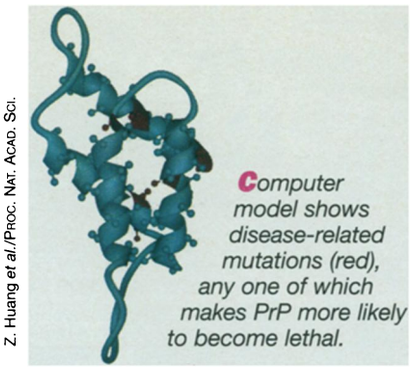
Prying into Prions

A twisted tale of an ordinary protein causing extraordinary neurological disorders

By ELIZABETH PENNISI



Z. Huang *et al.*/PROC. NAT. ACAD. SCI.

Computer model shows disease-related mutations (red), any one of which makes PrP more likely to become lethal.

When Stanley B. Prusiner coined the word "prion" for a new kind of infectious agent (SN: 12/5/81, p.359; 2/27/82, p.135), he unwittingly borrowed a term from ornithology. Nevertheless, his prions have proved as elusive to scientists as the seabirds of the same name are to bird-watchers.

In 1982, Prusiner — a researcher at the University of California, San Francisco, School of Medicine — proposed that an infectious protein caused scrapie and some other types of brain diseases. His idea seemed more science fiction than reality. Indeed, the feathered prions have been easier to track down in their Antarctic habitat than have molecular prions in nerve cells.

About 60 years ago, animal scientists realized that sheep could catch a disease, scrapie, that turned their brains into pitted sponges. They assumed the blame lay with a virus but never could find one.

Twenty years later, neurobiologists began pondering a strange movement disorder that affected a tribe indigenous to New Guinea. What seemed at first an inherited problem then proved transmissible to monkeys as well. These apparently contradictory observations left researchers confounded.

In people, the most common prion problem — a spongiform encephalopathy called Creutzfeldt-Jakob disease — sometimes runs in families and has been considered more prevalent in Sephardic Jews and in families from Chile and Slovakia. A second genetic disorder, Gerstmann-Sträussler-Scheinker syndrome, occurs much more rarely. So does a third, fatal familial insomnia. The discovery of a Chicago family with the progressive sleep disorder this spring by Pierluigi Gambetti of Case Western Reserve University School of Medicine in Cleveland brought the world's known total to just nine families.

In affected families, symptoms appear out of nowhere and affect succeeding generations. But prions also invade new hosts, much as an infectious virus or bacterium does. They can even jump from one species to another.

Physicians have traced about 40 cases of a prion disease in adults to injections of infected growth hormone administered during childhood. A few people have caught these diseases after receiving foreign tissue during brain operations or corneas from infected donors.

In England, an epidemic of mad cow disease (bovine spongiform encephalopathy) erupted in 1986, presumably caused by feed containing the processed remains of sheep with scrapie. To date, 140,000 cattle have succumbed, though the number diagnosed each year now seems on the wane. Also, reports of prion-related problems in mule deer, elk, antelope, and mink suggest that these animals consumed contaminated food, notes Charles Weissmann, a molecular biologist at the University of Zürich in Switzerland.

But contaminated by what, exactly?

The quest to answer this question has provided new insights into prions and the nature of genetic and infectious diseases in general.

Most pathogens package the instructions for replicating and infecting their hosts in nucleic acids such as DNA or RNA. Yet despite more than a decade of prying, researchers still have not associated any genes with infectious prion particles. As far as anyone can tell, a prion is nothing more than protein, says Byron Caughey, a biochemist with the Rocky Mountain Laboratories in Hamilton, Mont., part of the National Institute of Allergy and Infectious Diseases.

Therein lies the rub. "Nobody can understand how a protein can have that kind of information in it," says pathologist Colin L. Masters of the University of Melbourne in Parkville, Australia. Somehow, on its own, a string of amino acids manages to do what it typically takes several more complicated molecules to accomplish — and only if the molecules work as a team.

"It's a bizarre problem and clearly a very important theoretical problem, if not a practical one," he adds.

Even more bizarre, this villainous molecule — called the prion protein (PrP) — arises from the cells it may one day destroy. Normally, PrP sits anchored on the surfaces of nerve cells, minding its own business. But it definitely has a hidden, deadly side. Chemically, scrapie PrP isolated from infected animals seems the same as normal PrP. Yet normal PrP dissolves in water and falls apart when attacked by enzymes called proteases; scrapie PrP becomes insoluble and resists protease breakdown. The basis for this radical change lies in the three-dimensional arrangement, or conformation, of the amino acids of these two versions.

In general, much of normal PrP's amino acid string spirals into four helices, says Michael A. Baldwin, a chemist at the University of California, San Francisco. Baldwin and his colleagues have used various computer models to deduce a possible three-dimensional structure for normal PrP. The program considered PrPs from a dozen species to get a general sense of how the protein looks and chose four probable arrangements of the four helices, the group reported in the July 19 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

By looking at PrPs involved in inherited diseases, the San Francisco scientists settled on one form called the X-bundle structure. In this conformation, the helices arrange in two parallel layers, each layer itself made up of a parallel pair of helices. The lower pair is rotated out of line 45° relative to the upper one.

This conformation allows amino acids that shy away from water to interact to form close connections with each other, thereby stabilizing the protein's structure. Alterations in these connections may cause the protein to shift to another conformation, says Baldwin.

Chemical analyses indicate that in this altered state, sections of the protein line up as parallel zigzag strands. Weak links connect these strands into a pleated structure called a beta sheet (SN: 5/15/93, p.316). In theory, the existence of one sheet somehow triggers other peptide stretches to become beta sheets. This sets off a chain reaction that eventually may lead to the production of insoluble masses of abnormal PrP.

Caughey and research colleagues from the Massachusetts Institute of Technology have devised a way to make this transformation occur in a test tube. Because they know that the test tube contains a PrP solution, the researchers are fairly certain that no other molecules force or

participate in the transformation. Their results, reported in the Aug. 11 NATURE, bolster the notion that a molecular vampire is at work.

Just as Dracula's bite converted his victims into fellow bloodthirsty monsters, "we've shown that by mixing the normal form with the abnormal form, you can get conversion to the abnormal form," Caughey told SCIENCE NEWS.

In a single move, a prion both infects a new cell and replicates by transforming the cell's normal PrP into another version of its dastardly self. It does this by unwinding the protein strand, but without breaking apart any normal PrP, Caughey notes. Because this abnormal form resists degradation by enzymes, the number of nasty PrPs accumulates and snowballs.

"This paper is a tremendous milestone," Masters comments. "It opens the way for creating the infectious [form] in a test tube."

Caughey and others agree they need to show that this converted protein can infect cells. But first, the technique for making altered PrP needs refining. In their mixing experiment, Caughey and his coworkers added 50 times as much abnormal protein as normal protein, an excess not likely to occur in cells naturally. Also, this excess prevented the group from separating out the converted molecules and testing whether they cause disease. Finally, much as they tried, the researchers could not guarantee the absolute purity of the abnormal proteins they added.

Nevertheless, "it's a very important first experimental proof that one protein can change the conformation of another," says neurobiologist Jiri Safar of the National Institute of Neurological Disorders and Stroke in Bethesda, Md.

Other tantalizing hints of this have come recently from microbes. The yeast protein called Ure2p may sometimes alter its conformation, thus changing the yeast's metabolism, says Reed B. Wickner, a geneticist at the National Institute of Diabetes and Digestive and Kidney Diseases, also in Bethesda. The altered form of Ure2p is inactive, he reported in the April 22 SCIENCE.

In the July GENETICS, two other groups independently described another yeast protein with possible prionlike qualities. Oddly enough, the gene (sup35) that codes for this protein looks a great deal like PrP's gene, says Brian Cox from Cold Spring Harbor (N.Y.) Laboratory. Some of the mutations are similar, including the addition of extra, repeating nucleotides in the first part of the gene, Cox points out in the Aug. 1 CURRENT BIOLOGY.

No one really knows how Count Dracula, supposedly the father of all vampires, developed his hunger for blood. But scientists do know how at least some prion proteins wound up in their pathogenic state. About 10 percent of prion diseases have a genetic basis; that is, the gene for PrP mutates. This alteration leads to substitutions, additions, or deletions among the 253 amino acids that make up PrP. Somehow, these slight differences predispose the protein to switching its shape.

Depending on the makeup of the rest of the gene, different diseases can result. A gene sometimes has slight differences (polymorphisms) in its nucleotide sequences, which affect the amino acids in the resulting protein. For example, people with fatal familial insomnia and those with inherited Creutzfeldt-Jakob disease have an altered amino acid in PrP at position 178. But the PrP of these two groups differs at another position, 129. A valine at that position (in combination with the altered 178th amino acid) leads to the pitting of the brain characteristic of Creutzfeldt-Jakob disease. A methionine there ultimately makes the brain incapable of sleep.

"Here, the disease was caused not by the mutation alone, but by the combination of the mutation and the polymorphism [for] 129," Gambetti says. "It tells us that the idea that a mutation results in disease was too simple."

Ideas about how PrP leads to disease also may prove too simplistic. Some researchers assume that the accumulation of toxic forms of PrP results in the destruction observed, says Masters. But there's also evidence that imbalances in normal PrP concentrations can harm cells.

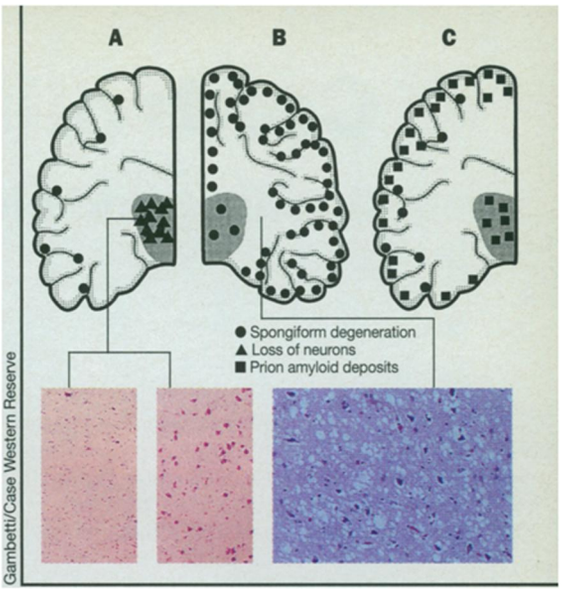
Weissmann thinks the data so far suggest that the known prion diseases develop after abnormal protein accumulates. As with the development of amyloid plaques in people with Alzheimer's disease, certain molecules take on a seemingly sinister configuration — the beta sheet — and accumulate as plaques.

"In some fashion, the nerve cells don't like it [the new shape]," Masters says. The narrow junctions between nerve cells, called synapses, decrease in number; eventually nerve cells disappear, gutting the brain. With prion diseases, this process takes months; with Alzheimer's, it takes decades, he notes.

Also, 2 years ago, Weissmann created a mouse strain lacking any gene for PrP. Those embryos seemed to develop normally and to function without problems.

But recently, when neurobiologists took a closer look at these animals, disabilities became evident. John Collinge and his colleagues at St. Mary's Hospital Medical School in London measured the electrical properties of nerve cells from the brains of mice. To do this, they removed thin slices of the hippocampus from normal mice and from mice with no PrP.

In cells missing the prion protein along their membranes, "there's abnormal electrical excitability," says Collinge. Also, PrP's absence altered the prolonged change in electrical properties that contributes to learning and memory, he and his colleagues report in the July 28 NATURE. They traced these changes to an impaired functioning of one kind of molecular docking site, the $GABA_A$ receptor. That receptor failed to shut down impulses as thoroughly and quickly as it does in normal mice.



Changes in brain tissue in (a) fatal familial insomnia, (b) Creutzfeldt-Jakob disease, and (c) Gerstmann-Sträussler-Scheinker disease. In fatal familial insomnia, normal thalamus (right) loses neurons (left), while in Creutzfeldt-Jakob disease the brain looks pitted.

"This electrical abnormality is very reminiscent of patients with Creutzfeldt-Jakob disease. Often their bodies undergo violent, involuntary jerks, and about 10 percent suffer from seizures," Collinge notes.

His team thinks that the loss of functional PrP can lead to the disintegration of the brain. They suspect, too, that normal PrP helps to form synapses, without which nerve cells most likely die. "What we think we're seeing [in these mice] is an arrested form of prion disease," Collinge concludes.

Weissmann is not so sure. Even after 2 years, mice lacking PrP are just too healthy, whereas "a scrapie [infected] mouse is really a sick mouse," he points out.

Meanwhile, Gambetti thinks PrP needs to exist in just the right amounts. Transgenic mice that make too much PrP eventually develop muscle disease, and last year other researchers found that people suffering from inclusion body myopathy, a muscular disorder, build up excess PrP in their muscles. Other, similar types of problems may remain to be discovered.

"There is a new chapter [in prion diseases] waiting to be opened," Gambetti predicts. □