



Beware the Shape-shifter

U.S. faces “mad cow-like” epidemic in deer and elk

First it killed sheep. Then it ate cannibals. In the past 10 years, it's created thousands of “mad cows” and warped a rare, age-related brain disease into a death sentence for teenagers who ate British burgers. And today, roaming the wide-open plains of the American West are emaciated deer and elk plagued by this incredibly contagious agent.

Its name: prion (pronounced preahn). Prion proteins naturally exist on the surface of nerve cells in the brain of every human and animal. In most of us this protein never reveals its mongrel potential, but when it does shape-shift into a fiend, it is inevitably fatal.

Glenn Telling at the University of Kentucky is facing this monster head-on in his current research to study chronic wasting disease, the prion protein's deer and elk killer. With grants from the National Institutes of Health, Telling is using basic science to shed some light on the least understood of the prion diseases in animals and trying to

answer the question of whether CWD, following mad cow's precedent, could spawn a disease that targets humans.

Sheep and cannibals

Three hundred years ago a Scottish farmer noticed something odd in his sheep and goats. They were scraping off their wool and hair by rubbing themselves against stone walls, fence posts, anything they could find, as if they were scratching at an itch they couldn't quite reach. Mysteriously, these animals soon suffered premature deaths.

This condition, dubbed scrapie, remained little more than a veterinary curiosity until the advent of a disease called kuru, recognized in the 1950s in a remote cannibalistic tribe, the Fore people in Papua New Guinea. Part of their funeral rituals involved cooking and eating the brains of dead tribe members, but beyond being gruesome, this ritual was killing the Fore.

Carleton Gajdusek, who received the 1976 Nobel Prize for his kuru research,

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discovered the time from first symptom to death was three to 12 months. In 1959, research veterinarian William Hadlow pointed out the remarkable similarities between scrapie and kuru, namely the destruction of brain tissue characterized by sponge-like holes.

This appearance of brain tissue destroyed by what was later identified as prions led researchers to dub this family of fatal diseases “transmissible spongiform encephalopathies.”

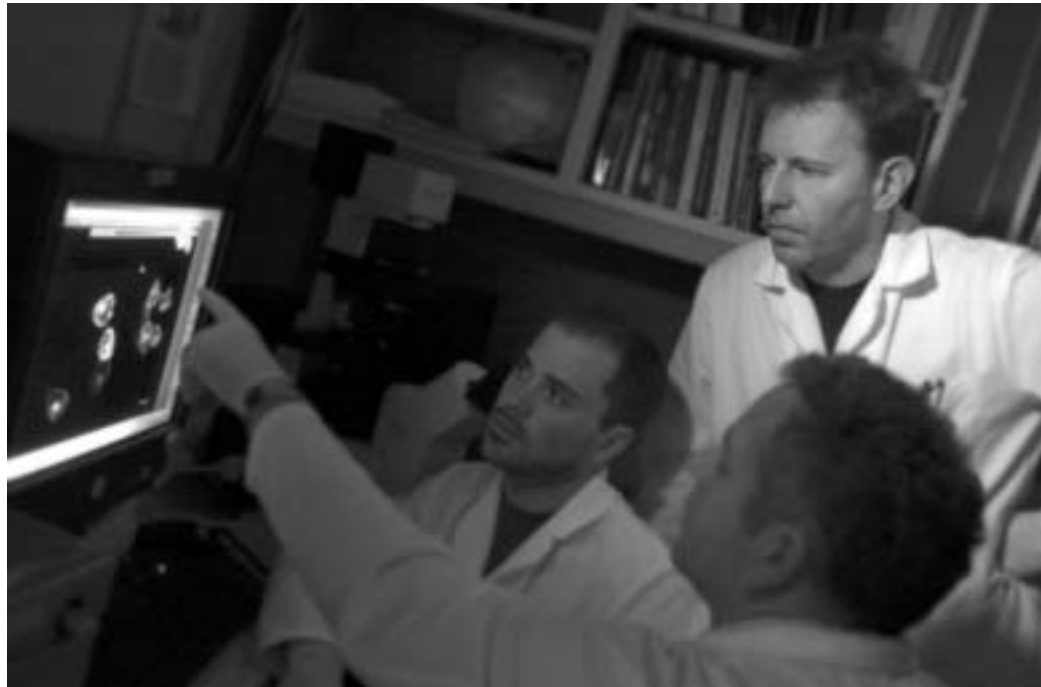
The trouble with proteins

But how do good proteins go bad? The prion protein, PrP for short, can somehow transfigure itself into a pathogen simply by changing shape. As PrP twists, bends and folds, it undergoes a transformation akin to Dr. Jekyll and Mr. Hyde.

The good and evil versions are chemically identical chains of more than 200 amino acids. The only difference in the evil protein—the one that can cause disease—is misfolded sections that take on a flat shape rather than the normal spiral. The rogue, misshapen protein then has the ability to warp other pure proteins into disease-causing shapes, which, in turn, lock together forming long chains that kill nerve cells, riddling brain tissue with holes.

Prions are an entirely different breed of evil. Coined by Stanley Prusiner in the early 1980s, and derived from “proteinaceous infectious particle,” the term “prion” marked a new paradigm of infection. Up to that point every infectious agent known to science fell into the well-defined categories of virus, bacteria, fungi, or parasite, and certainly anything infectious had to

Third-year grad student Michael Jernigan, research associate Adrian Centers (pointing) and Glenn Telling (standing) discuss images of PrP, the prion protein, injected with a fluorescent agent that allows the scientists to study living infected cells.



have a genetic component (some DNA or RNA) to drive it. Proteins couldn’t be infectious on their own—or could they?

Placing the blame

“Looks like a virus, smells like a virus, must be a virus.” Telling says this was the obvious conclusion for scientists searching for a causative agent in scrapie.

But Prusiner set out to test a sensational theory that the infectious agent lacked any nucleic acid (DNA or RNA). His work at the University of California-San Francisco revealed that the prion protein could fold into two distinct shapes (what scientists call conformations)—one normal (PrP^c: the amicable Dr. Jekyll) and one that caused scrapie (PrP^{sc}: the nefarious Mr. Hyde).

Deposited by disease-carrying animal carcasses, prions can lurk in soil for years, waiting to infect grazing animals that ingest them.

“He was the first to show that to cause disease this protein somehow goes awry, and it appears this happens when it shape-shifts,” says Telling, who worked with Prusiner for eight years in San Francisco, first as a postdoc and later as a faculty member.

Telling says Prusiner was regarded as a maverick at the time he proposed his prion theory. “He had trouble getting funding from NIH, and his views were not widely accepted. Since then, Stan has done a remarkable job to convince people, by doing excellent science and rallying the aid of a lot of very smart people.” Prusiner’s work was further vindicated with the Nobel Prize in 1997.

“Today nobody would argue that, at the very least, PrP plays some role in the diseased state,” Telling says. “At one extreme, skeptics contend that it acts as a receptor for some yet-to-be-identified virus. At the other extreme is the protein-only hypothesis, in which the abnormal protein itself is the infectious agent and converts normal pro-

teins into pathogenic ones.

“There are a lot of gray areas in between, which makes this at the same time extremely interesting and frustrating to work on,” says Telling. “No virus has been found and no virus-specific nucleic acids have been identified, in spite of exhaustive efforts to detect them.”

Some very basic questions about prions remain.

What is their natural function? Telling says that prion proteins seem to play a role in maintaining appropriate copper levels in the brain, but beyond that, scientists don’t have many good leads. (Copper helps supply blood to the brain, acts as a brain stimulant, and aids in nerve and brain function.)

Why are they so difficult to kill? Conventional means of sterilization (like cold, heat and ultraviolet radiation) that can successfully inactivate viruses have little or no effect on prions, which Telling says is yet another clue that this is an infectious agent unlike any other. Surgical instruments and lab equipment that may have come in contact with prions from brain tissue or lymph nodes are difficult to sterilize. As a result, European hospitals are using disposable surgical instruments as of-

ten as possible. It takes up to 1,080 degrees Fahrenheit to destroy prions. Deposited by disease-carrying animal carcasses, prions can lurk in soil for years, waiting to infect grazing animals that ingest them.

Why do these diseases take so long to incubate? Incubation periods ranging from months to decades frustrate scientists’ efforts to nail down a point of infection. “The long incubation has something to do with the rate of conversion of normal proteins to pathogenic ones,” says Telling. “If you are peripherally infected with these agents—say you ingest them—it takes time for them to infiltrate the central nervous system, and then it takes more time for replication and accumulation of prions to levels that cause permanent damage.” Symptoms include impaired muscle control, loss of mental acuity, memory loss, depression, insomnia, and emaciation.

Prions don’t cause Alzheimer’s disease, but prion proteins share similar features with the non-infectious proteins that accumulate in Alzheimer’s fatal plaque deposits. Future pharmacological interventions designed to prevent proteins from changing shape may be able to halt this domino effect and successfully treat prion diseases and other neurological disorders.

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prion
(pronounced pree-ahn)

Attacking prions in Telling's lab: (left to right) Rajgopal Yadavalli (a postdoctoral fellow), Shawn Browning (a grad student in the microbiology Ph.D. program) and Jifeng Bian (visiting scientist from Shandong Medical University in China)

Seeing pink elephants

The most common human prion disease is Creutzfeldt-Jakob Disease (CJD). Like Alzheimer's, CJD is normally a disease of old age. It most frequently occurs in people in their 60s. Unlike Alzheimer's, CJD is rare: it kills one in a million people.

There are three types of this disease. The first, sporadic CJD, accounts for 85 percent of cases. Scientists call it sporadic because it occurs infrequently and in scattered cases. The rarest type, acquired CJD, is caused by infection, usually accidentally. Only 250 cases have been documented worldwide of people who developed CJD after medical procedures such as injections of human growth hormone from cadavers, corneal transplants, and infected surgical instruments. The last type of CJD is inherited in families carrying specific mutations in the prion protein gene and accounts for 15 percent of cases.

Between 1994 and 1996, 12 British teenagers were diagnosed with CJD. "To see CJD in people under 30 was the neuropathologist's equivalent of seeing a pink elephant," Telling says.

The timing of the diagnoses eerily coincided with Britain's outbreak of bovine spongiform encephalopathy (BSE), aka mad cow disease. "About 180,000 cattle have been killed by this disease, and recent estimates suggest that a least two million cattle were exposed to the agent," says Telling, a native of Great Britain with firsthand knowledge. Although he did all of his training and spent the majority of his career in the United States, Telling spent two years with the Medical Research



Council in London at the height of the epidemic.

Scientists traced BSE back to prions in scrapie-infected sheep. Cattle were fed meat and bone meal made from sheep, and then cattle were fed meal from other cattle. "The disease was propagated by refeeding cattle to cattle in a cyclical process. Basically high-tech cannibalism," Telling says. "In 1996, Britain removed all animals over 30 months old from the food chain and established a ban on feeding ruminant-derived protein to other ruminants [deer, sheep, cows]."

"Cattle are extensively consumed by the general public, so it's likely that BSE prions jumped from cattle to humans via the food chain." Science has since confirmed that this "new variant CJD" is actually the human form of BSE, Telling says. To date, 129 people

in the U.K., six in France, and one in Hong Kong, Ireland, Italy, the United States, and Canada have been diagnosed with new variant CJD.

The threat across the pond

"Could this happen to us?" This is the question facing officials and hunters in North America in light of an emerging disease in deer and elk.

Eleven U.S. states (Colorado, Illinois, Kansas, Minnesota, Montana, Nebraska, New Mexico, Oklahoma, South Dakota, Wisconsin, and Wyoming) and two Canadian provinces (Alberta and Saskatchewan) have reported cases of chronic wasting disease (CWD), a prion disease that causes progressive weight loss, excessive salivation and urination, and inevitable death.

Affecting wild and captive mule deer, white-tailed deer and Rocky Mountain

Eleven U.S. states have reported cases of chronic wasting disease (CWD), a prion disease killing deer and elk.

elk, CWD is endemic in northern Colorado and southern Wyoming. Scientists have recognized CWD as a clinical syndrome for more than 30 years, but the disease may have been present in free-ranging deer populations for more than 40 years. One thing that's clear is that CWD is a highly contagious disease, particularly in captive settings.

Telling has partnered with Colorado State University and recently received a \$2.6 million share of a seven-year, \$8.4 million grant from the National Institute of Allergy and Infectious Diseases, part of NIH. Colorado State's portion will fund an emerging disease research center, in Fort Collins, focused on CWD. This is one of seven centers supported by NIH in a new push to explore emerging infectious diseases.

The Colorado Department of Wildlife facility in Fort Collins has been at the center of CWD research for a number of years. "We know that deer and elk that are introduced into the Fort Collins facility develop CWD at a very high rate," Telling says. "But we don't know exactly how." Scientists speculate bodily fluids like saliva or urine may transmit the disease, and animals that are confined have a greater chance of exposure to other animals. Efforts to sanitize the facility have been unsuccessful.

Shipping deer and elk across state lines for breeding purposes has most likely played a role in the spread of CWD. And it's possible that efforts such as Kentucky's aggressive program to reestablish a free-ranging elk herd in Eastern Kentucky, which started in 1997, may have brought the disease to the Bluegrass. No deer or elk in Ken-

tucky have been diagnosed with CWD, but elk were imported from two states (Kansas and Nebraska) currently battling the disease.

Telling has discussed strategy with the Kentucky Department of Fish and Wildlife Resources, and, according to Jonathan Gasset, wildlife division director, that department, in cooperation with the Kentucky Department of Agriculture and the Kentucky Alternative Livestock Association, created regulations in July 2002 to protect the state from possible CWD infection. In November, Kentucky Governor Paul Patton issued an executive order placing a temporary moratorium on the import or export of deer and elk. Gasset says deer and elk resources in Kentucky contribute more than \$300 million annually to the state's economy through hunting, wildlife viewing and employment related to those activities.

When the CWD threat came to light, hunters were told not to eat organs known to harbor prions, like the brain, spinal cord, tonsils, eyes, spleen, and lymph nodes; and they were warned to wear rubber gloves when processing animal carcasses.

"We don't know if this disease can transmit to other animals, in particular livestock that share pastures with deer and elk in the West," Telling says. "Could CWD cross the species barrier to create a BSE-like disease in cattle or a scrapie-like disease in sheep? And more importantly, we don't know anything about whether this disease will cause problems in humans, and this is a particularly important question in

light of the emergence of new variant CJD in Europe.

"The stakes are a lot higher than 15 to 20 years ago," he says. "The CDC, USDA and NIH are very concerned about this disease because they don't want a mad cow epidemic on their hands."

Mice as a model

One more question in the list of unknowns about CWD is the prevalence of strains. Viruses can manifest as different strains caused by slight genetic variations that result in different biological properties. Prion diseases appear to have similar variations, evidenced by longer or shorter disease incubation periods and differences in severity of symptoms. But how do strains work in prions—without DNA or RNA?

This was one of the questions Telling tackled in San Francisco with Prusiner. "Our work showed that different strains of prions had subtly different conformational states that appeared to correlate with strain properties. The strain of the agent is somehow enciphered within the conformation [shape] of the prion protein, and that conformation is imparted with a high degree of fidelity from one protein to another."

But how do you isolate strains of CWD? Telling's program, supported by grants from NIH, focuses on transgenic mice. He has inoculated these mice with CWD, and is in the "wait and see" stage. (The long incubation period means it will take a while for clinical symptoms to develop and his next round of work to begin.) Telling will compare CWD from deer and elk to look for strain-indicating differences in the prions.

"This transgenic animal model is important because, for the first time, we'll be able to test the infectivity of various tissues and bodily fluids," he says. "Is saliva the key to how this disease is transmitted from one animal to the next? We can't tell from infected deer and elk," Telling says. Besides the fact that deer and elk aren't ideal experimental models because of their size and expense, the main problem for scientists is the difficulty of maintaining CWD-free control groups.

In addition to making transgenic mice that are susceptible to deer and elk prions, Telling says one future goal of this work is to make use of transgenic mice that are susceptible to human, sheep and cow prions, a crucial step in identifying how prions jump between species. "This will allow us to explore the most important molecular determinants of the species barrier and prion strains. If, for instance, we saw transmission of CWD into mice that normally respond to human prions, it may indicate the potential for CWD transmission to humans."

Pro-active attack

"If CWD were to transmit to humans, we don't know whether it would look like new variant CJD. It could have an entirely different neuropathology and not exclusively affect younger people," Telling says.

U.S. doctors and scientists are looking for CJD more closely than before, he says, but it's a difficult task. "CJD is a rare disease, and it can look a lot like other neurological disorders. It's only definitively diagnosed with a brain biopsy after death."

Telling talks about the much publicized group of Wisconsin hunters who annually feasted on wild game, including elk from Colorado and deer from Wisconsin. Two of the men died in 1993 and the third in 1999; all were between the ages of 55 and 60. All appeared to have CJD and, because of the sheer numeric improbability that this was coincidence, speculation ran rampant that they got the disease from consuming prion-infected meat. "Tests have proven that one of them died from Pick's Disease, a related, non-prion neurological disorder. One of them had an inherited form of prion disease caused by a mutation." The third man's disease could not be scientifically linked to CWD, Telling says.

"Right now there's no convincing evidence that any of these cases in the United States acquired CJD as a result of being exposed to either BSE or CWD prions, but people tend to make those connections easily. Because the stakes are very high, it's important not to jump to premature conclusions."

Telling is seeking \$2.6 million from the Department of Defense for his next study in collaboration with colleagues at the University of Rochester in New York. This study is related to work by Adriano Aguzzi, a pathologist in Zurich, who has made transgenic mice that express a particular antibody and are completely resistant to prion infection.

"His results give evidence that immunotherapy for prions is a possibility," Telling says. "We'll test specific antibodies produced at the University of Rochester to see if those antibodies can protect against the development of CWD using our transgenic models."



Immunotherapy is one of the hottest topics in prion research right now."

This \$2.6 million is part of a \$42.5 million congressional appropriation to the National Prion Research Program, run by the DoD. The military is supporting prion research based on the potential threat to food and blood supplies.

"By being proactive the U.S. government is taking exactly the right approach," Telling says. "CWD is an emerging disease, perhaps the least understood of all of the prion diseases in animals. It's extremely contagious, and it's an open question whether it's going to be a major public health threat or transmit to other animals."

"There are still a lot of unknowns, and it's critical to keep an open mind. It's important to err on the side of caution, in light of the debacle that occurred in the U.K." ■

For more on prions, see "The Blood Threat" online at www.rgs.uky.edu/ca/odysey/spring03/shapeshifter.html.