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Experiments Establish "Protein-Only" Nature of Prion Infections

Two independent research groups have established conclusively that prions are proteins, and that they do not depend on genes or other factors for transmission of their traits. According to the scientists, the studies answer a nagging question that had raised doubts among some researchers about the validity of the so-called "protein-only" hypothesis of prion infectivity.

Scientists have grappled for years with one of the central tenets of the protein-only hypothesis, namely, that a single prion protein, when unaltered by genetic mutation, can give rise to different strains of prions with varying infectivity and other properties. The two research groups established that the strains could be accounted for by different misfolded conformations of the same protein. The researchers say this finding could contribute to better understanding of the functioning of disease-causing prions in animals and humans.

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Both groups published their findings in the March 18, 2004, issue of the journal *Nature*. Howard Hughes Medical Institute investigator Jonathan S. Weissman at the University of California at San Francisco led one group. The other effort was led by Chi-Yen King at Florida State University.

Both groups worked with yeast prions, which are similar to the mammalian prions known to cause fatal brain-destroying human diseases such as Creutzfeldt-Jakob disease and kuru, and the animal diseases bovine spongiform encephalopathy ("mad cow disease") and scrapie.

Scientists theorize that both yeast and mammalian prions transmit their characteristics via protein-protein interactions, in which an abnormally folded prion influences its normal counterpart to assume an irregular conformation.

In mammalian prion infections, abnormal, insoluble shapes trigger protein clumping that can kill brain cells. In yeast cells, the insoluble prion protein is

not deadly; it merely alters a cell's metabolism.

Both the mammalian and yeast prions adopt similar infectious conformations characterized by a high content of beta-sheet structures. These beta-sheet-rich aggregates, commonly referred to as amyloid, are also associated with a number of noninfectious neurodegenerative diseases including Alzheimer's disease and Parkinson's disease. In both yeast and mammalian prions, the generation of different strains can sometimes enable prions to jump the “species barrier”—to infect a species other than the one originally infected.

While considerable research had indicated that amyloids were a key component of prions, many researchers had suggested that other components, including perhaps RNAs, might underlie the differences in the various prion strains.

“I would say this puts to rest any question about whether the protein-only prion hypothesis as a general principle is true,” said Weissman of his group's findings. “And it also establishes that prion strains can be accounted for solely by the ability of the protein to misfold into more than one conformation. There might be other factors that influence it in mammalian prions, but at this point people have to prove that there are; there is no reason to suspect that there need be.”

The researchers from Florida State conducted experiments demonstrating that different strains of yeast prions can transmit their strain-specific characteristics simply through “seeding” by a prion protein.

“What we were looking for was a smoking gun,” Weissman said of the experiments in his laboratory. “We wanted to be able to take one protein, misfold it into two different self-propagating infectious conformations and show that you get two different strains, with no possibility of there being another molecule there at all.”

To do so, the lead author, Motomasa Tanaka, developed a technique to generate specific strains of yeast prion proteins simply by varying the temperature at which the newly produced proteins folded into their infective shapes.

“The use of temperature to influence folding was an elegant approach, because once you've changed the temperature, it leaves no trace in the solution,” said Weissman. “There are no other molecules that it might be argued are contributing to the differences.”

In test tube experiments, the researchers demonstrated that the protein conformations produced at different temperatures propagated themselves as distinct strains—providing templates for the folding of other proteins into the same shapes. Further structural analyses of two of the strains confirmed that the proteins were, indeed, folded differently.

When the researchers introduced the differently folded proteins into yeast cells, they found that inside cells, these proteins did indeed produce different

prion strains that passed their properties from generation to generation. Finally, they showed that extracting prion protein from subsequent generations of yeast cells yielded protein with the same properties as the strain with which the cells had originally been infected.

Weissman said that the ability to generate, manipulate and study distinct prion strains in yeast should lead to more detailed studies of how amyloid proteins form and propagate, which will be useful in guiding future studies of strain properties of the disease-causing mammalian prions.

“Clearly, it's technically much harder to work with mammalian prions, in large part because they are dangerous and because they take much more time to cause the disease,” said Weissman. “Nonetheless, I think some of what we are learning about how to make proteins misfold into different conformations will be directly relevant to understanding mammalian prions, and perhaps even to trying to understand the strain phenomenon in mammalian prions. This includes how strains can affect the virulence of a disease or how likely it is to jump a species.”