

# Clearing **Toxic** Clumps from the Brain

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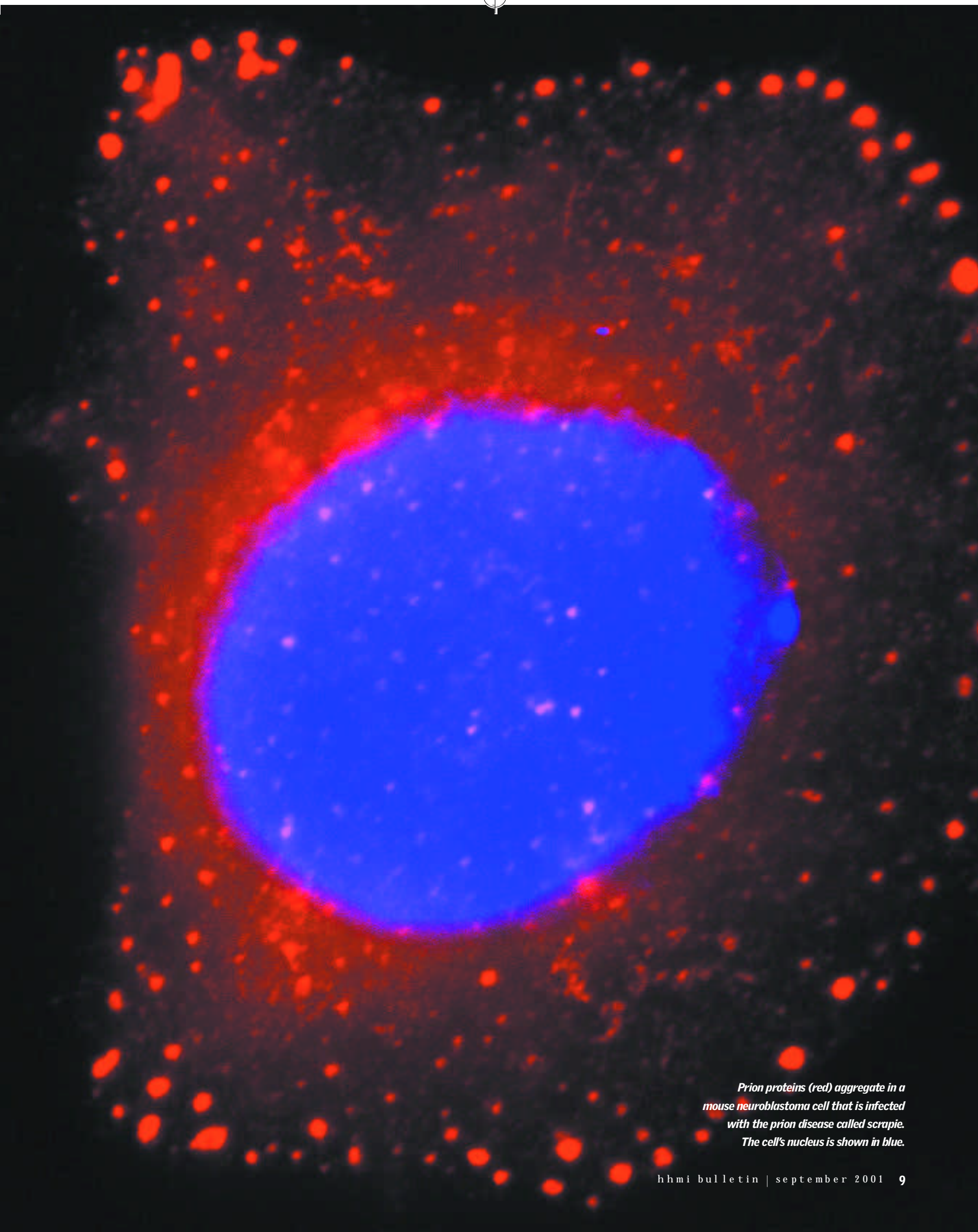
fter decades of pessimism about the chances of ever finding effective treatments for Alzheimer's disease, Huntington's disease, Lou Gehrig's disease and other progressive killers of the brain such as mad cow disease, scientists have produced a flood of new discoveries that link these outwardly unrelated ailments and suggest ways in which they might be reversed or prevented.

What these diseases have in common is a plague of abnormally shaped proteins that stick together and destroy brain cells. Different proteins are at fault in each disease. They affect different parts of the brain, and they produce diverse symptoms. Yet all these proteins are "misfolded," meaning they have strayed from their proper three-dimensional shapes; either they never reached these shapes as they emerged from tiny protein factories inside the cell, or they became corrupted. So, instead of doing their normal jobs, these proteins form aggregates of insoluble gunk, and in this process they devastate the brain. Some of them—"prions"—are even infectious; they cause mad cow disease and its human counterpart, new-variant Creutzfeldt-Jakob disease.

The big question now is how to clear up—or prevent—protein

New discoveries link seemingly unrelated, fatal illnesses such as Alzheimer's disease and Huntington's disease to misshapen proteins, providing hope for new treatments.

**BY MAYA PINES**



*Prion proteins (red) aggregate in a mouse neuroblastoma cell that is infected with the prion disease called scrapie. The cell's nucleus is shown in blue.*

clumps. Coming from several different directions, researchers have uncovered a variety of potential targets for drugs. Equally important, they have learned how to try out their new remedies on yeast, flies, worms or mice. “Things have really changed in the past few years,” says Susan L. Lindquist, newly elected director of the Whitehead Institute for Biomedical Research and former hhmi investigator at the University of Chicago, who works mostly with yeast cells. “The first meetings I attended about these diseases were so depressing. . . . But now we have several different kinds of strategies that look as if they might work, so everyone shares an optimism that just wasn’t there before.”

## Amyloids in Alzheimer’s

Some of the most promising research involves the dreaded Alzheimer’s disease, which robs so many older people of their ability to think or to remember, and then kills them. Alzheimer’s affects an astoundingly large number of people—10 percent of those over age 62 and roughly half the population over age 85.

The brains of Alzheimer’s patients are typically riddled with strange, insoluble “plaques,” which consist of amyloid (small protein fibers that form a hard mass). For a long time, there seemed no way to get at the amyloid—or the disease.

The “defining moment,” according to Sangram Sisodia, a researcher on Alzheimer’s at the University of Chicago, came with the discovery of specific mutations in the dna of certain unusual families. Not only

is Alzheimer’s hereditary in these families, but it also occurs very early in life: Members may develop the disease in their forties or fifties.

Certain families had errors in the gene that codes for amyloid precursor protein (APP), a large molecule that is cut up by various enzymes to release peptides of amyloid-beta—including the type that accumulates in Alzheimer’s plaques. In other families, two different genes, *presenilin-1* and *presenilin-2*, had mutations that also produce an increase in amyloid-beta.

With these genes in hand, researchers charged ahead, creating transgenic mice and other animals in which the genes’ effects could be studied rapidly. One of their first experiments showed that amyloid plaques were produced in the brains of transgenic mice with mutated APP genes, just like in those of Alzheimer’s patients. This experiment and others opened the door to the possibility of finding drugs that counteract the effects of the mutated genes. Several drug companies, including Bristol-Myers Squibb Co. and Amgen, Inc., are now racing to develop compounds that might prevent the enzymes from snipping the precursor protein and thereby stop the release of amyloid-beta. At the National Institute on Aging, in Baltimore, researchers developed an experimental drug called phenserine that simply decreases the production of the precursor protein, thereby lowering the level of amyloid-beta; the drug is now in clinical trials.

In 1999, researchers at Elan Pharmaceuticals in South San Francisco announced they had made a vaccine against Alzheimer’s disease—and that it worked in transgenic mice. The vaccine contains bits of amyloid-beta, prompting the mice to make antibodies against this substance that apparently prevent Alzheimer’s plaques from forming in the animals’ brains. Human trials of this vaccine began last year and demonstrated its safety. Now the company will test the vaccine’s efficacy.

Believing the vaccine too good to be true, two independent teams of scientists—one led by Peter St. George-Hyslop, an hhmi international research scholar at the University of Toronto, and the other by David Morgan of the University of South Florida—recently set out to challenge its effectiveness. Instead, they ended up supplying evidence in its favor. After mice with the equivalent of Alzheimer’s disease received the vaccine, their performance on memory tests clearly improved.

Another group at Tel Aviv University in Israel is betting on a different vaccine, AN-1792, to prevent or treat Alzheimer’s. Though nothing is yet certain, several researchers—including Lindquist—think that at the current rate of scientific progress there is a good chance of seeing treatments to slow down or prevent Alzheimer’s disease within five to ten years.

## A Clearance Mechanism

Another area “where there’s suddenly a lot of optimism,” according to Lindquist, is Huntington’s disease (HD), a rare inherited disorder that is best known for having killed the folk singer Woodie Guthrie. People with this disease slowly deteriorate both mentally and physically for more than a decade, suffering uncontrollable writhing movements along the way. Though the *huntingtin* gene was identified in 1993, after a long search, its normal function is still unknown and the mechanism of the disease remains a mystery. Nevertheless, the gene’s discovery has led to some interesting findings—and new approaches to treatment.

The key to whether the *huntingtin* gene is normal or defective turned out to lie in a kind of genetic stutter: a repetitive sequence of the dna triplet CAG, which codes for the amino acid glutamine. Stretches of CAG “repeats” appear in every human being’s *huntingtin* gene, but in varying lengths. Whereas the normal gene has a sequence of between 6 and 34 CAG repeats, the abnormal gene contains many more. In fact,

## Prospects for Treatment

Susan L. Lindquist can imagine several possible strategies for treating diseases caused by misfolded proteins: Scientists might find or design proteins or drugs that would bind to their sticky surfaces and prevent other molecules from getting trapped there. They might design proteins that could insert themselves between the aggregates and help break them up. They might alter the

chaperone balance of the cell to help ensure that the proteins attain their correct shape. Or they could try to increase the activity of the cell’s proteasome, a sort of garbage can in the cytoplasm that chews up misfolded proteins and gets rid of them.

A wide range of diseases might be treated with these approaches, she says. In addition to the 20 or so amyloid disorders and at least eight CAG-repeat diseases, Parkinson’s disease is a candidate. There are some helpful treatments for Parkinson’s today, but they do not get to the root of the disease, which involves aggregates of the alpha-synuclein protein. Amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig’s disease), too, has been shown to involve protein aggregates—in this case, the protein superoxide dismutase.

There is even hope of treating or stopping mad cow disease—bovine spongiform encephalitis, or BSE—before the disease spreads to large numbers of humans in the form of new-variant Creutzfeldt-Jakob disease. “I really do believe that most of these neurodegenerative diseases can be attacked,” Lindquist says. “It may be five or ten years away, but we will be able to make a real difference.” —MP



TODD BUCHANAN

**Susan L. Lindquist has new optimism that work in yeast, flies and worms will bring important insights into protein misfolding in several fatal diseases.**

any stretch of dna containing more than 40 of these repeats ensures that its bearer will develop Huntington's—and the greater the number of repeats, the earlier the disease strikes and the greater its ferocity. Researchers also discovered that the abnormal form of *huntingtin* produces misfolded proteins, which then stick together in toxic clumps inside the patients' brain cells.

People used to believe that once Huntington's disease begins, it is irreversible—that the gunk in the brain cells cannot be dissolved and that the brain cells are doomed to die. But when Ai Yamamoto and Rene Hen of Columbia University and their colleagues tested this idea last year, they found otherwise. Working with mice that had been engineered to carry an HD gene, they devised a way to turn the gene on or off at will. Then they showed that the disease progressed only as long as the *huntingtin* gene kept churning out more of the abnormal protein. Stopping this production, they reported, “not only halts progression of the disease, but can reverse aggregate formation and progressive motor decline.”

This was tremendously good news—not only for Huntington's patients and their families but for people concerned with any of the

other diseases caused by misfolded proteins. “It proves that mammals have a clearance mechanism that normally removes this junk,” says Arthur L. Horwich, an hhmi investigator at Yale University who studies how proteins fold. He points out that HD and other diseases might be reversible if one could find ways to shut off the production of the abnormal proteins. The cells that are dead could not be revived, of course, but those that are sick might be cured. “It could happen,” he says, “if one could intervene rapidly enough.”

## Prodding Proteins into Line

One of the greatest puzzles in biology is precisely how the body's billions of proteins fold into their correct three-dimensional shapes. When amino acids are first strung together to make new proteins, they flop around inside the cell like cooked spaghetti. Then they rapidly contort into various partially folded states before adopting their final, active form.

Because scientists knew that a special class of “chaperone” proteins guide nascent proteins toward their proper structure, they wondered if an extra supply of chaperones would help to *prevent* protein misfolding. For instance, could it prod emerging huntingtin proteins sufficiently into line to prevent them from becoming toxic to neurons?

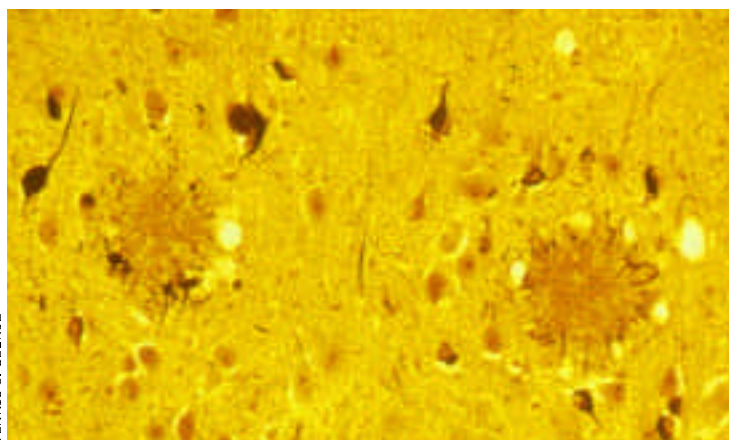
Nancy M. Bonini, an hhmi investigator at the University of Pennsylvania, decided to try this idea out in *Drosophila* because, as she explained, “late-onset, progressive diseases such as Alzheimer's or Parkinson's can take months or years to develop in mouse models, but in fruit flies they take only 10 days.” Instead of working directly with Huntington's, however, she focused on spinocerebellar ataxia type 3 (SCA3, also known as Machado-Joseph disease), one of at least eight diseases caused by too many CAG repeats.

After Bonini and her colleagues inserted the human SCA3 gene into fruit flies, they found that the flies' retinas, which contain nerve cells, rapidly degenerated. The researchers then produced a new strain of flies that had both the SCA3 gene and a gene for a human chaperone protein, Hsp70, in the hope the chaperones would come to the cells' rescue. They did. The chaperones suppressed the disease, and the flies' eyes remained normal.

Other researchers, such as Huda Zoghbi, an hhmi investigator at Baylor College of Medicine in Houston, are working with mice as well as flies. Zoghbi is working to find molecules that help suppress the damage caused by CAG repeats in the fly, then use the mouse model to examine how these molecules produce their effects.

## The Workhorse: Yeast

Lindquist's favorite model organism, however, is *Saccharomyces cerevisiae*, or baker's yeast, which can be manipulated easily and grows rapidly. Eventually, she hopes, models of HD in yeast will provide a route for “first-level screens for pharmacological agents that might reverse some of the damage” from CAG-repeat diseases. “Obviously, something that works in yeast might not work in mammalian cells,” she says, “but you can screen through many, many more compounds much



DENNIS J. SELKOE

**Two senile plaques from the brain of a person with Alzheimer's disease contain deposits of amyloid-beta protein surrounded by degenerating nerve endings. The black objects are neurofibrillary tangles within degenerating neurons.**

# The Mad Cow Connection

The epidemic of mad cow disease that erupted in British herds in 1986 has slowed down recently, probably because the country's meat industry changed its methods of recycling animal byproducts into cattle feed.

Yet an unknown number of apparently healthy people who ate infected meat in Britain long ago may be incubating a fatal brain disorder, new-variant Creutzfeldt-Jakob disease (vCJD), which seems to be caused by the same type of infectious proteins, or prions, as mad cow disease. At least 105 people have been infected, mostly in Britain, and 98 have already died.

Mad cow disease and vCJD kill so many brain cells that they leave holes in the victim's brain, making it look like a sponge—which is why these disorders are called “spongiform” diseases. In sheep, the equivalent disease has been known for about 300 years as scrapie, a fatal illness that makes its victims tremble and wobble frantically, often rubbing themselves raw against the fences of their pens as they try to stay upright. Their brains, too, are riddled with holes.

Was it scrapie that infected British cows? Maybe. But how could infectious prions overcome the usual barrier between species? Recent studies by Jonathan S. Weissman, an hhmi investigator at the University of California, San Francisco (UCSF), and graduate student Peter Chien provide a possible answer. Working with two different species of yeast, they showed that a yeast prion, Sup35, can misfold into several “dramatically different” infectious shapes; this property enables abnormal prions from one yeast strain to interact with normal prion proteins from other strains, inducing them to adopt similarly abnormal shapes. In yeast, abnormal prion proteins clump together and lose their normal activity. In mammals,

clumps of abnormal prion proteins may damage the brain.

“Other aggregation diseases are either sporadic, like most cases of Alzheimer's, or inherited, like Huntington's,” says Weissman. “What makes mad cow disease so frightening and unusual is that it's infectious. But that also gives you a chance to eliminate it.”

All mammals, including humans, carry the prion gene *PrP*, which was discovered and named by Stanley Prusiner of UCSF in the 1980s. Prusiner and others showed that transgenic mice lacking the prion gene were totally resistant to infection with scrapie. “One of the keystone experiments in the prion hypothesis was to delete the prion gene from mice and show that now they weren't capable of acquiring the disease,” comments Arthur L. Horwich, an hhmi investigator at Yale University. When these mice were given a normal prion gene, they became susceptible to scrapie again.

Several research teams have been looking for ways to eliminate PrP-scrapie aggregates or to prevent them from forming in the first place. “We work with molecules that denature PrP protein,” says Fred Cohen, at UCSF, where he collaborates with Prusiner. “We learned that branched polyamines work well in cell culture. But they don't cross the blood-brain barrier and don't get into the brain, so they cannot be used on animals. However, they could still be quite useful as disinfectants.” There are no good disinfectants against abnormal prions, and cases of people contracting Creutzfeldt-Jakob disease from surgical instruments or from corneal transplants have been reported.

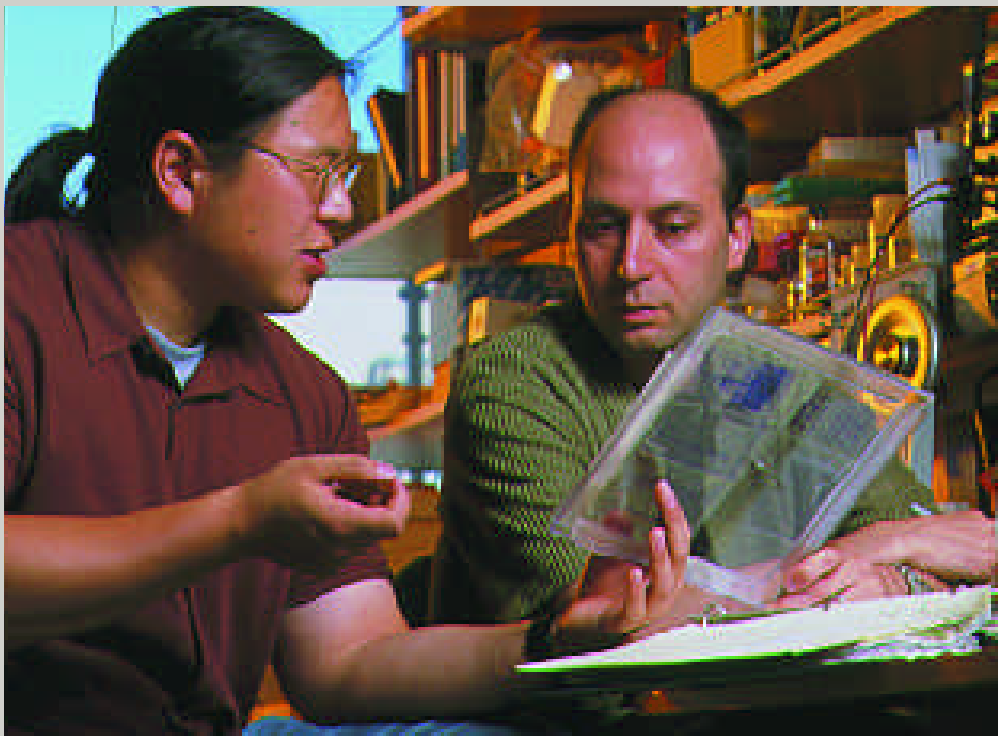
“We also found that some antibodies actually clear prions from mouse cells infected with scrapie,” Cohen says. “This argues that prion diseases may be treatable with antibodies.” Most recently, Prusiner's team examined a large number of compounds that are known to cross the blood-brain barrier and discovered that two older drugs—chlorpromazine, an antipsychotic, and quinacrine, an antimalarial agent—prevent the formation of PrP-scrapie. They are so encouraged by this finding that they suggest the drugs are “immediate candidates” for treating Creutzfeldt-Jakob disease and other prion diseases, and have begun testing them in people dying from CJD.

Still, the best approach would be prevention, Weissman points out. The new-variant CJD is “a very aggressive disease,” he says. “Once you have the symptoms of vCJD, it will be difficult to treat.” Because there's no blood test for the disease, the American Red Cross announced in May that it would stop accepting blood from anyone who has spent as little as three months in the United Kingdom or six months elsewhere in Europe during the past two decades.

Weissman would like to know what makes cows or people susceptible to these diseases. Since some sheep are naturally resistant to scrapie, it might be possible to find bulls that are naturally resistant to prion diseases and use them to breed more-resistant cattle herds. “If we can learn where mad cow disease came from and how it is transmitted,” he says, “we may be able to ensure that the cattle supply is completely free of mad cow disease—and to prevent future outbreaks.”

—MP

**Jonathan S. Weissman (right) and grad student Peter Chien determined how prions can jump from species to species.**



BARBARA RIES

more rapidly and much more cheaply in yeast than you could in mammalian neuronal systems.”

Even more important, perhaps, research on yeast may lead to some basic insights. “The problem of why proteins misfold—and why that is associated with toxicity—is very complex,” Lindquist says. “It’s such a complex problem that I think it’s really important for lots of people to be working on it, and to be approaching it from different angles.”

One reason for the complexity is that “there seem to be a variety of different ways in which proteins can misfold,” she explains. “In addition, there are different kinds of aggregates.” To make matters worse, “aggregated proteins are really miserable to work with,” she says. “You can’t crystallize something that’s an aggregate, so you can’t use x-ray crystallography. You can’t use any of the typical tools for the study of protein structure, which require the protein to be either in solution or free. So we’re reduced to using some fairly primitive tools, and when you see a big blob inside a cell you can’t tell whether it’s the same as another blob. Yet one blob might be toxic and the other not. One might provide a surface for other proteins to bind on, while others don’t.

“It’s not even clear,” she adds, “whether it’s the aggregate per se that’s toxic. Could it be some earlier misfolded intermediate? The large aggregates might be the cell’s way of sequestering material to protect itself. . . . This fundamental problem has not been solved.”

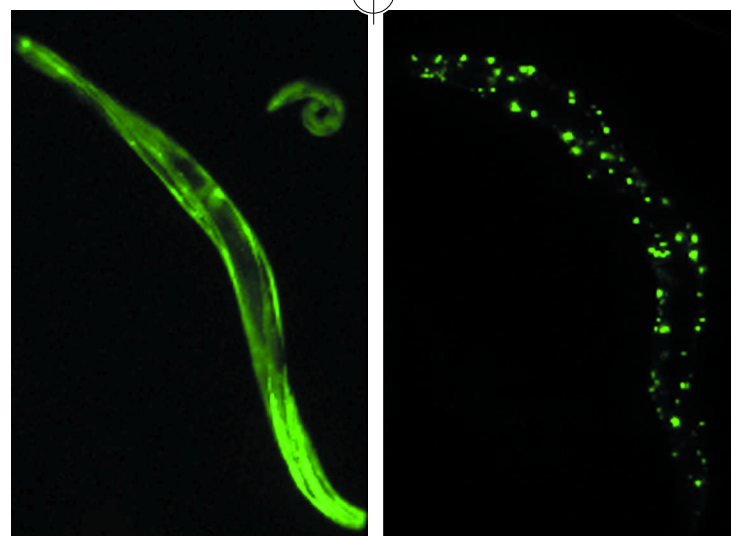
At least in Huntington’s disease, which Lindquist has been studying in yeast cells, she believes that the really dangerous state is the small aggregates. “But we don’t know how much that applies to other diseases,” she says.

## The Influence of Prions

Yeast may in fact provide the key to understanding not only amyloid diseases and CAG-repeat diseases, but also “spongiform encephalopathies” such as mad cow disease, which have been attributed to prions. The very idea of prions makes many scientists uneasy because it violates the fundamental principle that the presence of nucleic acids, such as DNA, is essential both to inheritance and infection. Yet prions produce clumps in the brain that are infectious even though no nucleic acid seems to be involved. In addition, prions can pass their traits across generations.

Lindquist came to prions by accident. “We just happened to be working on a protein called Hsp104, which is a heat-shock protein—it’s very important for thermal tolerance in yeast,” she says. “Other chap-

**Arthur L. Horwich believes that Huntington’s disease and similar disorders might be reversible, if abnormal protein production could be shut down.**



**Muscle cells in a worm’s body wall show the effect of an excess of CAG repeats, as occurs in Huntington’s disease. A normal *C. elegans* whose DNA contains no more than 19 CAG repeats produces protein that is distributed evenly throughout the muscle cells (left). With too many CAG repeats, the protein forms uneven clumps (right).**

erones just bind to partly folded proteins and keep them from getting into trouble, but Hsp104 can actually take a structure and change it. It can take apart newly formed aggregates that are caused by heat stress. So I prefer to call it a remodeling factor rather than a chaperone.

“Then we got a call from Yury Chernoff,” Lindquist recalls. Now at Georgia Tech, Chernoff was then a scientist in Susan Liebman’s lab at the University of Illinois who was studying [PSI], a genetic trait in yeast that seemed to be disobeying Mendel’s laws of inheritance. (Such traits used to be labeled “non-Mendelian,” but now they are indicated by brackets: [PSI].) “He had been trying to clone genes that control [PSI]’s inheritance,” Lindquist recalls, “and he said the one clone that really had a big effect was the gene for this protein we had been working on, Hsp104. We hadn’t yet published much on its molecular mechanisms.

“Susan Liebman had been working on [PSI] for a long, long time. So we collaborated with her and Yury, and we showed that you could cure yeast cells of this trait just by transiently changing the level of Hsp104. The normal state was then passed on from one yeast generation to the next. This was a very strong argument that the trait was inherited through a protein structure”—in other words, through a prion.

A little later, Lindquist discovered that Hsp104 also controls the aggregation state of the huntingtin protein, at least in yeast. “So now we’re using Hsp104 in yeast to change the aggregation state of huntingtin and to test some models of how its toxicity might arise,” she says. Next, she plans to look for “agents that might interfere with the toxicity,” first in yeast, then in mammals.

Lindquist believes prions arise when certain normal proteins accidentally produce folding intermediates that have a sticky surface. “That surface provides a template for other partially unfolded proteins, which bind with it and wind up getting trapped,” she explains. “Little aggregates of protein are then passed on from mother cell to daughter cell through the cytoplasm, and when the daughter cell starts making her own proteins, these have the same capacity to get trapped on larger aggregates.

“It’s very heritable. It’s very reproducible,” Lindquist says. “If you cross a cell that has a prion trait with a cell that doesn’t, the prion trait is always dominant because it’s got that sticky surface, and the other proteins join up to it. Then all the cell’s progeny will have that trait in them.”



HAROLD SHAPIRO