volved in egg-laying that normally is only found in females.

Project manager Tom Muir cautions that the various soups of chemicals at the sites seem to produce a wide range of effects. "There's a lot of fuzziness to the national data set," Muir says. In some rivers, for instance, female fish had high levels of 11-ketotestosterone, while in others, both sexes had depressed levels of male and female hormones. More research is needed to establish cause-and-effect relationships, Muir adds.

That's just what the British study, sponsored by the U.K. Environment Agency, set out to do. The research followed on earlier work by Sumpter's group, which had shown that male rainbow trout kept in cages near sewage outfall pipes produced high levels of vitellogenin. To tease out what compounds might be responsible, researchers collected sewage effluent from three treatment plants and, using a variety of analytic techniques, isolated compounds that were likely to act like estrogen in the fish.

To their surprise, the researchers discovered that the estrogenic compounds were not industrial pollutants but three hormones found in women—17β-estradiol, estrone, and ethynyl estradiol. The last, which was present in vanishingly small amounts, is a potent synthetic hormone in birth control pills. One reason the researchers didn't expect to find these substances in the water is that before they are excreted in urine, the kidneys tack on a chemical group—a glucoronide or sulfate—that renders the compounds biologically inactive. Sumpter speculates that during sewage treatment, enzymes from bacteria may be clipping off the chemical group. The researchers then showed in the lab that the tiny amounts of hormones found in the effluent can cause male fish to produce vitellogenin. They are now exposing young fish to effluent to see whether it will cause them to develop into hermaphrodites.

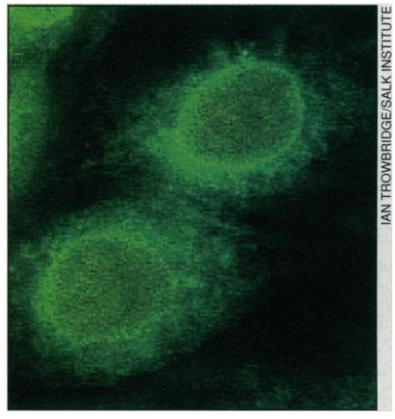
Sumpter says their result doesn't mean industrial chemicals aren't also harming fish in some heavily polluted rivers. For example, he believes that high vitellogenin levels found in male fish in some U.K. rivers will turn out to be caused primarily by nonylphenol, a chemical discharged by textile factories. But, he says, because sewage is the dominant source of pollution in U.K. rivers, these substances in urine are probably largely responsible for the country's hermaphroditic fish. And that's a lesson for other researchers studying endocrine disrupters, Sumpter says: "I would almost certainly have voted for synthetic man-made chemicals, and that would have turned out to be wrong." Toxicologist Steven Safe of Texas A&M University in College Station agrees: "This points out that we have to be pretty careful in jumping to conclusions."

–Jocelyn Kaiser

ALZHEIMER'S RESEARCH

Dissecting How Presenilins Function—and Malfunction

In Alzheimer's disease research, one small protein has long claimed a large share of attention. Known as β amyloid (Aβ), it is a major constituent of the abnormal structures called plaques that stud the brains of Alzheimer's patients, and mutations in the gene that produces it account for some inherited cases of the disease. But β amyloid is now having to share the stage with two other proteins, the presenilins, that play an even larger role in hereditary Alzheimer's. Discovered barely 2 months apart in 1995, the two presenilin genes, now called *Presenilin 1* and *-2* (*PS1* and *-2*), are mutated in about half of all inherited cases, compared to a few percent for the gene that makes Aβ. And now researchers are beginning to glimpse the normal roles of these proteins—and how they might go awry in the disease.



IAN TROWBRIDGE/SALK INSTITUTE

Localized. The yellow staining indicates that the presenilins are present in the endoplasmic reticulum and the Golgi.

New results, presented just last month at the annual meeting of the Society for Neuroscience in Washington, D.C., indicate that the protein produced by the *PS1* gene is apparently needed for the proper operation of a major developmental regulatory pathway. Known as the Notch pathway after a protein that is one of its key members, its jobs include transmitting the signals needed to make cell-fate decisions—such as whether to develop into nerve or muscle cells—in species ranging from the fruit fly to mammals. Just how the presenilin contributes to Notch signaling is unclear, but some researchers think it may play a role in the cell's internal protein-handling systems, helping bring Notch to its normal location, the external cell membrane.

If so, the finding would dovetail with other work, presented both at the neuroscience meeting and in a raft of recent publications. It suggests that the mutations in *PS1* and *PS2* somehow alter the way that cells handle another protein—the larger molecule from which Aβ is clipped—and thus cause increased production of a particular variant, called Aβ42 because it contains 42 amino acids, that is thought to be especially prone to forming plaques. "I think it's all really quite exciting. The amyloid work and the presenilin work are coming together rather nicely," says Alzheimer's researcher Dennis Selkoe of Harvard University. The hope is that if researchers can understand how the gene mutations lead to Alzheimer's, they will be that much closer to finding the causes of the much larger number of nonhereditary cases.

Selkoe cautions, however, that despite the rapid progress, some major gaps remain. Any role for the presenilins in protein trafficking is, for the moment, conjecture. But the work is telling researchers which aspects of cell physiology are likely to hold the answers, as well as providing a good model organism that ought to aid the studies. This is the tiny nematode, *Caenorhabditis elegans*, one of developmental biologists' favorite creatures, which turns out to have a presenilin of its own. Indeed, it was a discovery made about a year ago in *C. elegans* that originally tipped researchers off to the possibility that the presenilins might play a role in Notch signaling.

Molecular biologists Diane Levitan and Iva Greenwald of Columbia University College of Physicians and Surgeons were looking for mutations that suppress the effects of a mutation in a worm gene, called *lin-12*, that is the equivalent of the *Notch1* gene of the mouse. The idea was that any gene containing such a suppressor mutation might work in the same signaling pathway as *lin-12/Notch1*. Levitan and Greenwald found what they were looking for, and when they cloned one of the affected genes, which the researchers called *sel-12* (for *suppressor and/or enhancer of lin-12*), its sequence turned out to be 50% identical to that of *PS1*. That high degree of resemblance suggested that *PS1* might also be involved in Notch signaling. Further work in both *C. elegans* and mice has borne that out.

At the neuroscience meeting, Philip Wong, who works with Sam Sisodia at Johns Hopkins University School of Medicine, provided the first public description of a mouse in which the *PS1* gene has been knocked out. The disruption of the gene produced results much like those that Janet Rossant's team at Mount Sinai Hospital in Toronto found when

they deleted a large portion of the *Notch1* gene itself, implying a functional link between the two genes. In both cases, the gene changes proved lethal because of disturbed development; all the embryos died during the gestation period. For the Sisodia team's knockout mice, which were generated in collaboration with Hui Zheng and Lex Van der Ploeg of Merck Research Laboratories, brain hemorrhages were the main cause of death—even though, puzzlingly, the brains otherwise appeared normal.

In addition, Wong said, the *PS1* knockouts showed a "dramatic defect" in their skeletons. The original cartilage of their spinal columns did not form true bone, and the vertebrae were fused and disorganized—a defect that Wong, Sisodia, and their colleagues traced to disorganization of the somites, the knots of embryonic tissue that give rise to skin, muscle, and the vertebral column. Instead of being arranged in pairs along the spinal cord, the somites in their mice were misaligned or irregularly shaped along the neural tube. The Rossant team saw similar somite derangements in their mice.

Further evidence implicating the presenilins in Notch signaling comes from experiments done in *C. elegans* itself by two teams, one led by Greenwald and Sisodia and the other by Christian Haass of the Central Institute for Mental Health in Mannheim, Germany, and Ralf Baumeister of the University of Munich. (The Greenwald-Sisodia group's results appear in the 10 December issue of the *Proceedings of the National Academy of Sciences*, and Haass described his team's at the neuroscience meeting.)

The researchers began with a *C. elegans* strain having a mutated *sel-12* gene. The mutant worms suffer from a number of abnormalities, including the inability to lay eggs, presumably because of disrupted Notch signaling. If the human presenilin proteins really do serve the same function in the signaling pathway as the Sel-12 protein, then a normal *PS* gene should reverse the effects of the *sel-12* mutation. And when the researchers introduced the normal human genes into the mutant worms, that's exactly what they found. "The animals looked totally normal," says Haass. Introducing mutant *PS* genes that had the same defects found in Alzheimer's patients, on the other hand, did little to reverse the effects of the mutant *sel-12*.

Of course, the big question now is what the presenilins' role in Notch signaling might reveal about the causes of Alzheimer's disease. To answer it, researchers will first have to pin down how the presenilins take part in Notch signaling. There are two leading possibilities: Either the proteins have a direct role in signaling, perhaps by relaying Notch signals to the nucleus, where they are acted upon, or they may have an indirect role such as building or maintaining the signaling pathway. They might, for instance, help convey the Notch protein to the cell's external membrane so that it can do its job.

Haass notes that this second idea, that the presenilins are somehow involved in protein trafficking, would "fit perfectly" with the other results now pouring out about these proteins. For one thing, the presenilins are ideally situated in the cell to play such a role. Several groups have shown that the presenilins are present in two interior compartments, the endoplasmic reticulum and to a lesser extent the Golgi apparatus, that are centers of protein processing and trafficking.

What's more, a large body of evidence now shows that mutations in the presenilins somehow alter the way the cells handle amyloid precursor protein (APP), the larger protein from which Aβ is cleaved. When APP is processed in cells, it can release Aβ fragments of varying lengths, but the Aβ42 variant appears particularly dangerous. Not only is it the predominant variant in Alzheimer's plaques, but it is also deposited early, perhaps acting as a seed around which other Aβ peptides gather to form a full-grown plaque. And Aβ42 is precisely the variant increased by *PS* mutations.

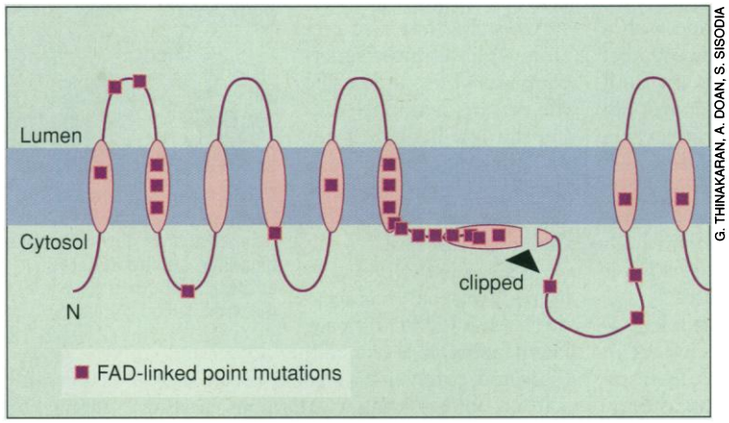
In the August *Nature Medicine*, for example, a team led by Steve Younkin of the Mayo Clinic in Jacksonville, Florida, reported that Alzheimer's patients with presenilin mutations have higher levels of the Aβ42 variant in their bloodstreams than do normal controls or people with nonhereditary "sporadic" Alzheimer's. And that result has since been buttressed by work on transgenic mice by David Borchelt of the Sisodia lab, and independently by Younkin's team working with those of Karen Duff and John Hardy of the University of South Florida in Tampa.

When the researchers introduced either wild-type or mutant genes into mice, they found that only the mutant versions increased Aβ42 production in the animals' brains. "It's consistent across the board that mutant presenilins raise Aβ42," Sisodia says. (The Duff-Hardy-Younkin team's results appear in the 24 October issue of *Nature*, and the Sisodia group's are in the November *Neuron*.) So far, however, the mice carrying the mutant genes have not developed plaques or any of the pathological changes of the neurodegenerative disease. Indeed, they are, as Selkoe puts it, "disgustingly healthy." The hope is that they are still too young to develop Alzheimer's-like changes.

Although researchers have a long way to go to figure out how mutant presenilins alter APP processing, there are some clues. For example, like many other proteins, newly synthesized APP is folded and processed in the endoplasmic reticulum and Golgi for transport to the cell interior. And ordinarily, proteins that fold improperly should be destroyed. But Dora Kovacs, Rudy Tanzi, and their colleagues at Harvard Medical School have evidence that mutated *PS1* allows misfolded APP to accumulate in cells, whereas the wild-type protein doesn't.

Misfolding, in turn, might be what causes the APP to be cut in the wrong place, releasing extra Aβ42. Such misfolding might make the protein susceptible to cutting by an enzyme that is less able to attack properly folded APP. Or it might lead the cell's protein-handling machinery to shunt the misfolded protein into the wrong cellular compartment—and into the clutches of an Aβ42-promoting enzyme. Evidence that such an enzyme exists comes from Selkoe and his colleagues, who found that Aβ42 is clipped out of APP by a different enzyme from the one that releases a shorter Aβ fragment.

That may sound like the outlines of a coherent picture. But there is still the possibility that mutations cause the presenilins to go astray in a quite different way—not by losing a normal function in protein trafficking, but by gaining some toxic function. In last week's issue of *Science* (p. 1710), for example, Benjamin Wolozin, who recently



G. THINAKARAN, A. DOAN, S. SISODIA

New weave. Presenilin 1 threads through the endoplasmic reticulum membrane, so that both ends and the large loop project into the cytosol. The point mutations associated with familial Alzheimer's (FAD) are concentrated in the transmembrane regions and the loop, indicating that these regions are important for PS1 function, as their disruption has such devastating consequences. The protein is also clipped where indicated, although the significance of this is not yet known.

moved from the National Institute of Mental Health in Bethesda, Maryland, to Loyola University Medical Center in Mayrood, Illinois, and his colleagues provided what might be a gain of function for mutant *PS2*.

They found that the mutant protein induced apoptosis, or programmed cell death, in cultured nerve cells under conditions in which normal PS2 had no effect. If mutant PS2 has a similar effect in the brain, the enhanced apoptosis could lead to the nerve cell loss of Alzheimer's. Other researchers are skeptical, however, primarily because mice carrying mutant *PS* transgenes have so far remained healthy. The animals "have no problem even if you grossly overexpress the presenilins," Selkoe says. Wolozin responds that while he understands the skepticism, the transgenic mice made so far may not be optimal for seeing apoptosis problems because the methods used for generating them may have selected for cells that can withstand the *PS* mutations. Like many others, he is now turning to *PS* gene constructs that can be turned on after development to see what effects they have.

So despite all the recent progress on the proteins, researchers still have plenty of work to do in trying to understand exactly what the normal and mutated presenilins do. But their apparent involvement in an important developmental pathway, combined with their link to a major feature of Alzheimer's pathology, guarantees that their turn on stage is just beginning.

–Jean Marx

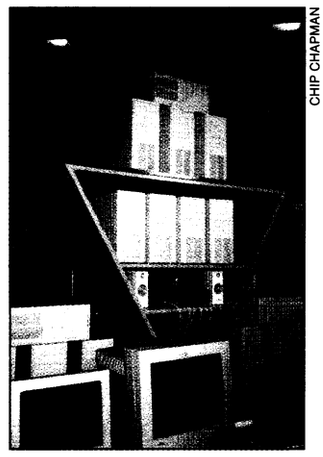
COMPUTING

Do-It-Yourself Supercomputers

The November Supercomputing '96 convention in Pittsburgh was a showcase of flashy technologies of the future. So what were a 3-meter pyramid and a rack of industrial shelving, both containing what looked like—and indeed were—piles of personal computers, doing in this setting? The two displays constituted two homemade supercomputers, built only of Pentium processors and other components that anyone with a link to the Internet and a credit card number could purchase for less than $60,000. Both machines, called Hyglac and Loki after figures in Norse mythology, can run over a billion operations a second (gigaflops)—a realm of processing speed formerly open only to supercomputers based on clusters of workstations and costing several hundred thousand dollars or more. The future, it seems, is not only here, but it's also affordable.

Called "pile of PCs," the technology is the poor scientist's version of clusters of workstations, says the California Institute of Technology's (Caltech's) Thomas Sterling, who is the father of what he is calling the Beowulf class of supercomputers because they are "lean and mean, and fighting something bigger." The strategy takes advantage of the extraordinary improvement in personal-computer processors over the past half-dozen years. PC processor performance has increased four times as fast as that of the workstation processors that power most supercomputers today, and high-end Pentium Pro chips now rival workstation processors for many types of calculations. At Supercomputer '96, both Hyglac and Loki, the former a product of Caltech and the latter from the Los Alamos National Laboratory, matched or exceeded the performance of a cluster-based supercomputer costing six times as much.

Sterling notes that users seeking raw computational power may still do better with the workstation-based machines, and that limitations in the networks linking the chips mean that not all software will run well on a pile of PCs. But he and others expect the strategy to catch on. While some computer scientists think piles of PCs are ripe for commercializing, Sterling himself is developing software infrastructure and a how-to manual, so that scientists can put together their own low-cost systems. Because the creators of cluster-based supercomputers like to describe their efforts as akin to picking the low-hanging fruit, says Sterling, "what we're akin to is digging up potatoes. We can stoop lower than anything else."



CHIP CHAPMAN

Piling them on. Sixteen Pentium Pro processors, linked by a local area network, make a gigaflops supercomputer, a strategy embodied here in a machine called Hyglac.

The approach sprang from a 3-year-old program at the NASA Goddard Space Flight Center in Greenbelt, Maryland, to develop a gigaflops-scale workstation, able to analyze enormous satellite data sets, for under $50,000. Sterling, who was at Goddard at the time, took on the challenge, and the Beowulf machines were born. When Sterling moved to Caltech last summer, he joined computational astrophysicist John Salmon, who was collaborating with Mike Warren and Wojciech Zurek at Los Alamos on large simulations of galaxy evolution. The astrophysicists had been thinking of building a commodity-based supercomputer at Los Alamos, and so Loki was born there, inspired by Sterling's machine, while Sterling and Salmon put together Hyglac at Caltech.

"What we're akin to is digging up potatoes. We can stoop lower than anything else."

—Thomas Sterling

Both groups started in the early autumn, says Salmon, "scouring the networks and computer magazines for the best price and performance we could lay our hands on." By mid-November, they had put together the two computers from the same basic ingredients: 16 200-megahertz Pentium Pro chips, the kind of connections used for local-area networks, and Ethernet software written by Don Becker of NASA Goddard. "It's hopefully nothing terribly unique," says Sterling—"a system anyone can put together."

That should appeal to scientists hoping to get supercomputing into the labs on a shoestring budget, says Rick Stevens, a computer scientist at the Argonne National Laboratory. These first two demonstration machines "prove you can take commodity stuff—freeware software, freeware compilers—and anybody in any country can get on the Web, order the parts, have them shipped air express, and build this ensemble and get performance comparable to a high-end supercomputer."

Salmon, who plans to use Hyglac for his galaxy-evolution studies, adds that the advantages go beyond price. Each Beowulf-class supercomputer can be constructed from technology only weeks old, while vendor-bought machines can suffer a technology lag of years. And by building their own machine, scientists can have exclusive access to it 365 days a year. "Personally," says Sterling, "I'm old enough to believe that gigaflops is still supercomputing, and I believe that any scientist can afford $50,000. If you accept those two statements as true, it's fair to say that the pile of PCs has come of age."

–Gary Taubes