

A SILVER LINING

Sure, some prions can cause diseases, but others are turning out to be beneficial.

by Richard Saltus

illustration by Deth P. Sun



EVEN THE WORST

gang of rogues may reveal surprising qualities that cast them in a more favorable light. In fact, evidence is mounting that a class of proteins called prions—despite the fact that their best-known member causes a rare, deadly brain-wasting scourge in humans—can also be good citizens in the life of a cell.

In 1982, Stanley Prusiner, a biochemist at the University of California, San Francisco (UCSF), isolated the first prion as the cause of lethal scrapie in sheep. In 1997, he won a Nobel Prize for the achievement and for characterizing a mechanism for protein aggregation and self-perpetuation, or as the Nobel committee wrote, “for his discovery of prions, a new biological principle of infection.”

In the intervening years, a variety of proteins with primitive self-sustaining properties have been found in yeast, fungi and bacteria, snails, flies, turtles, frogs, birds, mice, and other mammals, including cattle, sheep, deer, elk, and people. The functions of these prions are far more diverse and complex than anyone imagined.

Prions are proteins that have converted from a normal configuration to a “misfolded,” self-perpetuating form that reproduces even though it lacks a genetic blueprint like DNA or RNA. Like one bad apple spoiling the barrel, the prion sets off a cascade, converting other proteins of the same kind from which it was formed into prions as well.

To be sure, some prions are nothing but bad news. That first one, termed PrP^{Sc} by Prusiner, forms toxic amyloids, abnormal proteins that aggregate to form tough, fibrous deposits in cells. Amyloids spread through the brain and spinal cord, decimating nerve cells in animals and humans.

A string of recent findings, however, shows that some prions can serve beneficial functions—among them: helping maintain long-term memories in snails, mice, and fruit flies; fast-forwarding evolution in yeast to equip them with survival traits; aiding synthesis of the pigment melanin in mammals; forming biofilms that give adhesive properties to bacteria; and storing hormones inside endocrine cells.

“The disease prions are just a minor idiosyncrasy,” says HHMI investigator Susan Lindquist, a leading researcher on protein folding and prions at the Whitehead Institute for Biomedical

Research at the Massachusetts Institute of Technology. “Prions are a really deeply rooted part of normal biology.”

She developed this silver-lining view of prions back when most scientists saw nothing but their destructive side. In a review written in 1997, Lindquist wrote: “My contention is that yeast and mammalian prions are not oddities in a biological freak show, but actors in a larger production now playing in a theater near you.”

That prediction is being borne out by a stream of discoveries from her Whitehead laboratory and others in the United States and abroad. “The real excitement now is to determine how widespread this biology is,” Lindquist says.

THE GOOD GUYS

The first hint that prions might be advantageous in multicellular organisms came in 2003 in a series of studies in the giant marine snail *Aplysia californica*. A major question in brain research is how memories are stored. Research in *Aplysia* had shown that memories are stored at specific nerve synapses that undergo long-term strengthening that lasts as long as the memory lasts. In the first of two papers published in *Cell* in 2003, HHMI investigator and neuroscientist Eric Kandel at Columbia University, and his former postdoc, Kausik Si, now at the Stowers Institute for Medical Research, found that the long-term maintenance of this process at nerve synapses requires continuous production of proteins, which is regulated by a protein called CPEB.

But how did CPEB keep this process going? Si noticed that one end of CPEB resembles the prion domain found in yeast prions. He suggested that the protein could convert to a self-perpetuating form that was distinctively prion-like. He and Kandel wondered if, when stimulated repeatedly (as in learning), the CPEB in synapses could become a self-sustaining protein that spurs the ongoing translation of messenger RNA (mRNA) into memory.

“In principle, this could be how you remember things for the rest of your life,” says Kandel.

To test this idea Kandel and Si joined forces with Lindquist. In the second 2003 *Cell* paper, they observed that CPEB proteins from the *Aplysia*, when inserted into yeast, formed self-perpetuating units. In a follow-up paper in *Cell* in February 2010, Si and Kandel showed the same in *Aplysia*’s own sensory neurons:



Evidence that prions have been conserved over long periods of time suggests to Jonathan Weissman that some proteins can form beneficial prions.

CPEB acts like a self-sustaining prion and the prion form is essential for maintaining the synaptic strengthening that forms a memory.

Si is working on the *Aplysia* CPEB homolog in the fruit fly and is using the model system to probe the role of prion-like features in learning and memory. Kandel's lab group has found a homolog of *Aplysia* and fly CPEB in mice. A homolog of CPEB also exists in humans.

Most prion studies have been in the fast-growing yeast *Saccharomyces cerevisiae*, which yielded the first clues to the potential benefits of some prions. In 1994, Reed Wickner of the National Institutes of Health reported that certain yeast proteins could exist in a normal form and a misfolded prion form. A case in point, the protein known as Sup35p can switch back and forth between a normal and a prion version—PSI+ and psi-. The PSI+ form is self-replicating and forms fibrous amyloids, just as in mammals.

Lindquist entered the field around the time that Wickner published his landmark observations. She was working on a "heat-shock" protein, Hsp104, which, she showed in a 1998 paper, helps yeast adapt to changes in temperature by dissolving aggregated proteins. She and others observed that Hsp104 plays an essential role in the maintenance of yeast prions.

"Later, my lab reported that PSI+ was an 'evolvability factor' that had a beneficial effect in allowing cells to try out genetic variation hidden in their genome," Lindquist says.

"When the proteins switch into the prion state, their function is switched," Lindquist explains. "They change the pattern of gene expression in the cell, and we think that this provides immediate new biological states that are potentially beneficial and could help the organism to evolve more quickly."

When the yeast is suddenly confronted with changing conditions, says Lindquist, the organism doesn't switch the whole colony to prions. Just a small number of cells make the conversion—turning on previously silent genes to test the waters, so to speak. "We think of this as a 'bet-hedging' strategy," she explains. The more stress the yeast is under, the more likely its proteins will misfold and become prions—"like gamblers who put money on more numbers on a roulette wheel."

For example, Lindquist explains, if a grape dusted with yeast falls from the vine and into a puddle, the yeast is now in a drastically different environment. Various prions in the waterlogged yeast cells will switch from inactive to active, or active to inactive, to ensure that some of the cells survive underwater. Some of those activation switches help the yeast, but some do not.

Jonathan Weissman, an HHMI investigator at UCSF, has studied survival functions of the PSI+ prion independently and also in collaboration with Lindquist. He agrees that, while the PrPSc is almost certainly toxic, "that doesn't mean that other proteins can't form prions that are beneficial,"—especially because, as he has shown, they have been conserved in microorganisms for hundreds of thousands of years.

Lindquist and colleagues reported in *Cell* in 2009 that they had surveyed the yeast genome and uncovered 19 proteins that have prion states. The collection of new phenotypes that can be selected by prion switching potentially helps the yeast adapt to environmental variables such as salt concentration, oxidative stress, and changes in carbon sources.

The results reinforce Lindquist's suggestion that prions may have evolved through the selective pressure of stress.

"I think the prion principle is a wonderful way to distribute information in a non-genetic way," says Adriano Aguzzi, a prion expert at the University Hospital of Zurich in Switzerland, who credits Lindquist with building a convincing argument that the prion phenomenon is an alternate evolutionary pathway.

Susan Lindquist, Eric Kandel, and colleagues have collaborated to show that proteins involved in normal cell processes can convert to a self-perpetuating, prion-like form to maintain memory, for example.



A KILLER INTRODUCTION

The 1982 discovery of prions by Stanley Prusiner, a biochemist at UCSF, was a stunning answer to a longstanding and stubborn problem: what was the mysterious agent responsible for transmissible spongiform encephalopathies (TSEs)—progressive, incurable, and lethal diseases that destroy nerve cells and leave a sponge-like trail of destruction?

The earliest known TSE was scrapie, an affliction of sheep. Other TSEs now known to be prion diseases are bovine spongiform encephalopathy (BSE, or mad cow disease) and some very rare but lethal neurodegenerative diseases in humans, including Creutzfeldt–Jakob disease (CJD), variant CJD, kuru, and familial fatal insomnia. These diseases have incubation periods measured in years.

The BSE epidemic of the 1980s and early 1990s killed 170,000 head of cattle and led to the slaughter of more than 4 million cows, mostly in England, before it subsided. During this epidemic, a form of BSE called variant CJD spread to humans who ate meat products from the infected herds. Mad cow disease killed about 200 people.

Scientists had determined that the agent carrying scrapie was very small and extremely hardy: it couldn't be inactivated by heat, ultraviolet radiation, or denaturing, which would destroy any genetic material used in reproduction.

Prusiner reported that the cause of scrapie was a novel infectious particle, which he dubbed a “prion,” consisting solely of misfolded proteins, devoid of genetic material. This was hard to swallow for many scientists, since even the smallest bacteria and viruses have some genetically encoded instructions for replication.

For years afterward, some skeptics rejected the “protein-only” model of prions, arguing that they must contain *some* genetic material that had escaped detection. A strong refutation of that view came in 2000 when Weissman at UCSF reported that he had created synthetic prions in a test tube directly from a pure yeast protein, Sup35.

To understand what makes a prion, recall that cells manufacture proteins by assembling chains of amino acids according to a “recipe” contained in a specific gene. Then the protein folds into a three-dimensional configuration like a complex piece of origami.

If for any reason the protein fails to fold correctly, it may not work at all, or its function may be different from what was specified.

PrP^{Sc}, the scrapie prion isolated by Prusiner, is a misfolded form of a naturally occurring cell-membrane protein, PrP, which is expressed in nerve tissue and elsewhere in healthy people and many animals.

REASON FOR BEING

Researchers have spent many years in a frustrating hunt for the function of the normal PrP protein. Surely this protein must have some other reason for being than to form lethal prions. Studies showed that mice in which the PrP protein was “knocked out” were resistant to infection with the misfolded PrP^{Sc} prions. Otherwise, lacking the PrP protein seemed to have little effect.

A piece of the puzzle seems to have fallen into place. Aguzzi and other scientists reported in January 2010 in *Nature Neuroscience* that the protein helps maintain the myelin sheaths that surround and protect neurons in the peripheral nervous system. In mice engineered to lack PrP, peripheral nerves suffered progressive damage as a result of demyelination. Aguzzi said in an interview in the journal that he suspects PrP plays a similar role in higher mammals.

The finding may also bear on another longstanding enigma: are prion diseases caused by a “gain-of-function” process—the misfolded protein itself damages nerve cells—or a “loss-of-function”

change, in which conversion of the normal protein to prions robs the nerves of something they need to stay healthy?

Outside the nervous system, another hint of PrP's normal function came in a 2006 report from the Lindquist lab. The scientists found that expression of the protein in stem cells is necessary for the self-renewal of blood-manufacturing tissues in bone marrow. The study showed that all long-term hematopoietic stem cells express PrP on their surfaces and that blood-forming tissues lacking PrP in stem cells exhibited increased sensitivity to cell depletion.

STRUCTURE AS BLUEPRINT

HHMI scientists have begun to decipher another prion riddle—the existence of distinct “strains” of prions that produce different phenotypes. For example, the PrP^{Sc} prion causes several distinct neurological diseases in animals and humans. Each disease is slightly different in the length of its incubation period, the typical symptoms, and the areas of the brain that are damaged.

“This was one of the most intriguing and challenging questions,” explains Weissman. In viruses or bacteria, strain differences are specified by instructions in their genetic code. If prions lacked any genetic material at all, where were the blueprints that determine strains?

Weissman carried out a series of experiments demonstrating that prions could propagate as distinct strains, each specified by different arrangements of atoms in the protein's structure. The structural configuration of a prion strain *was* the blueprint for making more copies—no DNA required.

Using the powerful yeast system, Weissman and colleagues created synthetic prions with different configurations that generated phenotypically diverse strains. In another experiment, they analyzed two distinct strains of the yeast protein Sup35, one of which was strongly infectious and the other weakly infectious.

The analysis required a special application of nuclear magnetic resonance spectroscopy, which enabled him to identify structural differences at the atomic level underlying these different phenotypes. The results, published in *Nature* in 2007, confirmed that the same protein can misfold into dramatically different prion conformations—which is why the phenotypes they produce can vary so significantly.

Some of the newest prion work takes a deeper look at multiple strains. The goal is to figure out how the amino acid sequence of

a prion determines its configuration and how the resulting structures differ at the atomic level.

HHMI investigator David Eisenberg, a protein structure specialist at the University of California, Los Angeles, uses various types of x-ray crystallography techniques to study the structure of the amyloid proteins produced by prions.

In 2005, Eisenberg showed that when a protein converts to a prion, it polymerizes into an aggregate made up of tightly packed layers known as beta sheets (as opposed to the coiled alpha helices that dominate in the normal protein structure). The firmly bound beta sheets, held together by an interlocking of their side chains that Eisenberg has termed a “steric zipper,” give the prion its stable, tough properties. In fact, the sheets are so closely packed that they exclude water, making prions insoluble.

In a 2009 paper in *Nature Structural & Molecular Biology*, Eisenberg proposed an explanation of how a single protein gives rise to varying shapes of amyloids that specify different strains. Instead of DNA alterations, as in living organisms, prion strains are specified by structural changes that are sufficiently stable to perpetuate the strain identity in successive prions—and when they jump from cell to cell and animal to animal.

In one type of strain-determining mechanism, the same segment of a protein's amino acid sequence can specify different “packing” arrangements of the beta sheets. In an alternative mechanism, distinctive beta sheets are produced by different segments of the protein.

“Our hypothesis is that the differences in packing produce diverse amyloid fibrils, and these fibers cause different disease types,” says Eisenberg.

In fact, these variations that Eisenberg is finding at the most fundamental, atomic level may come full circle to explain the array of functions—some harmful, some helpful—found in prions ranging from yeast to humans.

Meanwhile, he and other scientists, including Si, Kandel, and Lindquist, are searching for evidence of the positive side of prions.

If that search is fruitful, Si has a radical proposal: “Prions have been so tightly linked to the diseases that if we find more of these positive functions, I think eventually we might have to come up with a new name for them.” ■