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Amyloid Fibers Sprout One Step at a Time

Researchers have combined sophisticated biochemical and imaging techniques to get a glimpse of the stepwise assembly of amyloid fibers in a yeast prion protein. Their findings suggest that these structured fibers form in competition with the amorphous globules that some believe may cause toxicity in amyloid diseases such as Alzheimer's and Parkinson's. The researchers say this may have important implications for those designing drugs to prevent formation of the brain-damaging proteins in those diseases.

The researchers reported their findings in the October 2004 issue of the *Public Library of Science Biology*. They were led by Howard Hughes Medical Institute investigator Jonathan S. Weissman at the University of California, San Francisco. HHMI investigator Ronald D. Vale, also of UCSF, was a co-author of the article.

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Working in yeast, Weissman and his colleagues investigated the mechanism by which a prion protein assembles individual polypeptides into long amyloid fibers. These fibers are similar to the amyloid plaques that clog the brains of patients with Alzheimer's or Parkinson's disease.

Unlike bacteria and viruses, prions consist only of aberrant proteins that misfold into forms that, in turn, induce normal proteins to misfold. In mammalian prion infections, these abnormal, insoluble proteins trigger protein clumping, producing a plaque that can kill brain cells. In humans, clumping causes fatal brain-destroying diseases such as Creutzfeldt-Jakob disease and kuru; in animals it causes bovine spongiform encephalopathy (mad cow disease) and scrapie.

In the yeast cells Weissman and his colleagues used as research models, however, the insoluble prion merely alters a cell's metabolism. Besides offering a model for studying prions, the yeast system also provides an excellent model for the growth and aggregation of amyloid protein, said

Weissman. Studying this process could have important implications for understanding amyloid diseases, he said.

Initial efforts to understand amyloid formation compared the process to the formation of the cell's cytoskeleton, - a better understood mechanism known as nucleation-polymerization, in which the cytoskeletal proteins (actin and tubulin) coalesce into long fibers. Experiments from a number of labs, however, revealed that this process could not explain amyloid formation.

Amyloid formation was also associated with the transient accumulation of intermediate molecules that have been implicated in causing disease. "The process of forming amyloids seems to be implicated in disease perhaps as much as the actual aggregates themselves," said Weissman. "So understanding why some proteins form amyloids and aggregate, and under what conditions that occurs, and the intermediate processes involved, is critical in determining what is toxic about amyloid and how it might be possible to affect the pathology it causes. Yet despite the importance of this process, we know little about the underlying mechanism by which amyloid forms and grows."

For example, said Weissman, evidence is accumulating that it is not the plaque itself that is toxic, but rather the smaller and more amorphous oligomers that typically accompany plaque formation. But why such oligomers form and what role they play in making amyloid plaques was unknown.

Weissman and his colleagues sought to understand the dynamics of how the amyloid puzzle pieces assemble themselves. They analyzed the timing of the yeast prion protein assembly, and how that varied with different concentrations of the fiber and of the individual units, or monomers, that add to the growing fibers. The researchers also explored a particularly puzzling feature of amyloid formation: the fact that agitation dramatically accelerates the process.

Drawing on expertise in the Vale laboratory, the scientists complemented these indirect studies with "single-molecule fluorescence technology" to observe fiber growth directly. In this technique, an immobilized fiber is first tagged with one fluorescent molecule. When shorter amyloid segments or monomers are tagged with a fluorescent molecule of a different color and added to the immobilized fiber, researchers can watch the growth of the fiber.

The analytical approaches revealed that the yeast amyloid fibers grow by the addition, one by one, of individual monomers - rather than assembly of amorphous, globular oligomers. Thus, said Weissman, if the oligomeric globules are, indeed, the toxic molecules, they form in competition with the structured fibers, rather than being key intermediates in fiber formation. Such a possibility could have implications for treating amyloid diseases such as Alzheimer's and Parkinson's, he said.

"Investigators are now screening for drugs that would prevent amyloid from forming, to treat these disorders," said Weissman. "While it is quite

speculative at this point, if such drugs favored the production of more oligomers, which are toxic, then those drugs could actually have the opposite effect than was intended. Conversely, drugs that encourage the rapid formation of a relatively inert and stable amyloid might deplete the toxic oligomers and therefore be beneficial," he said.

Weissman emphasized that basic studies of amyloid formation must be extended beyond the yeast prion model before the monomer-addition mechanism can be considered a general one. Thus, he and his colleagues are now studying the mechanism of formation of other amyloid proteins, including the molecular details of how individual monomers bind to a growing fibril.