

# Ironing Out the Wrinkles in The Prion Strain Problem

The prion hypothesis—the famously heretical notion that naked protein particles, without a stitch of nucleic acid, can cause transmissible disorders of the nervous system such as mad cow disease and the similar Creutzfeldt-Jakob disease (CJD) of humans—has gained increasing acceptance in recent years. But even true believers acknowledge a major stumbling block: the existence of multiple strains of prions with different, and apparently inheritable, characteristics. “This was the fly in the ointment for the prion hypothesis,” says Glenn Telling, a molecular biologist who works at the University of California, San Francisco, with Stanley Prusiner, the leading proponent of the hypothesis. “How can you have stably inherited information—strain differences—without a genetic component?”

Within the past year or two, a possible solution to that quandary has begun to emerge. Prions may consist of a cellular protein that has misfolded into an abnormal three-dimensional structure, so researchers have speculated that different prion strains consist of the same protein misfolded in different ways. The idea is that each one can impose its brand of misfolding on the normal protein by simple contact, thereby transmitting disease with unique strain characteristics. On page 2079, Telling, Prusiner, Pierluigi Gambetti of Case Western Reserve University in Cleveland, and their colleagues—working with prions from two human diseases—provide compelling new evidence for that idea.

They show that a single type of normal prion protein in the brains of living mice can be converted into two different forms, depending on the type of abnormal human prion that initiates the conversion. The result is two different patterns of pathological changes, as would be expected for different prion strains. Dennis Choi, a neurologist at

Washington University in St. Louis, calls the findings “a very powerful piece of support for the idea that an abnormal protein can confer its abnormal configuration onto the host prion protein.”

The puzzle the UCSF workers are addressing dates from 1985, when Prusiner’s lab and others found that prions, the protein particles found in the brain in neurodegenerative disorders like CJD and kuru in humans, mad cow disease, and scrapie in sheep, are misfolded or “flipped” versions of cellular prion protein (PrP), a normal component of brain cells. That flipping, or conformational change, may occur in the brain spontaneously for unknown reasons or because of mutations in cellular PrP, and cause buildup of abnormal prions and nerve cell damage. But because some of the diseases are transmissible, Prusiner also proposed that infection with one of the misfolded versions can induce disease by forcing healthy PrP molecules to refold themselves into abnormal prions.

But at the time, few other researchers would consider the idea of an infectious agent composed only of protein, a problem compounded by the existence of prion strains. They insisted that the disease-causing agent had to have some kind of genetic material, perhaps a virus lurking in the prion preparations.

Then, in the December 1994 *Journal of Virology*, Richard Bessen and Richard Marsh of the University of Wisconsin, Madison, reported some of the first evidence that different misfolded conformations of the protein might explain the strains. Studying mink transmissible encephalopathy, a prion disease, they found two strains when the disease was transmitted to hamsters, each one associated with different incubation times, symptoms, and distributions within the brain—as well as with a distinct conformation of the hamsters’ PrP. In a follow-up study in *Nature* in June 1995, Bessen, who had moved to the National Insti-

tutes of Health Rocky Mountain Laboratories in Hamilton, Montana, Byron Caughey, and their Rocky Mountain colleagues showed that the two prion conformations could be spread to one type of cellular PrP when the proteins were mixed together in a cell-free system.

Now, Telling, Prusiner, and their colleagues have extended the work to human prions in a mouse model. They used a strain of transgenic mice carrying a chimeric human/mouse PrP gene that was developed in their lab for susceptibility to human prions. They injected the animals’ brains with extracts from the brains of patients who died either from CJD or from fatal familial insomnia (FFI), an inherited disorder caused by a mutation in the PrP gene.

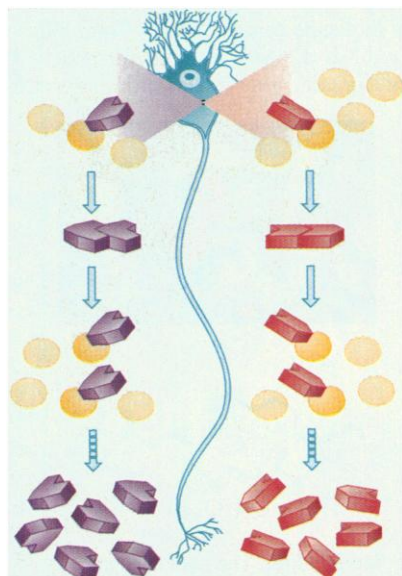
Prusiner and his colleagues knew that the prion proteins causing the two disorders differ in the way they fold because when they are cut with a protein-splitting enzyme, the CJD material gives a 21-kilodalton fragment, while that from the FFI patients yields a 19-kilodalton fragment. The question then was, would the prions produced in mice infected with the human prions yield the same protein fragments as the parent molecules? And indeed, the animals produced PrP fragments matching those of the original inoculants. “Clearly, you can impart two different conformations to the same primary structure,” Prusiner says. “We don’t know at this point whether we can keep propagating them in these mice. We suspect that we can, and that’s the ongoing experiment.” He also suspects that many more conformations are possible.

No study involving prions goes undebated, however, and this one is no exception. At the heart of the criticism lies a problem that has dogged prion research since it first began: No one has yet been able to take the definitive step of generating purified prions in a system that would rule out the possible presence of viruses and show that they transmit scrapielike diseases. “The group has taken a relatively crude [brain] extract,” says Michael Harrington, who studies nervous system diseases at the California Institute of Technology in Pasadena. “Therefore, it’s not defined.” The paper, he adds, is evidence that conformational differences yield strains, “but it doesn’t confirm it.”

To Prusiner and his colleagues, the failure of years of research to turn up a virus is powerful evidence that none is involved in these disorders. But Telling says, “there are people who will go to their graves believing these diseases are caused by viruses.” Prusiner concurs: “Some will say that forever, and there’s nothing to say to them except that the evidence is overwhelming. ... They can think what they want. I can’t help them.”

—Denise Grady

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**Bad seeds.** Prion strains may consist of differently folded abnormal versions of the protein (red, purple) that transmit their characteristics to the normal prion protein (yellow) by forcing it to fold the same way.

SOURCE: S. PRUSINER