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Researchers Identify Key Elements Controlling Prion Formation

Howard Hughes Medical Institute researchers have identified small regions within a yeast protein that control the protein's conversion to an infectious agent known as a prion. Yeast prions are proteins that are conceptually similar to the mammalian prions that have gained notoriety for their roles in such fatal brain-destroying human diseases as Creutzfeldt-Jakob disease and kuru, and in the animal diseases, scrapie and bovine spongiform encephalopathy, or mad cow disease.

No one knew that prion conversion was controlled by such a small region, and in such a specific way, said HHMI investigator Susan Lindquist at the Whitehead Institute for Biomedical Research. Lindquist and postdoctoral fellow Peter Tessier published a research article describing their findings on May 10, 2007, in the journal *Nature*.

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— Susan L. Lindquist

The precision of this process offers key insights into the mysterious behavior of prions, Lindquist observed. Different configurations of the recognition region cause the prion to assume different shapes, or variants. We've been able to understand some fundamental questions about how prions form different strains and how they establish and overcome species barriers, said Tessier.

First isolated and purified in the early 1980s, prions are proteins that can fold into self-templating configurations, so that proteins of the same type adopt the same configuration. In their prion state, such proteins accumulate into masses known as amyloid, which can cause such human disorders as Creutzfeldt-Jakob disease. The conversion of proteins to a prion state also plays a role in normal biological functions, including cell adhesion, skin pigmentation, adaptation to environmental stress, and maybe even long-term memory.

For more than a decade Lindquist has been investigating a prion known as Sup35 in the yeast *Saccharomyces cerevisiae*. Sup35 normally helps regulate the flow of information from DNA into the cellular machinery that produces proteins. When Sup35 converts to a prion state, its activity is reduced, which changes the expression of the yeast's DNA and alters biological function.

Two parts of Sup35 control its conversion to a prion state: a middle region (M) and a region toward one end of the protein (N). But how these regions function and interact remains unknown. To probe the mechanisms of prion formation, Tessier and Lindquist applied a new technique to the problem. They attached segments of the M and N regions, each 20 amino acid residues long, to a glass slide. Each successive peptide overlapped the preceding one by one to six residues, allowing 136 different segments of the protein to be displayed on the slide.

They then exposed the peptide segments on the slide to a solution containing fluorescently labeled copies of the Sup35 protein in its non-prion state. The soluble protein accumulated over a very small set of the peptides, which corresponded to two regions of the M and N sections that had been found previously to be sites of protein-protein contact in assembled fibers.

When the protein that had accumulated over the peptides was scraped from the slide and examined under an electron microscope, it revealed fibers characteristic of amyloid prions. Though Tessier and Lindquist do not yet know the exact process leading to the accumulation of the prions, they hypothesize that specific peptides are causing the protein to adopt a prion configuration. We think the peptides are nucleating the amyloid conformation on the surface of the arrays, says Tessier. Now we're exploiting that method to identify how these prions initially form.

Tessier and Lindquist also applied their technique to the Sup35 protein from a different yeast species, *Candida albicans*, which has been evolutionarily separate from *S. cerevisiae* for at least 800 million years. Remarkably, says Lindquist, the *C. albicans* protein also had two regions that controlled the formation of prions, suggesting that the same mechanism has been operating in prions since before the evolution of multicellular organisms. We had postulated previously that prions serve an important biological function, Lindquist said. To me, this finding suggests that these functions really have been selected by evolution to enhance self-perpetuation.

To probe the ability of prions to adopt different configurations and possibly to cross species barriers, Tessier and Lindquist used a chimeric Sup35 protein that contains the active segments of both *S. cerevisiae* and *C. albicans*. This chimeric protein interacted with the peptide segments of *S. cerevisiae* at one temperature and with the peptide segments of *C. albicans* at a different temperature. Furthermore, alterations of specific amino acids within the active regions of the proteins caused them to adopt different configurations or strains. We can identify conditions, like temperature or mutations of the protein, that selectively promote the assembly of one prion strain rather than another, says Tessier.

This finding suggests the circumstances that could cause a prion from one species to cause disease in a different species. If the active element of a prion could cause proteins in another species to convert to a prion state, the prion could be infectious in both species. This helps us understand how you can cross the species barrier, says Lindquist.

Lindquist and Tessier point out in their paper that the technique they used could be adapted for high-throughput drug screens. Peptides from the active regions of prions could be used to look for compounds that prevent prion assembly, promote it, or redirect the formation of prions into alternate configurations. More generally, insights into the folding of proteins into different configurations offer possibilities to fabricate materials and devices with complex, nanostructured features, says Lindquist. That's been an unexpected consequence of this research, she said.