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## New Model Suggests How Prions Take Shape

Howard Hughes Medical Institute (HHMI) researchers at the University of Chicago have identified a new mechanism by which the infective proteins in yeast called prions replicate their structures. The finding may provide a general model for understanding how protein aggregation occurs in several human diseases, including Alzheimer's disease.

In an article published in the August 25, 2000, issue of the journal *Science*, HHMI investigator Susan L. Lindquist and her colleagues at the University of Chicago report that prions produce self-perpetuating amyloid fibers by a model that Lindquist's team calls nucleated conformational conversion.

Lindquist's team studied the critical domain of Sup35, a yeast prion that it had previously discovered could transmit heritable characteristics from one generation of yeast to the next. Yeast prions 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."

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Both yeast and mammalian prions transmit phenotypes via protein-protein interactions in which an abnormally shaped prion protein influences its normal counterpart to assume an abnormal shape. In mammalian prion infection, such abnormal, insoluble shapes cause protein clumping that kills brain cells. In yeast cells, the insoluble prion protein is not deadly; it merely alters a cell's ability to function.

Although there has been a great deal of interest in prions, researchers do not yet know how prion assembly occurs. "There have been general theoretical models developed over the last 40 years to describe various protein self-assembly processes, but nobody really had any idea how prion proteins assembled into amyloid fibers," Lindquist said. The general models suggest that Sup35 might convert to amyloid fibers by growing from single protein elements, called monomers; by assembling on a template; or by collecting on a "nucleus," like crystals growing from a seed. Understanding the amyloid assembly mechanism would have considerable benefits, said Lindquist.

"Mammalian prions constitute a serious health threat, so it's important to understand how they propagate. If our work extends to mammalian prions, such knowledge could offer potential pathways to treatment," she said. "Also, while it is far from certain, at least some other amyloids involved in diseases such as Alzheimer's disease might assemble in this way.

"Finally, the yeast prion represents an entirely novel mode of inheritance that does not depend on the genome, but on proteins. Such a mechanism will likely have a wide influence in understanding some basic biological phenomena."

In their experiments, the scientists used a number of analytical techniques such as atomic force microscopy and biochemical studies to understand the basic structural changes that were occurring as Sup35 self-assembled. The scientists also measured the time-course of the assembly process, as well as the effects that varying concentrations of the soluble Sup35 protein had on Sup35's ability to assemble into fibers. Initial experiments quickly eliminated the monomer-directed conversion mechanism from contention, leaving templated subassembly and nucleated formation as possible models.

One particularly startling finding, said Lindquist, was that the rate of assembly seemed not to be dependent on the concentration of the soluble protein. "This lack of concentration dependence was very unusual," she said. "It just didn't fit with either of the remaining models. If we hadn't had three people in the lab doing the same experiment and coming up with the same results, I don't think I would have believed it."

Results from additional experimental manipulations of the assembly conditions led the scientists to propose a new model to explain their results. They call their model, nucleated conformational conversion (NCC), and it postulates that individual soluble Sup35 prion domains wobble between different structural conformations in solution and cannot by themselves find the right way to fold. "But when a group of these wobbling proteins gets together, it's a different environment and the proteins help each other reach the proper fold," Lindquist said. "Once a group of these proteins has formed a stable seed or nucleus, new proteins join the bandwagon and acquire the same structure. The initial complexes are like globs of goo, like a plastic resin that hasn't hardened yet." Once they do harden, they can collect other globs and turn them into amyloid fibers, she said.

"We're suggesting that this particular protein—and likely at least some other amyloids involved in human diseases—is unusual in that it remains unstructured for very long periods of time," said Lindquist. "It can't figure out how to get into a stable structure until it joins in with other proteins in a kind of molten complex. And within that complex, the structure becomes stabilized."

The blobs of protein are so fragile, in fact, that if they grow too large they may fall apart, which may help explain why higher concentrations of protein have little effect on the speed of fiber assembly, said Lindquist.

Lindquist and her colleagues now plan future work to explore other amyloid-producing proteins to determine whether they use the same assembly mechanism. "My guess is that there will be a class of amyloid-forming proteins that also has this same tendency to exist in solution as random coils," she said. "These might include such proteins as those involved in Parkinson's disease."

Also, she said, detailed understanding of the NCC mechanism might be applied to creating protein-based "nanodevices," molecule-sized machines that could be used as components of computers, sensors or other infinitesimally small machines.