

# A Story Still Full of Holes

The maddening complexity of prion proteins

by Jonathan Frohnmayer

Who could have guessed that a protein—the very molecule that is essential to virtually every function of every organism on the planet—might actually be the cause of one of the most mysterious and virulent diseases known to man as well as a host of other mammalian species? As foreign as this concept may sound, research within the last few years provides compelling evidence that this is exactly the case for a certain class of proteins known as prions, the cross-species agents behind such brain wasting syndromes as Mad Cow Disease in cows, Scrapie in sheep, and Creutzfeldt-Jakob Disease and Kuru in humans.

With the recent discovery of Mad Cow Disease in Washington State last December and the looming possibility that Mad Cow could become a worldwide health concern, more interest, research, and money than ever before are being devoted to the subject of prions. Nevertheless, it remains one of the most fascinating and perplexing topics in contemporary pathology. What is this molecule? How does it turn the brains of young, healthy, normal human beings and animals into veritable gray mush full of holes? What can modern science do about it? Questions abound in the burgeoning field of prion research and answers to some are just emerging.

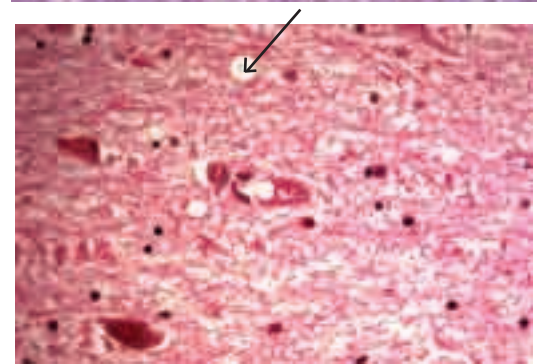
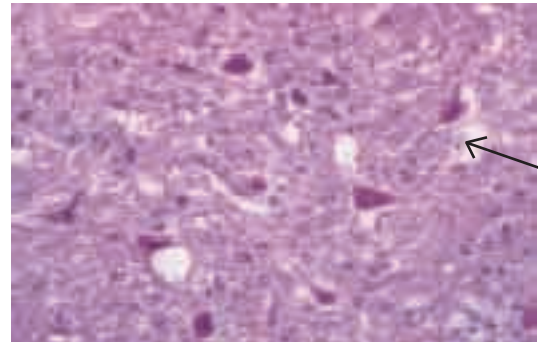
## A protein that can cause disease?

Although brain-wasting disorders have been documented for centuries, the idea that a protein can be the cause of an infectious disease was not on anyone's scientific radar until Stanley Prusiner introduced the concept in 1981. Until then, scientists assumed the only way for diseases to spread was through self-reproduction, which requires an information-containing sequence of DNA or RNA such as a virus, bacteria, or fungus. But in contrast to other disease-causing agents, prions are merely strands of amino acids, containing no genetic material. How, then, can a prion possibly be infectious? And how can it reproduce itself? Clearly, something about prions is fundamentally different from other proteins.

Every protein in the body has a specific shape and conformation. Many proteins have amino acid receptor sites that allow them to induce changes in the conformations of other proteins. Prions have two separate conformations— a normal, harmless “wild type” version that we all possess, and a densely-folded infectious form. The infectious form induces the wild type form to misfold, causing it to become infectious as well. Thus, even a single infectious prion obtained from eating tainted beef can catalyze a massive chain reaction that renders virtually every prion in the brain misfolded, destructive, and infectious.

Like Altoids, infectious prions are curiously strong. In their natural wild-type form, prions denature when heated or exposed to a change in pH just like most

other proteins, but the infectious version is among the most stable and resilient proteins known. An infectious prion can be heated to 500 degrees Fahrenheit, doused in formaldehyde, or centrifuged to 500 mph while maintaining its infectious conformation. From what little scientists have deduced about the protein itself, we



Small holes throughout the brain tissue are characteristic of prion diseases. Above we see a mad-cow disease infected cow brain and CJD infected human brain.

know that infectious prions are rigidly crumpled up, with numerous amino acid groups unnaturally shielded from the outside environment. This allows them to survive the hostile digestive enzymes in the stomach before moving into the bloodstream, and makes the process of finding a cure quite complicated.

Although the mechanism behind the folding of prion proteins is not well understood, perhaps the greater mystery is the nature of their neurotoxicity. The course of prion disease symptoms can last anywhere from one month to six years in humans, depending on the type and source of the prion as well as the amount ingested. Over the course of the illness, victims experience memory loss, subtle personality changes, loss of motor coordination, dementia, and eventually slip into a coma. The final stage of prion disease is death, reached when the victim's brain stem has been sufficiently destroyed so that it can no longer sustain the body's autonomic functions such as breathing and heart rate. The brains of humans, cows, mice, and chimps that died from prion illnesses all bear



Courtesy of Duke University

One of the diseases that result from a pathogenic form of the prion protein is Kuru. This young boy is experiencing its symptoms of neurodegeneration.

a strikingly similar—and truly frightening—characteristic. They are riddled with visible holes called vacuoles, presumably in places where large quantities of neurons died, creating a sponge-like appearance. For this reason, prion diseases are known as transmissible spongiform encephalopathies.

### How can a prion destroy the brain?

In numerous experiments over the last decade, when the gene encoding the normal, endogenous prion protein (PrP) was suppressed in mice, the mice became immune to deleterious health effects when injected with infectious prions. This likely explains why prion diseases occur only in the brains of victims, since that is where the majority of wild-type prions reside in mammalian species.

Why does the gradual accumulation of the infectious form of a prion make the brains of disease victims look like Swiss cheese? Nobody knows for sure, but researchers such as Dr. Gregory Barsh, professor of pediatrics and of genetics in the Stanford School of Medicine, have an idea. Evidence exists that spongiform disease results from a defect in prion protein turnover. At the end of a protein's life, it is often degraded by enzymes that target specific sites of the protein for destruction. In the wild-type conformation, prions may have these specific target sites exposed. On the other hand, the misfolded, infectious prions have a densely-folded conformation that may hide these specific sites from enzymes, making them resistant to destruction and leading to a buildup of the prion protein in the brain. Vacuoles may form in the brain when cell lysosomes swell from accumulating prion aggregates, eventually causing the neuron to rupture. Nevertheless,

a clear, tested, and universally agreed upon explanation remains elusive.

### What is the function of normal prions in the brain?

What is the purpose of the normal, endogenous prion protein in the brain? The mice in the experiment whose genes for prions were removed were visibly identical to normal mice, showing no significant abnormalities in behavior, health, or lifespan. Only upon very close scrutiny were scientists able to pinpoint subtle differences in the neuronal electrostatic interactions between the two types of mice, yet the implications of those differences appear obscure. It seems paradoxical that a gene would have evolved in many species for the sole function of making possible the contraction of an irreversibly fatal disease, but no concrete evidence to the contrary exists. Some researchers speculate that wild-type prions are involved in cell-to-cell recognition. Evidence also indicates that prions, rather than having any intrinsic activity themselves, merely have receptor sites that modify the function of other proteins. Recently, a team led by Eric Kandel

at Columbia University in New York has found that a prion-like protein may help nerve cells store memories. Regardless, the exact function of normal prions remains to be discovered.

### Should we care about Mad Cow and other prion-related diseases?

The most common question facing the average person regarding prion diseases is how much of a risk they pose. At this point, the answer is not clear. One of the many unique facets of transmissible spongiform encephalopathies is their ability to cross species barriers. Slightly differing forms of infectious prions exist in different species. They are believed to be most transmissible among animals of the same species, but they can also cross-infect other species.

Another unique facet of prions is their status as both infectious and genetic diseases. A human form of the disease—Creutzfeldt-Jakob—arises sporadically from a genetic trait in approximately one in one million people in the United States every year, making it one of the rarest and most mysterious illnesses in existence.

Potentially more alarming, however, is the possibility for the animal forms of the disease, especially bovine spongiform encephalopathy (BSE or Mad Cow Disease), to seep insidiously into food supplies around the world. At first glance, one may wonder why the government of the United Kingdom has spent over eight billion dollars since 1986 to quarantine a disease that has, so far, claimed the lives of fewer than 200 individuals, or why the discovery of

a single diseased cow in the state of Washington launched the beef industry into an international panic. The reason is that Mad Cow Disease is an invisible epidemic. Prions accumulate in the brain at an exponential rate, meaning that exposure to only one or two infectious prions could take decades before a critical mass is reached and the victim shows clear signs of brain cell death. In theory, then, the single case of a visibly ill cow could mean that 100,000 more are silently incubating the disease, doomed along with consumers of their meat to a tragic fate.

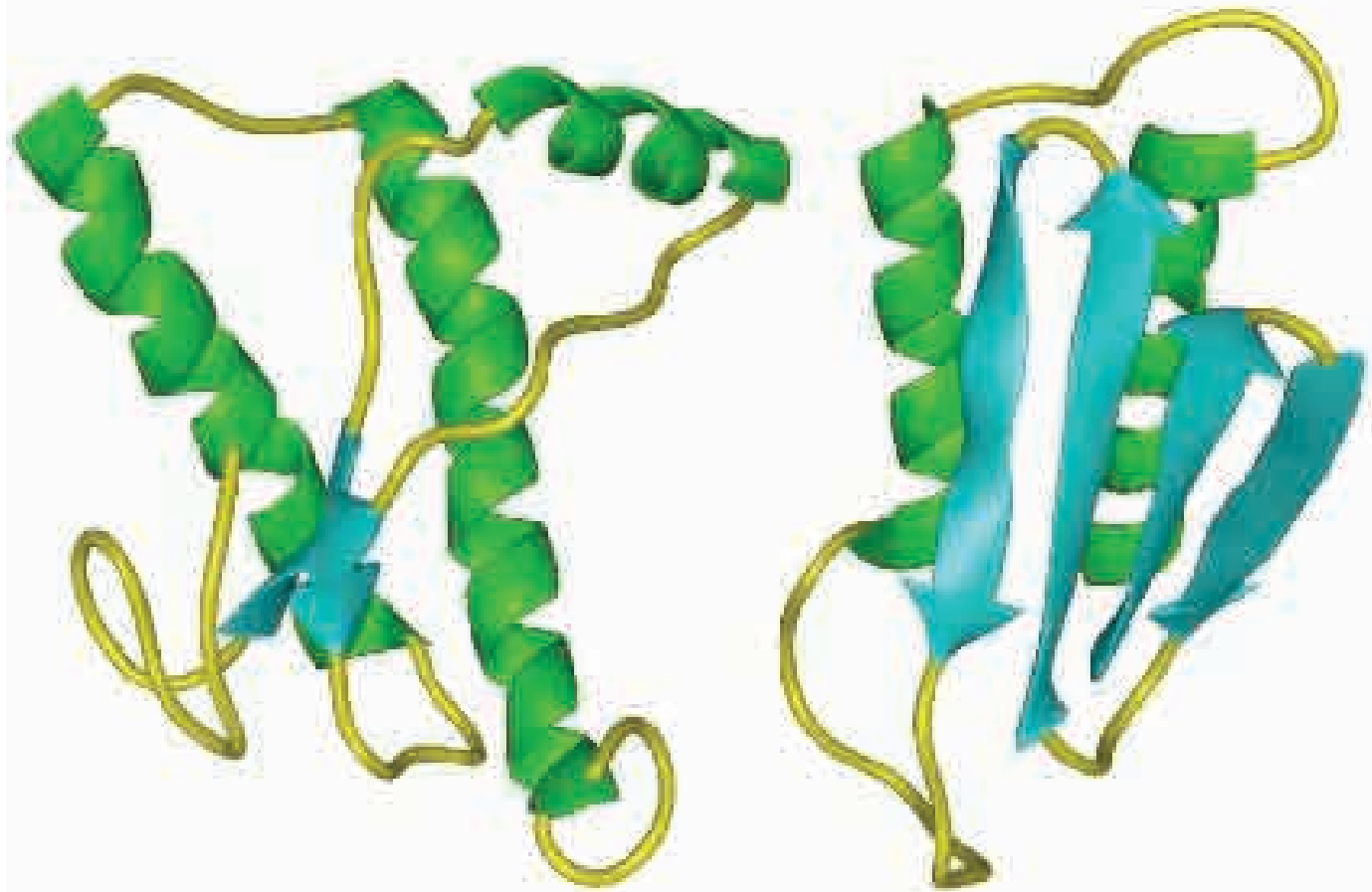
Is this cause for alarm? Perhaps, but fortunately, new methods for detecting the disease are emerging rapidly. Scientists at the University of Jerusalem, for example, recently found a technique to identify prions in a diseased animal's urine with over 90% accuracy. This and other diagnostic measures provide some grounds for optimism, but what about a cure? So far, not a single effective treatment exists, but that does not mean there are no ideas on the horizon.

### What is the future of prion research?

Since not only infectious prions but also a number of host wild-type prions need to be present in order to cause brain damage, it may not actually be necessary to destroy

the infectious prion in order to prevent the onset of the disease. The disease would effectively be neutralized if researchers found a way to disable the receptor site that allows infectious prions to cause wild type prions to misfold. Much current research now involves applying powerful enzymes to break off successive chunks of the amino acid chain of the infectious prion. By observing at which point the prion loses its shape-shifting ability, researchers hope to identify the root of its infectious nature and develop chemical or antibody-based resistance to it.

Prion diseases have increasingly been thrust into the public consciousness. Most people fear their tragic consequences, while scientists continue to be tantalized by the enigmatic and poorly understood biology of the prion protein. Although much light has been shed on the subject in the decades since Stanley Prusiner introduced the protein-only hypothesis, many of the most fundamental questions in prion science remain unanswered. What role does the dormant prion play? How do prions destroy the brain? Is there anything modern science can do about it? With luck and dedication, researchers will hopefully answer these and other questions surrounding this mysterious protein. **S**



<http://www.cnpfarm.ucsf.edu/~wallacea/nobel.html>

A normal prion protein (left) and a disease-causing form (right) exhibit two protein motifs, called "alpha helices" and "beta sheets". Alpha helices (shown in green) consist of linked amino acids that spiral around like a coiled spring. Beta sheets (shown in blue) form when amino acid chains line up in a flat plane.