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A Proliferation of Amyloid Arrangements

Four years ago, Howard Hughes Medical Institute investigator David Eisenberg provided the first glimpse of the atomic structure of amyloid fibrils – long filaments of abnormal proteins stacked up inside cells. Amyloid fibrils are found in more than 20 human diseases, including type II diabetes, Alzheimer’s disease, Lou Gehrig’s disease, and Creutzfeldt-Jakob disease (CJD), the human version of mad cow disease. Yet their precise role in these ailments remains a mystery.

Now, Eisenberg has extended his earlier work, discovering that each fibril-forming protein can nestle against its neighbors in a variety of arrangements, much like identical Lego blocks can interconnect in different patterns. Further, Eisenberg proposes that the variation in these structures represents a protein-based system of inheritance between cells that parallels the genetic code. The basic unit of protein-based inheritance – the analog of the nucleotide base pair in the genetic code - is the “steric zipper,” says Eisenberg, which is a specific arrangement of interconnecting proteins.

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Eisenberg’s earlier work on fibrils led to his discovery of steric zippers, and in new work published August 16, 2009, in *Nature Structural & Molecular Biology*, he adds more zipper patterns to the catalogue of possible amyloid structures. The findings point out how identical proteins can form amyloids of different shapes – namely by packing next to their neighbors in different orientations.

“Many investigators have noted that amyloid fibrils from the same protein have different morphologies,” says Eisenberg, who is the director of the University of California, Los Angeles - Department of Energy Institute of Genomics and Proteomics. “They’re either ribbon-like or spaghetti-like or

they're twisted. My guess is that different steric zippers define these different morphologies.”

In earlier work, Eisenberg's colleague Melinda Balbirnie discovered that a short segment of an amyloid protein – a peptide only seven amino acids long – could form a steric zipper. When Balbirnie added the short peptides to solution, they clumped into crystals. However, Eisenberg says “we began to notice that these segments formed more than one type of crystal. And when we determined the structure via x-ray crystallography, the packing of these peptide segments into steric zippers looked different.”

By studying peptides from 10 different amyloid-forming proteins, Eisenberg found three different types of variation in the steric zippers. Sometimes, the peptides shifted relative to each other. That is, the teeth of the zipper shifted relative to the teeth of the neighboring peptide, much like the fingers of two folded hands can interdigitate in different ways. Eisenberg calls this a registration polymorphism. Next, he found that some amyloid-forming peptides were flipped relative to each other – much like two hands folding palm to back-of-hand, rather than palm-to-palm. He calls this a facial polymorphism. Finally, Eisenberg found that different amino acid sequences of the same protein could form different steric zippers. He calls this segmental polymorphism.

The existence of this wide variety of amyloid stacking patterns may explain one of the perplexing features of human prion disease, says Eisenberg. The human prion protein, known as PrP, causes two forms of CJD. In the spontaneous version of CJD, the PrP protein kinks into an abnormal shape and forms fibrils. The disease, which is genetic and develops in about 1 in a million people, causes the brain to become spongy. This leads to dementia and, after a decade or longer, death. In contrast, people who suffer from variant CJD (vCJD), such as people in Britain and elsewhere who contracted the disease after eating tainted beef, die much more quickly. The same protein, PrP, is implicated in both diseases, but researchers who broke apart the fibrils and analyzed them found that the fibrils from CJD and vCJD form different patterns.

“If you get CJD, it takes years and years and years for your brain to get holes,” says Eisenberg. “But the people who ate those bad burgers, who get vCJD, they get demented within about two years and die. A pathologist can tell from looking at the brain after death whether the person had CJD or vCJD. It's exactly the same protein, the same sequence that forms fibers in the brains of these people, and yet the diseases are different. The hypothesis produced from our paper is that these two prion strains are caused by different steric zippers, different stacking arrangements.”

At the end of the new paper, Eisenberg speculates that steric zippers form the basic unit of information transfer in an as-yet unexplored system of protein-based inheritance. Some

amyloid proteins, such as PrP and a protein called sup35 that is found in yeast, are infectious and can spread to other cells. The “message” conveyed by these different strains depends on which steric zipper lies at the core of the fibril, Eisenberg suggests. In the new paper, he estimates that a large number of steric zippers is possible in nature.

“This is a frontier area, and many of my colleagues will probably accuse me of engaging in speculation,” says Eisenberg. “That’s fine. I’m simply interested in offering up a new idea supported by our research.”