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Structural Studies Reveal New Clues to Prion Infectivity

Detailed structural studies have revealed new insights into why the same prion protein can have different properties and be either weakly or strongly infectious. The researchers said their observations in prions that infect yeast are likely to hold true for the sorts of prions that infect humans and animals.

A research team led by Howard Hughes Medical Institute investigator Jonathan S. Weissman analyzed the structures of two unmodified yeast Sup35 prion proteins in two infectious conformations. They identified key structural differences that explain the different behaviors of these prions. The researchers published their findings online September 2, 2007, in the journal *Nature*. Weissman and his colleagues are at the University of California, San Francisco.

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— Jonathan S. Weissman

The scientists studied yeast prions, which are similar to mammalian prions in that they act as infectious proteins. In recent years, mammalian prions have gained increasing notoriety for their roles in such fatal brain-destroying human diseases as Creutzfeldt-Jakob disease and kuru, and in the animal diseases, bovine spongiform encephalopathy (mad cow disease) and scrapie.

Yeast and mammalian prions are proteins that transmit their unique characteristics via interactions in which an abnormally shaped prion protein influences a normal protein to assume an abnormal shape. In mammalian prion infections, these abnormal 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. Prions propagate themselves by division of the insoluble clumps to create seeds that can continue to grow by causing aggregation of more proteins.

One of the unexplained questions facing prion researchers is how a single prion can apparently assume different conformations — with each conformation having different disease or phenotypic properties. Previous structural studies of prions had not yielded a clear understanding of the basis of strains because the prion protein is large and complex. Due to the size and complexity of prions, studies utilizing x-ray crystallography, a technique commonly used to determine the structure of proteins and other molecules, have been limited to short peptide fragments of the prion protein.

There have been a number of fairly low-resolution pictures of prions that more or less proved that these different strains were in different conformations; but they really hadn't established the nature of the different conformations, Weissman said. It was really a big black box. We basically didn't have the conformation of any single prion, let alone the two prion protein strains in two different conformations.

In their experiments, Weissman and his colleagues used the yeast prion Sup35 because two of its strains, termed weak and strong, can be unequivocally produced in pure form in the test tube for structural analysis. Rather than using x-ray crystallography, they employed hydrogen/deuterium (H/D) exchange measured by nuclear magnetic resonance spectroscopy (NMR). In this technique, molecules are first placed in a deuterium buffer and their hydrogen atoms are allowed to exchange to deuterium. Using NMR to see which hydrogen atoms exchange and which do not, researchers can then deduce structural information since highly structured regions resist exchange while unstructured regions quickly exchange.

In their analyses, the researchers allowed two strains of the yeast prion to exchange over the course of a week. By comparing the extent of exchange for each residue in both conformations, the researchers identified the well-structured regions responsible for prion activity and were able to clearly map out the structural differences between the two strain variants.

The scientists confirmed their NMR-derived structural information of the two prion variants by conducting *in vitro* studies. They selectively altered the prion protein through mutation in ways they predicted would and would not disrupt prion function based on the H/D exchange data. Then, they tested the effects of those mutations on prion conversion *in vitro*, and observed remarkable consistency between the two techniques.

Both the NMR and *in vitro* studies yielded evidence that each prion strain had the same kind of tightly packed core that was critical to their ability to form amyloid clumps. However, the two strains differed in the nature of a long molecular extension from this core. The strong strain had a less structured extension that might be more easily recognized by chaperone proteins in the cell that help the prion generate new seed prions to propagate the infection, said Weissman.

In contrast, the weak strain had a more structured extension, probably less conducive to chaperone recognition. Importantly, said Weissman, the findings confirmed theories of strain properties based on earlier findings by

other researchers who used different methods to study pieces of prions or altered prion proteins.

In our minds, our findings brought to a certain level of closure the understanding of the structural differences underlying strains, said Weissman. Now we understand the structural differences. We also have an idea how those differences lead to the differences in physical properties, and, in turn, how these differences in the physical properties lead to the phenotypic differences. We are starting to go all the way from the structural understanding of the different strains up to in vivo understanding of why they cause different behaviors inside the cell.

Weissman noted that the findings offer a broader lesson to researchers studying prions and other proteins whose misfolding can cause disease. Certainly, a bottom line from this study is that the rules of protein folding and the rules of protein misfolding are fundamentally different, he said. In many ways, we have to relearn basic principles of how proteins misfold. We have to forget many of the rules we learned from textbooks about protein folding because they are not necessarily applicable.

We now appreciate that the same polypeptide can misfold into dramatically different conformations. These different conformations can lead to dramatic changes in the structure of the prion form. This difference in structure, in turn, can change the physical properties of prions, which can affect their ability to propagate.

Ultimately Weissman hopes a better understanding of prion misfolding will lead to therapeutic approaches to diseases caused by misfolded proteins.

We know that some protein conformations—not only for prion diseases but for the far more common diseases of misfolding like Alzheimer's and Parkinson's—seem to be very toxic, whereas others seem to be relatively neutral or even protective, he said. We would like to understand the structural basis for the difference between the infectious, toxic or dangerous forms of misfolded proteins, and the neutral or protective forms. And then ultimately, we could try to develop therapies in which we push proteins down the pathways of the less pathogenic or less dangerous conformations.