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Tipping the Balance of Prion Infectivity

Two important questions face biologists studying the infectious proteins called prions: What stops prions that infect one species from infecting another species and what causes the invisible transmission barrier between species to fail sometimes?

In experiments with yeast prions reported in this week's issue of *Nature*, Howard Hughes Medical Institute researchers have shown how point mutations in prions—which do not compromise their infectivity—can nevertheless cause prions to alter the specificity of the yeast strain that they infect.

According to the researchers, their findings point the way to studies that could begin to clarify the factors that determine whether a prion specific to cattle that causes bovine spongiform encephalopathy (BSE), or mad cow disease, might become infectious to humans.

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- Jonathan S. Weissman

The studies also suggest a new approach for treating disorders such as Alzheimer's disease that involve aberrant protein folding, said the researchers. It might be possible to develop drugs that would influence toxic proteins that aggregate into brain-clogging plaque to fold into less toxic versions, they said.

The researchers, which included Howard Hughes Medical Institute (HHMI) investigator [Jonathan Weissman](#), Peter Chien, HHMI predoctoral fellow Angela DePace and Sean Collins at the University of California, San Francisco, reported their findings in the August 21, 2003, issue of the journal

Nature.

Unlike bacteria and viruses, prions consist only of aberrant proteins that misfold themselves into forms that, in turn, induce their normal counterparts to misfold. In mammalian prion infections, these abnormal, insoluble proteins trigger protein clumping that can kill brain cells. In humans, clumping causes fatal brain-destroying human diseases such as Creutzfeldt-Jakob disease and kuru, and in animals it causes BSE and scrapie.

In the yeast cells used as research models by Weissman and his colleagues, the insoluble prion merely alters a cell's metabolism. In previous studies of yeast prions, Weissman and his colleagues created a “chimeric” prion consisting of stitched-together pieces of prions that infected either of two yeast strains—*Saccharomyces cerevisiae* (*Sc*) or *Candida albicans* (*Ca*). The researchers found this chimeric protein to be “promiscuous”—capable of infecting either strain of yeast, depending on which one it was introduced into. The chimeric protein gave the researchers an opportunity to explore in detail why transmission barriers exist in yeast prions, which may help researchers understand the basis of species barriers that affect mammalian prions.

“It was known that very small mismatches, only a few amino acids, in a prion protein could cause a transmission barrier,” said Weissman. “It was also known that some proteins can misfold into multiple different types of prions, and that the specific shape of a prion is a key determinant of transmission barriers. But what wasn't understood was why, when you change the sequence, you would get a new transmission barrier.”

In their initial experiments, working with pure proteins, the researchers found that even changes in temperature could affect which infective form their chimeric prion assumed. Thus, they theorized, subtle mutations could cause species specificity by favoring one folded form over another.

“We hypothesized that if something as minor as a slight temperature change could affect which misfolded form the prion went into, if we could slow down which folding route the prion took, we could change the specificity of its infectivity,” said Weissman.

“It's like the Pachinko game in which a ball flipped into play can fall into one of a number of wells,” said. “A mutation in the prion produces a preferred misfolding—like tipping the Pachinko ball one way or another so that it affects which well the ball tends to fall into.”

To explore their hypothesis, the researchers created subtle mutations in the chimeric prion. These mutations caused the prion to be slower in adopting the folded conformation that infected either the *Sc* or *Ca* strains of yeast. They found that these mutations created a transmission barrier—such that for example, the chimeric prion mutated to favor the *Sc*-infecting form no longer

infected the *Ca* yeast strain. Importantly, the researchers found this effect both in test tube mixtures of the prions and in the yeast cell cultures themselves.

The findings emphasize the importance of looking beyond just the sequence of a prion protein in asking whether species barriers might be crossed. “Practically speaking, these findings mean that you can’t just ask the question of whether people are protected from mad-cow disease because cows are different from people,” Weissman said. “Rather, the answer depends on which type of cow prion it is. Studies must focus as much on the strain of the misfolded form as on what animal it is coming from.

“Our studies of yeast prions argue in a very concrete and definitive way—together with the extensive animal studies of mammalian prions—that this mutational effect on conformation is a major mechanism driving the origin of species barriers. And these findings begin to answer some of the questions of why new species barriers arise so quickly,” said Weissman.

Since the aggregation of misfolded amyloid proteins into pathological plaques also causes Alzheimer's and Parkinson's disease, said Weissman, the studies may suggest a new route to treating such disorders. Rather than seeking to prevent formation of amyloid plaques, drug treatments might aim at influencing the amyloid proteins to form less toxic products.

“The thinking in the field has now evolved to recognize that not all misfolded proteins are equally bad,” said Weissman. “So, a general strategy for treating or preventing diseases of misfolding might concentrate on small-molecule compounds that influence protein folding to favor non-toxic over toxic misfolded forms.”