Putting Prions to the Test

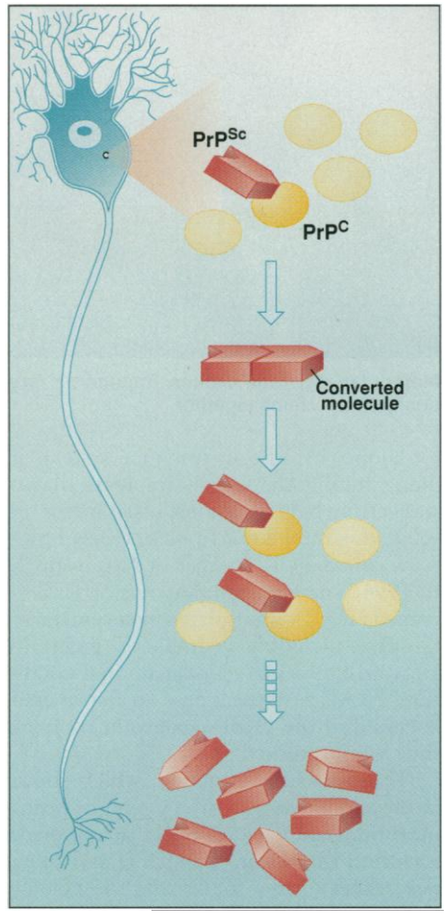
Infectious proteins—once a biological heresy—have been blamed for mad cows and dead people, and there is plenty of supporting evidence. Still, nobody has proven that these prions really exist

This spring, "mad cow disease" triggered economic and political upheavals in Britain. But the shake-up has roots in another upheaval—a scientific one—that began 3 decades ago, when radiation biologist Tikvah Alper posed a heretical question: Might the agent behind scrapie, a similar disease of sheep, lack nucleic acid? Pathogens, of course, aren't supposed to be able to perpetuate themselves without DNA or RNA to serve as blueprints, so this flew in the face of biological dogma. But Alper had subjected infectious tissue to radiation that would destroy DNA and RNA, and it remained infectious. She concluded that the scrapie agent was indeed nucleotide-free, like a protein. Physicist J. S. Griffith of Bedford College, London, tried to calm his biologist colleagues. "There is no reason to fear," he wrote in a September 1967 letter to *Nature*, "that the existence of a protein agent would cause the whole theoretical structure of molecular biology to come tumbling down."

That's a reassuring thought today, because while no protein has yet been proven to cause scrapie, a number of researchers say they are close to showing that one does. And similar aberrant proteins, they say, may cause mad cow disease, or bovine spongiform encephalopathy (BSE), which panicked Britain following reports of human cases that may be linked to eating BSE-tainted beef, as well as a number of other human and animal neurological disorders. The putative agent is called a "prion," a malfolded version of a normal cellular protein. Once prions (for "proteinaceous infectious particles") get into cells, in theory, they replicate by recruiting normal proteins to their cause, "flipping" them into a rogue prionlike shape that can go on to infect other cells and animals.

"The evidence for the prion hypothesis is getting more and more solid," says Charles Weissmann, a molecular biologist at the University of Zurich in Switzerland. For instance, infectious samples from victims of these diseases—which group under the banner of transmissible spongiform encephalopathies (TSEs)—do contain large quantities of a malfolded protein whose amino acid sequence matches that of a normal, cellular protein, known as PrP. And when samples containing those malfolded molecules are injected into animals they get sick, unless the animals don't have normal PrP—then they are resistant to scrapie, presumably because they have no PrP proteins for the infectious prion to flip. At the same time, no one has found a virus for any of the TSE ailments.

An entirely new disease process would be no small discovery, and Stanley Prusiner of the University of California, San Francisco, the neurologist who spearheaded the prion hypothesis and conducted many of the crucial experiments, won the prestigious Lasker Award 2 years ago for his work; today his name is mentioned frequently in connection with the Nobel Prize. "I think the power of his contributions has been enormous," says Allen Roses, chief of neurology at Duke University Medical Center. "I'm not on the Nobel committee, but if I were awarding a prize—a 'Roses' prize, let's say—I'd give it to him." (Prusiner declined to be interviewed for this article.)



SOURCE: S. PRUSINER

Making converts. One hypothesis about how infectious protein particles, or prions, cause disease is that an abnormal protein—PrP^{Sc}—contacts its normal twin—PrP^{C}—and changes it to the abnormal form, eventually harming neurons.

Yet the definitive test for prions—what Weissmann calls "the experiment of the decade"—has not been successful. To dispel lingering doubts that a virus might somehow be responsible for these diseases after all, researchers have to synthesize normal PrP in conditions guaranteed to be free of viral nucleic acid, and then show that the protein can fold into its rogue state and infect animals. Although many have tried, no one has succeeded in making a prion de novo.

In the absence of this clinching evidence, there are still many—even staunch prion supporters—who say prions are not a done deal. "I would say that the prion hypothesis looks very good in the absence of any other candidate—but it ain't formally proved," says Paul Brown, a neuroscientist at the Laboratory of Central Nervous System Studies at the U.S. National Institutes of Health (NIH) who studies TSEs. Prions, he says, could actually be receptors that usher a still-unknown virus or other infectious agent into the cell: "There isn't an experiment on the books in the past 10 years that disproves that hypothesis." Indeed, there are a few controversial experiments that suggest that the infectious agent is not the protein after all, but some unknown agent that is caught up along with it.

Some critics go further, charging that such inconsistencies haven't received enough attention, while prion advocates have oversold their data. "They've been advertised," says Laura Manuelidis, a neuropathologist at Yale Medical School in New Haven, Connecticut. "The prion hypothesis is really the 'cold fusion' of infectious disease—it's a very radical idea, and just like cold fusion it has some very appealing aspects," says Robert Rohwer, director of the Molecular Neurovirology Unit at the Veterans Administration (VA) Medical Center in Baltimore. "But because it's so radical it deserves a very very high level of skepticism and scrutiny before it's adopted."

Because of the power of the prion hypothesis, its potential importance, and the enduring doubts at its edges, *Science* took a detailed look at the state of the evidence, interviewing 24 researchers on both sides of the prion aisle—and some in the middle—to find out just what is and isn't known about prions and their role in disease.

An odd route to infection

By now prions have been linked to a range of rare, fatal human ailments, including Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), Gerstmann-Sträussler-Scheinker disease (GSS), and kuru (historically contracted in Papua New Guinea's Fore highlanders through ritual cannibalism), as well as BSE, scrapie, and a motley collection of other neurological maladies in mammals. In these afflictions, brain function typically withers as neurons die and the tissue develops spongelike holes, while other brain cells—glia—proliferate. Their infectious nature was demonstrated back in 1966, when Nobelist D. Carleton Gajdusek's group at NIH showed that kuru could be given to chimps by injecting them with diseased brain tissue. Since then, his team has shown that CJD and GSS are also infectious, and last year his group and others proved the same thing for FFI.

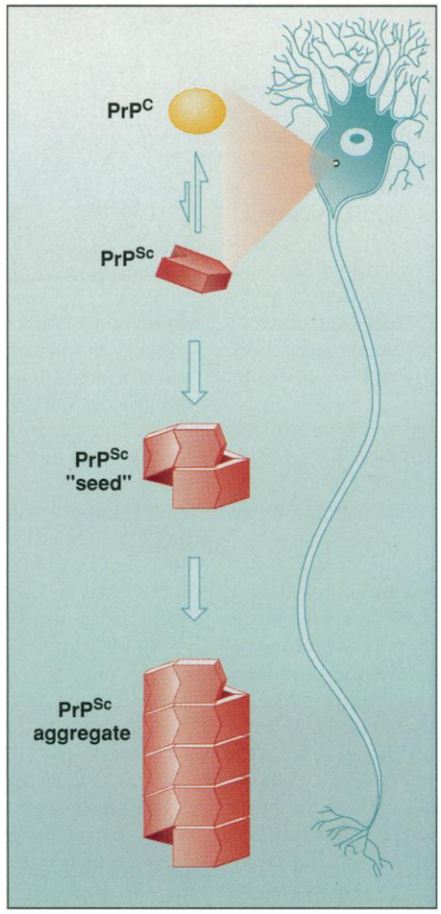
In the 1960s, the leading theory was that an unidentified, slow-acting virus was to blame. But Alper's radiation work suggested otherwise, and in the following years evidence continued to mount that the agent resisted treatments that destroy nucleic acids, but fell prey to ones that damage proteins.

Scientists got their first, tentative glimpse of a protein that might be responsible for the damage in 1981, when Pat Merz of the Institute for Basic Research in Developmental Disabilities in Staten Island, New York, reported that scrapie-infected mouse brains contained strange fibrils. One year later, in *Science*, Prusiner reported that he had found a protein in scrapie brains that didn't seem to be present in healthy brains. He posited that it might direct its own replication and suggested it be called a prion.

Despite initial resistance from a virus-focused field, Prusiner won converts, because the protein—called PrP—appeared key to infectivity. "Up until the mid-'80s I was still looking for a virus," recalls Richard Marsh, professor of animal health and biomedical sciences at the University of Wisconsin, Madison. "Yet when I started trying to purify the infectious agent using methods to purify the prion protein I got more infectivity than I'd ever gotten with any other method. Today I am convinced that the prion protein is an essential component of the agent—if not the only component."

Any "protein-only" model, however, would have to explain the initially perplexing discovery that PrP protein isn't restricted to diseased brains. In 1985, two groups—Weissmann, Prusiner, and Leroy Hood (then at the California Institute of Technology), reporting in *Cell*, and Bruce Chesebro and colleagues at NIH's Rocky Mountain Laboratories in Hamilton, Montana, writing in *Nature*—reported that they had found a gene encoding the PrP protein in healthy hamsters and mice. The amino acid sequences of both the normal and pathological proteins—called PrP^{C} and PrP^{Sc}—are identical. But PrP^{C} differs structurally from the protein found in diseased brains. PrP^{C} contains lengthy coils known as α helices, while in PrP^{Sc} many of the same parts of the amino acid chain are folded into structures known as β-pleated sheets. And unlike its counterpart, PrP^{Sc} tends to form insoluble aggregates and fibrils in brain cells.

In fact, those structural distinctions dovetailed neatly with a scenario for a protein-based infection first laid out by Griffith in his 1967 letter. Perhaps, he wrote, a protein that wouldn't normally adopt a particular folding pattern (because it was energetically unfavorable) could be catalyzed to do so by a protein that had already assumed that shape. Prusiner now argues just that: When PrP^{Sc} enters the brain and PrP^{C} molecules are exposed to the rogue templates, their structure flips, transforming them into more PrP^{Sc}. To account for most cases of CJD, where victims get the disease without an obvious infection, Prusiner argues that the PrP^{C} molecule very occasionally flips into the lethal conformation independently, without a template.



SOURCE: P. LANSBURY

Seed of destruction. An alternate view of the prion disease process is that a "seed" is needed: Infection by PrP^{Sc} seeds rapid polymerization of the molecules, leading to self-replication of the infectious agent.

This implies that if cells lack PrP^{C}, TSEs can't develop. And in 1993, a team led by Weissmann and University of Zurich neuropathologist Adriano Aguzzi reported in *Cell* that "knockout" mice lacking the PrP^{C} gene and protein didn't get sick when they were injected with scrapie. "This experiment had the chance to totally stultify the protein-only hypothesis," says Aguzzi. "If those knockout mice had become sick, then you could forget all about prions."

Aguzzi and Weissmann took these experiments a step further this January, reporting in *Nature* that they had grafted neurons that make PrP^{C} into brains of knockout mice lacking PrP^{C} and then infected those mice with scrapie. The grafted tissue became a mess of degenerating nerves, while the rest of the brain remained happy and healthy. Other evidence suggests that in mice genetically engineered to make varying levels of PrP^{C}, animals that make more of the protein get sick faster when inoculated with scrapie.

There are, of course, some basic questions about this proposed route to disease. For one, scientists don't know how PrP^{Sc} engineers the transformation of its healthy doppelgänger. Presumably it binds with PrP^{C}, which then refolds into a new and lethal shape. Just why that new shape should be lethal is yet another question. PrP^{Sc} might be actively toxic, or its proliferation might rob neurons of the normal PrP^{C} they need to survive—although the healthy PrP^{C} knockouts argue against that second explanation. (Some scientists report that knockouts do have defects, but these defects aren't scrapie.) Nor do scientists even know what PrP^{C} normally does in cells. "There are very basic things which are completely unknown at this point," says Peter Lansbury, a protein chemist at Harvard Medical School.

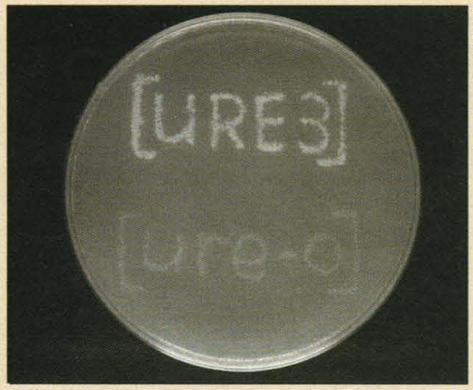
Only a protein?

The biggest unknown, however, is whether PrP^{Sc} is really the infectious culprit, or whether a virus is hiding out among all that protein. Beyond Alper's radiation experiments and other nucleic acid–destroying treatments, prion advocates stress that so far, attempts to find traces of a virus—viral nucleic acid sequences—have come up empty. Nucleic acid is present in infectious scrapie extracts, but no one has yet found one that is unique to infected brain, as would be expected of a TSE virus. For instance, scientists in several labs have tried to dig out DNA or RNA sequences that show up in infected brains, but not in normal ones, with a technique called subtraction hybridization analysis. The method removes the bulk of sequences that the two extracts have in common, enriching for unique ones. "Nobody has ever come up with an

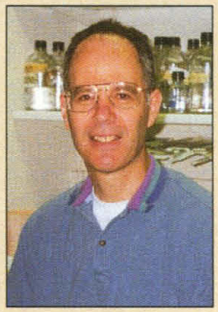
Flipping Yeast

The trail of "flipping" proteins that mark prion-associated diseases started with mammals—sheep—and hasn't strayed from the fold, as investigators have studied these maladies in hamsters, mink, people, and others. But while the leading explanation for these diseases holds that a flipped or malformed protein—a prion—from one creature can infect another animal and flip its proteins, scientists have struggled to prove this hypothesis in mammals (see main text). Recently the prion road has taken a decidedly nonmammalian turn, and researchers think the new direction could be a rewarding one.

The turn is toward yeast. In 1994, geneticist Reed Wickner of the U.S. National Institute of Diabetes and Digestive and Kidney Diseases proposed in *Science* that the spontaneous development of new nitrogen metabolism properties in certain yeast cells—a condition known as [URE3]—could best be explained if a protein called Ure3 had "flipped" into an abnormal configuration, in a spontaneous version of the process said to cause prion diseases. It seemed that yeast might provide a simple, easy-to-manipulate model system for studying how proteins undergo these flips—and how the process could be forestalled. "The yeast stuff is spectacular," comments David Westaway, a molecular biologist at the University of Toronto. "Even if it's not exactly the same as the mammalian situation it should really propel the whole field forward."



R. WICKNER



Prion clue. Reed Wickner *(right)* found that yeast could grow if the strain developed [URE3], a prionlike condition.

Already yeast experiments have pointed toward a particular class of "helper" molecules, known as chaperones, that may aid such a protein flip. Still, some researchers stress that entirely different proteins are involved in the yeast and mammal conditions, so the systems might be less comparable—and useful—than Westaway and others hope.

Wickner bolstered the analogy between yeast's flipping proteins and mammalian prions last October, when he and his postdoc, Daniel Masison, reported in *Science* (6 October 1995, p. 93) that the Ure3 protein from [URE3] cells is much more resistant to a protease, or protein-digesting enzyme, than is the normal protein. Protease resistance is a hallmark of PrP^{Sc}, the abnormal prion protein in mammals, and it indicates that a real structural change has taken place. Moreover, the more normal Ure3 a cell contains, the more likely it is to change to a [URE3] state—which makes sense if this change is indeed due to a chance protein flip, because a greater number of proteins increases the chance that one will flip. Wickner's team has also proposed that another state, [PSI], involves the spontaneous flipping of a protein called psi^-.

Several lines of reasoning favor a prion model for both conditions. For instance, cells can shed the [PSI] and [URE3] states, then spontaneously revert to them—a flip-flop that would be unlikely if either state were due to a viral infection or a mutation. What the researchers haven't yet shown is that the abnormal proteins can actually "infect" normal yeast and replicate when they are electrically pulsed into cells, as prions are proposed to be capable of doing. But Wickner is planning an experiment to do just that.

Yeast is producing insights into one of the basic prion mysteries: the reason these proteins—and perhaps mammalian prions—flip. A year ago, molecular geneticist Yury Chernoff, then a postdoc with Susan Liebman at the University of Illinois, Chicago, and co-workers reported that a protein, known as hsp104, may be aiding these flips in PSI yeast.

Hsp104 is a member of the chaperonin family—a molecule that guides other proteins as they fold up into their proper, three-dimensional configuration. When Chernoff and co-workers deleted the hsp104 gene from yeast cells, they found that hsp104 is crucial to the [PSI] condition: Without it the [PSI] condition (and presumably the flipped psi proteins) disappears from the cells. The scientists found, however, that too much hsp104 has the same effect, implying that the precise level of hsp104 in the cell is all-important.

How much relevance will these yeast findings have for scrapielike diseases? For those who suspect that a virus—not a prion—is behind these ailments, the answer is of course precious little. And some prion backers have their doubts too. Says Fred Cohen, a structural biologist at the University of California, San Francisco: "The philosophical similarity is intriguing, but the experimental condition may be sufficiently distant that it may not be the best system to pursue to learn more about human prion diseases."

Still, it is possible that yeast prions could open the way to filling a crucial gap in the experimental case for the prion hypothesis, says Wickner. Scientists have not been able to produce an infectious mammalian prion under conditions where they can rule out any role for a virus. But engineering the gene for the normal protein, PrP^C, into yeast, then manipulating the yeast's chaperone levels, might do the trick. Susan Lindquist of the University of Chicago, a co-author on the hsp104 work, is setting up a collaboration with prion champion Stanley Prusiner of the University of California, San Francisco, to study this. Lindquist is also collaborating with Byron Caughey of NIH's Rocky Mountain Laboratories in Hamilton, Montana, and Peter Lansbury of Harvard Medical School, who have produced flipped PrP proteins in the test tube, but haven't been able to make enough of them to prove that the newly flipped proteins are infectious. The hope is that chaperones will make this possible by boosting the efficiency of the test tube reaction.

It is also possible that if the yeast model is borne out, it may have clinical implications. Wickner suggests that one could use yeast as a setting for tests of drugs or other therapies that stop the prions from forming. If such treatments—which might combat prions by reducing chaperonin levels or chemically destabilizing prions once they have formed—work for several prionlike yeast proteins, they might be nonspecific enough to work for human prions too. **–R.M.**

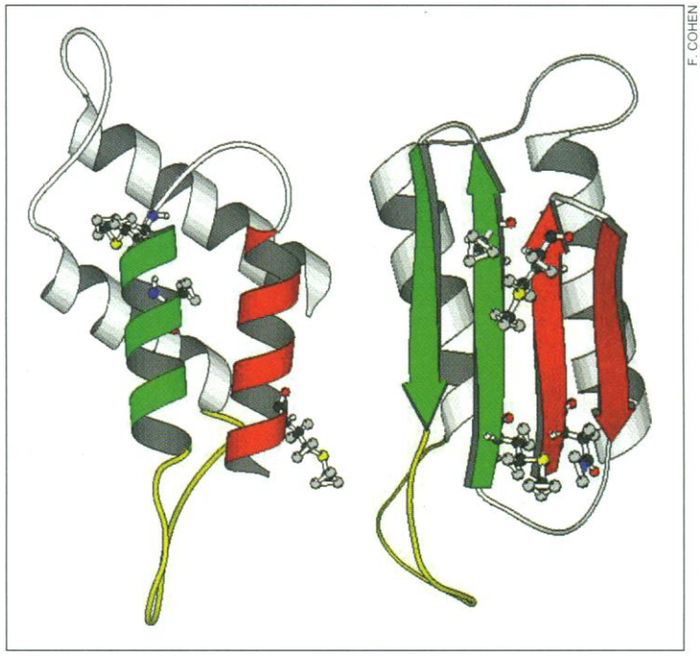
infectious nucleic acid, period," says Brown. "And it's been looked for. It's been looked for by the most modern techniques you can use."

There are other clues pointing away from a virus. Many come from the rare, human, inherited scrapielike diseases, which appear to involve mutations in PrP^C. They include GSS, in which patients lose coordination, then develop dementia and die; FFI, which first presents as sleeplessness; and a small percentage of CJD cases, in which dementia is generally the first sign. In 1989, Prusiner's group reported in *Nature* that a victim of GSS carried a mutation in his PrP gene. That same year, Jun Tateishi's group at Kyushu University in Japan reported a similar finding. Since then, Gajdusek's lab, Pierluigi Gambetti at Case Western Reserve University in Cleveland, and a bevy of other groups have found PrP mutations in CJD, FFI, and GSS families around the world. They have proposed that such people have inherently unstable PrP^C proteins that sooner or later "flip" and trigger disease; just which disease a victim succumbs to depends on which amino acids are altered.

When diseases run in the family, it's hard—although not impossible—to envision an infectious agent. "The familial cases make it much more difficult to argue on the side of a virus," says Aguzzi. "You can have close to 100% penetrance in these families—meaning if they have the mutation, they usually get the disease. So then you'd have to argue that there's some ubiquitous virus which infects everybody but will produce disease only in the patients who have the mutation. It's kind of acrobatics."

Indeed, once the genetic diseases arise, the victims' brains become infectious, as Gajdusek's group and others discovered in conditions such as GSS. Prusiner's group claims to have replicated this scenario by producing a familial GSS-like condition in lab mice. The mice, if they were engineered with a large number of PrP genes containing the human GSS mutation, spontaneously developed neurological sickness, complete with telltale spongiform changes in their brains. When Karen Hsiao, then a postdoc with Prusiner, took brain extracts from these mice and injected them into both normal hamsters and mice, as well as healthy mice carrying just a few copies of the GSS mutation, she found that some animals died of what seemed to be a scrapielike disease. The original transgenic mice, the scientists reported in a 1994 paper published in the *Proceedings of the National Academy of Sciences*, seem to have made infectious prions from scratch.

But many scientists, such as Brown and Weissmann, were troubled by these results. "They're terribly weird," Brown says. Skeptics wonder why normal mice did not succumb, only the mice harboring low numbers of the same GSS mutant gene. Another problem was that the hamsters were more prone to infection than the normal mice; because the putative infection came from mice, and these infections generally transmit more efficiently within species, the opposite should be true. Brown suspects that the animals may not have been infected by the mice at all, but by stray hamster scrapie particles in the lab. If so, that negates the crucial transmission part of the experiment, and the original transgenic mice may have sickened simply because they were making too much PrP. (There is evidence that even normal PrP can be toxic if overproduced.) Hsiao, while agreeing that there are aspects of the experiment that she does not understand—including strangely low levels of PrP^{Sc} in the transgenic mice and the animals they supposedly infect—believes that the finding is real. "We did very rigorous controls," she says.



F. COHEN

Pathological change? In a scrapie protein model, the normal form *(left)* has helices (red and green arrows) that change shape in the prion form *(right)*.

The ultimate prion test

Doubts such as these have driven the prion hunters to try and prove protein infectivity through a more direct route: by creating brand-new infectious prion particles on the lab bench. "The experiment of the decade in this field will be to take biosynthetic PrP^C which you have made under conditions where there is no possible [infectious] nucleic acid around and then wave your magic wand over the test tube, inject it, and find that it has become infectious," Weissmann says. "If that experiment is done, I think that 99.999% of the people in the field will agree, 'That's it.' "

But that test is easier described than done. In 1994, biochemist Byron Caughey and co-workers at the Rocky Mountain Labs, working with Lansbury, took a step toward making prions in the test tube. They reported in *Nature* that they could mix PrP^{Sc} purified from scrapie-infected hamster brains with normal PrP^C and form new molecules in the test tube that resist destruction by the enzyme protease. (Protease resistance is a PrP^{Sc} hallmark; PrP^C doesn't have it.) Scrapie researchers were delighted: "Quite sensational" is Aguzzi's assessment.

Still, everyone—Caughey and Lansbury included—is quick to point out that they can't separate the newly minted material from the original hamster PrP^{Sc} and then prove that this protein can transmit disease on its own. That's important, because while everybody agrees that scrapie brains accumulate aggregates of PrP^{Sc} which may cause disease symptoms, this does not mean that PrP^{Sc} itself is the infectious agent; the insoluble protein that accumulates in Alzheimer's brains, for instance, doesn't transmit disease. Part of the problem is that Caughey and Lansbury have to drive the reaction with large amounts of hamster scrapie material. Any new prion particles born on their lab bench would be undetectable because of the mass of infectivity already in the tube. "Until we or someone else can measure new infectivity the proof just isn't there," says Caughey.

In fact, other—and unsuccessful—attempts to create infectious prions have fueled doubts that PrP^{Sc} on its own can drive the creation of new infectious particles, as the purest form of the prion hypothesis proposes. Various other labs have tried to mass-produce normal or human familial mutant PrPs in *Escherichia coli* or mammalian cells, hoping that some of the molecules will flip and make prions. "We have managed to create material which is rich in β structure, and we've created material which is protease resistant—but to date we have not created material that is infectious," says Fred Cohen, a structural biologist at the University of California, San Fran-

Prusiner and the Press

A lot of people have voiced a lot of opinions about prions in this story—except for the one researcher most closely associated with the prion hypothesis, neurologist Stanley Prusiner of the University of California, San Francisco (UCSF). Prusiner twice refused requests from *Science* for an interview—indeed, he rarely speaks to reporters from any publication. He explained in a letter that he'd had "many bad encounters with the press."

One encounter stands out, according to several of Prusiner's colleagues. It was a profile of the prion researcher entitled "The name of the game is fame. But is it science?" which appeared 10 years ago in the popular science magazine *Discover*. The author was journalist Gary Taubes (now a contributing correspondent for *Science*).

Taubes reported in his story that Prusiner's forceful personality and a flair for public relations, rather than the scientific merit of his work, was behind the growing prominence of the hypothesis, and that Prusiner was loath to share credit with colleagues and tried to suppress publication of competitors' work. "It's one thing in science not to have your ideas accepted," comments Allen Roses, chief of neurology at Duke University Medical Center and a friend and colleague of Prusiner's. "It's quite another thing to be personally attacked."

UCSF

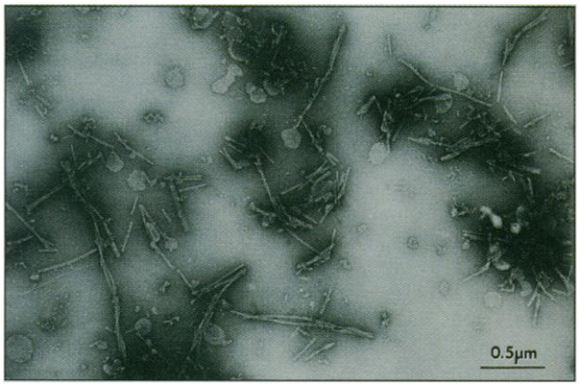
No comment. Prion researcher Stanley Prusiner isn't talking.

Today, Taubes still feels that his article was fair and accurate (although he says he wishes its tone had been less sardonic). He says he interviewed close to 40 scientists for the story, including a number of researchers who had worked for Prusiner, and that the criticisms came from the scientists: "Few in the field will deny that Prusiner's personality—for good and bad—has had a huge effect on how the field has progressed. So to do a story about it leaving Prusiner's personality out of it would be—stealing from [the writer] A. J. Liebling—like trying to make clear pea soup by leaving out the peas." And today, he notes, many scientists still maintain that crucial experiments proving that prions are infectious agents have not been done (see main text).

Prusiner's UCSF collaborator, structural biologist Fred Cohen, declined to comment on the *Discover* article but says he understands why Prusiner clammed up to reporters. He adds, "It's our loss from the standpoint of finding the best person to communicate the story about prions." Nor is there much chance that Prusiner will open up in the future, says Roses: "Prusiner has been tremendously stubborn in his scientific beliefs. I see no reason to suspect that he won't be equally stubborn about this." **–R.M.**

cisco, who collaborates with Prusiner. "Our suspicion is that there is some other molecule that is required for the really proper folding of PrP^{C}" into a prion state.

The growing suspicion that the malfolding of PrP is aided and abetted by some fellow traveler has gathered further support from studies suggesting that PrP^{Sc} may need to bind to lipids or molecules known as sulfated glycosaminoglycans in order to keep its infectious shape. Other research suggests that proteins called chaperones, which help fold other proteins into their proper shapes, might be needed for PrP^{C} to flip into the scrapie form (see box on p. 186). Still other experiments indicate that the flips may be a group effort. In a paper published last year in *Chemistry and Biology*, Caughey and Lansbury provide evidence that individual PrP^{Sc} molecules may not be sufficient to catalyze a flip; larger aggregates of PrP^{Sc} may be needed instead. (This may explain why certain fractions of PrP^{Sc} don't appear to be infectious.)



P. MERZ

Brain damage. Scrapie-infected mouse brains contain fibrils, or agglomerations of the protein.

Viruses, strains, and strained credulity

Yet there are those who charge that these theories about helper molecules are intellectual "acrobatics" themselves—and that the real fellow traveler is a nucleic acid–based pathogen, the true cause of infection. Yale's Manuelidis, for instance, is unimpressed by talk of additional molecules and different classes of protease-resistant PrP molecules, some that are infectious and others that aren't. "This is getting into medieval thought processes," she says. "If you can't distinguish structurally between the two, then how do you know that they're really different?"

Manuelidis thinks the real culprit is a virus. Never mind that no scrapie-associated virus or nucleic acid has ever turned up. Viruses, she argues, can be very hard to find: "It took 10 years—with hundreds of people working on the problem—to find the hepatitis C virus." What's more, she says, some viruses are resistant to treatments that normally destroy nucleic acids.

She does have hints that one might be lurking in TSE samples. In 1989, Manuelidis reported in the *Journal of Virology* that when she separated different molecules in infectious brain samples, the samples that contained the most infectious material were not those with the most PrP^{Sc}—a finding that has been reproduced in other labs. The most infectious samples, it turned out, had a density partway between that of protein and a nucleic acid—just what one expects of a virus, Manuelidis says.

And she is by no means a lone dissenter. Maurizio Pocchiari, a neurologist at the Istituto Superiore di Sanitá in Rome, also favors a viral culprit. Pocchiari, working with Heino Diringer of the Robert Koch Institute in Berlin, has reported finding tiny, viruslike particles in the brains of human CJD patients; Diringer earlier reported similar particles in hamsters with scrapie.

Pocchiari has other doubts about the disease-causing role of PrP^{Sc}. His reason? Pathogenicity of brain tissue is measured by how easily it infects other animals, and this measure normally tracks the buildup of PrP^{Sc} quite closely, as if the infectious agent and the protein were one and the same thing. But in a 1992 *Nature* paper, Pocchiari reported treating scrapie-infected hamsters with a drug—amphotericin B—and finding that the buildup of PrP^{Sc} lagged well behind the buildup of infectious pathogen. Somehow, the drug was interfering with the accumulation of PrP^{Sc}, but it wasn't interfering with subsequent infectivity—implying that PrP^{Sc}

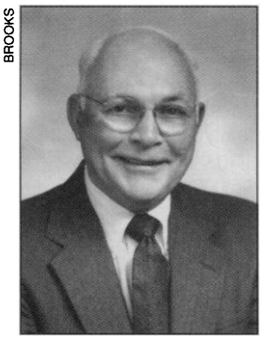
and the infectious agent are distinct.

For many, including Pocchiari, the most shaky part of the prion hypothesis is the phenomenon of scrapie strains. In mice alone, maybe 20 different prion strains have been isolated so far, defined by how rapidly infection takes hold in different mouse strains, and the different patterns of symptoms and brain lesions. It's easy to see how a virus could be so variable: It could simply accrue mutations. It is much harder to ask a single protein to be so plastic—and subsequently to be stable, for the protein would have to pass its particular characteristics on to new recruits. "I find this very hard to believe," says Pocchiari. "This is the only really weak part of the prion hypothesis. And so I still think it's a virus—although I might be wrong in the end."

"[Some parts of the prion hypothesis are] getting into medieval thought processes."
—Laura Manuelidis

Taken together, the ambiguities in prion experiments and the hints of other culprits add up to some reasonable doubts, says Chesebro. "There are four people in my group, and each one thinks something different," he says, noting that he leans in favor of a virus himself.

What irks some prion critics is that weaknesses in the theory aren't widely known. The reason, they charge, is that the weak points have been papered over by prion advocates in something akin to a public-relations campaign for the hypothesis. "This idea has been very, very assiduously promoted," says the VA's Rohwer. Both he and Manuelidis say that Prusiner is apt to pass quickly over data that do not fit his theory in popular articles, such as pieces in *Scientific American*, or in meeting presentations. Even terminology in the Medline scientific database, says Rohwer, is slanted in favor of prions: "The ... vocabulary has all been converted to 'prion,' 'prion,' 'prion.' Terms like 'scrapie agent,' which are less prejudicial in their characteristic of the disease, are footnotes."



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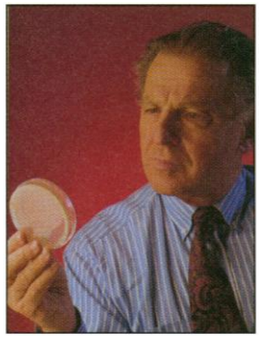
"We're going on ... 30 years almost. And nobody's come up with a substitute for the protein yet."
—Clarence J. Gibbs

Bending prions into shape

For those who favor the hypothesis, the idea that it's riding a public-relations wave quickly raises hackles. "I don't buy that at all," says Clarence J. Gibbs, an NIH virologist who works with Brown and Gajdusek. "I mean, for goodness' sake, when you try everything possible using the most modern technology that you have to your hand and you still can't show anything other than the protein associated with infectivity, I think you have to admit that the prion hypothesis is very strong. I think, frankly, that you have to keep an open mind and continue to look. But it's now, we're going on—what? 30 years almost? And nobody's come up with a substitute for the protein yet. That's pretty strong evidence."

His colleagues on the prion side are quick to rebut the evidence that seems to hint at a viral agent. Both Gajdusek's and Marsh's groups have attempted to repeat Pocchiari's amphotericin B experiment, so far with no success (although Pocchiari believes that this is due to differences in experimental protocol). As for Diringer's viruslike particles, Detlev Riesner, chair of biophysics at the Heinrich-Heine University in Düsseldorf, thinks they are just agglomerations of PrP protein. In a March 1996 paper published in the *Journal of Virology* in collaboration with Prusiner, he took hamster scrapie brain extracts and treated them with a detergent in an effort to solubilize and thus more easily analyze PrP^{Sc}. That treatment produced spherical particles composed of a few PrP molecules that Riesner says are suspiciously similar to Diringer's particles.

"The evidence for the prion hypothesis is getting more and more solid."
—Charles Weissmann



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In that same experiment, Riesner reproduced Manuelidis's 1989 finding—that infectivity can be separated from the bulk (but not all) of PrP protein. But unlike Manuelidis, he says, he studied the structure of the PrP proteins in both the infectious and noninfectious samples—and found that the noninfectious protein had lost its β sheet structure, while the infectious material retained it. His best guess is that treating PrP^{Sc} with detergent renders it impotent by stripping some lipid from the protein that usually keeps it in its infectious shape. "We find the same thing as Manuelidis—but our interpretation is different," he says.

Nor are different prion strains a body blow to the hypothesis, Lansbury says; in fact, they are a distinct possibility. He, Caughey, and colleagues have studied a pair of TSE strains, each causing a different pattern of disease in hamsters, in their test tube reaction. The two PrP^{Sc} proteins associated with these strains can be distinguished by the sizes of protein fragments left after they are attacked by enzymes. (This presumably reflects different folding patterns.) In a *Nature* paper last year, the scientists reported that these cleavage patterns were faithfully re-created in new converts when one PrP type or the other was used as a starting seed—as if not only flips, but strain-specific flips, could be faithfully replicated in vitro.

Until the elusive experiment of the decade finally comes up trumps by making an infectious prion, or an equally elusive virus is found, these disputes will continue to simmer. And this leads, ironically, to the one area of agreement among all concerned: Being a prion researcher (or a scrapie agent researcher, depending on one's point of view) isn't likely to become boring. "It's a really exciting field—there are lots of mysteries; the questions are not all settled," says Chesebro. "I think for too long this field has been dominated by a very few labs, and in the last few years it's beginning to break out of that mold—which is good, because that way you generate new ideas. And as we can see from the problem with BSE in England, this is anything but just a lab exercise. It's a serious public health problem."

–Rosie Mestel

Additional Reading

S. B. Prusiner, "The Prion Diseases," *Scientific American*, January 1995, p. 48.

S. B. Prusiner, Ed., *Current Topics in Microbiology and Immunology: Prions Prions Prions* **207**, 1996.

L. Manuelidis, "The Dimensions of Creutzfeldt-Jakob disease," *Transfusion* **34** (10), 915.

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