimer's. That doesn't necessarily mean that the mutation doesn't cause Alzheimer's, Younkin says. He notes that β-amyloid varies in length from 39 to 43 amino acids and his assay can't discriminate among the variants. It's possible, he speculates, that this other mutation causes more of the longer β-amyloid variants to be produced even while the total amount goes down, and that the longer variants are more prone to form amyloid deposits than the shorter ones. That has yet to be demonstrated, however. Meanwhile, says Rudolph Tanzi of Harvard Medical School, whose work focuses on the neurogenetics of Alzheimer's, the discordant findings with the two mutants raise a question about whether the production of β-amyloid is part of the mechanism of Alzheimer's pathology. It may well be that it is, he says, "but the jury is still out."

Everybody in the field agrees that one way to bring that jury to a verdict would be to insert the APP gene with the Swedish mutation into mice to see if the animals develop Alzheimer's pathology. But previous difficulties with using APP gene transfer to develop mouse models for Alzheimer's suggest this may not be such an easy thing to do (*Science*, 6 March 1992, p. 1200). Many groups are trying to accomplish the feat, however. Another possibility, suggests Younkin, is to measure β-amyloid concentrations in spinal fluid from affected and unaffected members of the Swedish Alzheimer's family. Finding higher concentrations in family members with the disease would support the idea that increased β-amyloid production is important.

Although Selkoe concedes the importance of animal models, he doesn't think people should overlook the virtues of using cultured cells to study β-amyloid production. They could, for example, help resolve an issue that has just cropped up in his group's work, which has produced circumstantial evidence suggesting that the lysosomes may not be the site of β-amyloid formation after all. The cell culture system should also be useful for identifying the enzymes that release β-amyloid, which are potential drug targets, as well as screening for drugs to prevent β-amyloid formation. "Before we [Selkoe and Younkin] showed that cells continuously produce the β peptide, we had no way to study the dynamic aspects of β-amyloid formation" Selkoe says. "Now we can do that." And studying that process could help resolve the vexatious, chicken-or-egg status of β-amyloid.

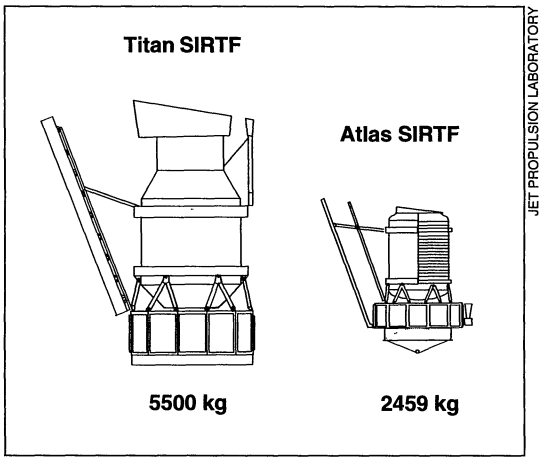
–Jean Marx

Astronomers Meet in Phoenix, Recount a Stellar Year

Despite a tightening of the National Aeronautics and Space Administration's budget and the trouble with the Hubble Space Telescope, astronomers were starry-eyed over their latest findings, presented at the major annual meeting of the American Astronomical Society, (AAS) January 3 to 7. New images and measurements of stars, galaxies, cosmic microwaves, and mysterious gamma rays, along with an exciting nova explosion, made it a bright year for those working with existing orbiting satellites and ground-based telescopes, though uncertain funding clouds the future.

A Leaner, Meaner Infrared Satellite

Last year things looked bleak for the Space Infrared Telescope Facility (SIRTF), says project scientist Michael Werner of the Jet Propulsion Laboratory. The proposed $1.3 billion satellite was a high piority for the astronomy community, promising to reveal hidden details of distant stars and galaxies. But Congress deemed it a lowly "technology activity" and denied funding to start building the spacecraft. NASA was left with $8 million to develop the infrared detection technology and only $150,000 for engineering.



Smaller, faster, cheaper. The new, version of the infrared observer, SIRTF, may cut costs by as much as half—and weight by an even larger fraction.

Now astronomers are back with a leaner proposal they hope will be more to Congress's liking. SIRTF planners combined the engineering money with money already approved by Congress in the previous year and went back to the drawing board. "We had to come up with a smaller, lower mass, less expensive mission," says Werner. At the AAS meeting they displayed their new conception: a smaller spacecraft designed to orbit the sun instead of the earth. The new proposal has the head of NASA taking notice. But the biggest hurdle—Congress—is still to come.

The key to the new, downsized mission was putting the satellite into solar orbit, explains project scientist Peter Eisenhardt of the Jet Propulsion Laboratory. Ironically, getting a satellite into solar orbit is easier than getting it to orbit the earth. To get into earth orbit, you have to fire your jets twice: once to get into orbit and once to make the path circular rather than elliptical (which is what it would tend to be with a single push). A solar orbit, on the other hand, needs only one push, which makes it possible to replace the cumbersome Titan rocket with the smaller Atlas.

Like many other missions, the old SIRTF was initially constrained into a low-earth orbit by the capabilities of the planned launch vehicle, says NASA director Daniel Goldin. Its planners later considered the possibility of a high-earth orbit, but that alternative presented problems of its own, Goldin adds. "There was a sub-conscious lock," he says. "The real breakthrough came when they said, 'Let's think freely.'" Out of that free thought came the idea of a solar orbit.

The SIRTF designers made a number of other changes to scale back bulk and cost, cutting the total equipment, launch, and operations cost in half. In the process they've tried to keep enough scientific capability to fulfill their promise of a new infrared scan of distant stars and planets. From its orbit, SIRTF will see past all but one-millionth of the background radiation that blocks our infrared view from Earth, allowing the satellite to carry out detailed studies of possible preplanetary disks surrounding stars and get a close look at the most distant galaxies.

Goldin, in a speech at the meeting, called the new plan "elegant." But he warns that while the SIRTF designers have sold astronomers and NASA, Congress may not be so easily swayed. Still, Goldin says, his agency plans to put the new concept before Congress in the not-too-distant future, and "if these people proceed with the wonderful things they are doing, there's a very good chance it will get started within this decade."