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THE DARK SIDE OF PROTEINS

Almost every human protein has segments that can form amyloids, the sticky aggregates known for their role in disease. Yet cells have evolved some elaborate defences, finds **Jim Schnabel**.

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Of all the ways that proteins can go bad, becoming an amyloid is surely one of the worst. In this state, sticky elements within proteins emerge and seed the growth of sometimes deadly fibrils. Amyloids riddle the brain in Alzheimer's disease and Creutzfeldt–Jakob disease. But until recently it has seemed that this corrupt state could threaten only a tiny fraction of proteins.

Research is now hinting at a more unsettling picture. In work reported in February, a team led by David Eisenberg at the University of California, Los Angeles, sifted through tens of thousands of proteins looking for segments with the peculiar stickiness needed to form amyloid[1]. They found, says Eisenberg, that "effectively all complex proteins have these short segments that, if exposed and flexible enough, are capable of triggering amyloid formation".

> **"Most proteins have evolved to fold in a way that effectively conceals their amyloid-prone segments."**

Not all proteins form amyloids, however. The 'amylome', as Eisenberg calls it, is restricted because most proteins hide these sticky segments out of harm's way or or otherwise keep their stickiness under control. His results and other work suggest that evolution treats amyloids as a fundamental threat. Amyloids have been found in some of the most common age-related diseases, and there is evidence that ageing itself makes some amyloid accumulation inevitable. It now seems as though the human body is perched precariously above an amyloidal abyss.

"The amyloid state is more like the default state of a protein, and in the absence of specific protective mechanisms, many of our proteins could fall into it," says Chris Dobson, a structural biologist at the University of Cambridge, UK. Several laboratories are now trying to find ways to supplement or boost these protective mechanisms, in the hope of treating or preventing a host of amyloid-linked diseases. "Advances in understanding amyloids could lead to a powerful new class of medicines for many age-related conditions," says Sam Gandy, a neurobiologist and clinician at Mount Sinai School of Medicine in New York.

Fibrils abound

The recent work on amyloids has partially confirmed a prediction made 75 years ago by the British biophysicist William Astbury. Proteins start as linear chains of amino acids, but most then fold into complex, three-dimensional, 'globular' shapes. Astbury proposed that almost any globular protein could be made to form dysfunctional fibrils by damaging — or 'denaturing' — it with heat or chemicals. By the 1980s, researchers had come to understand that these artificially induced fibrils had the same peculiar structure seen in disease-linked amyloids, such as the amyloid-β deposits in the brains of people with Alzheimer's disease. But the wider potential of proteins to naturally form this basic structure was not seen right away. "The previous paradigm was that the whole protein unfolded and then refolded into a fibrous structure," says Eisenberg.

By 1999, it was clear that numerous proteins could be made to form amyloids. Dobson proposed that unfolding exposes an essential stickiness in a protein's backbone of amino-acid chains[2]. Researchers were also linking more and more amyloid-forming proteins to disease, including tau proteins in Alzheimer's disease, α-synuclein in Parkinson's disease, polyglutamine in Huntington's disease, prion protein in Creutzfeldt-Jakob disease and amylin in type 2 diabetes[3].

Eisenberg and his colleagues studied such proteins using fibril-forming assays and X-ray diffraction techniques and found that their tendency to form amyloids came from specific segments within them[4]. These segments are typically about six amino acids long, and can be exposed when a protein partly unfolds.

These 'amyloidogenic' segments, Eisenberg's

team found, have a self-complementary 'steric zipper' structure that lets them mesh very tightly with an identical segment exposed on another protein[5]. Several of these segments are needed to seed, or nucleate, an amyloid. Segments stack atop one another to form sheets, two of which zip together to form the spine of the fibril. As it grows, the fibril is fringed by the remnants of the segments' host proteins. Eventually, this sprouting fibril breaks to form two smaller fibrils, each of which will grow from both ends again — and so on. "The nucleation event may be rare," Eisenberg says, "but once it starts, you can see how it would spread."

In their study[1], Eisenberg's team used a computer algorithm to determine when any short protein segment has sufficient steric-zipper-forming potential, based on its predicted three-dimensional structure. After calibrating against known amyloid segments, the team applied the algorithm to the genomes of human, budding yeast and the bacterium *Escherichia coli* and found that about 15% of the short segments coded by genes in these organisms had this property. "At that rate most proteins contain at least several of these amyloid-prone segments," says Eisenberg.

The work helps to clarify in a rigorous way why denaturing a protein often pushes it into the amyloid state, says Jeffery Kelly, a structural biologist and amyloid expert at the Scripps Research Institute in La Jolla, California. "It gives us a better idea of why some proteins have to partially unfold before they can start forming amyloids."

Eisenberg, Dobson and others have speculated that the self-complementary stickiness of these short segments might have made them useful building blocks in the earliest stages of life on Earth. Moreover, reports have started to emerge of proteins that function normally in the amyloid state, for example some pituitary hormones[6]. "We know by now of over two dozen native amyloids, so this state is clearly used by biology in a functional way as well as a dysfunctional way," says Eisenberg.

Even so, says Kelly, these native amyloids "are all highly regulated" by, for example, being tucked away inside membrane-bound compartments called vesicles. "That's why biology can use them and not suffer the consequences."

Most modern proteins fold into globular structures. But their folding patterns are so complex that they couldn't have evolved by accident. "If you had a machine that could generate protein sequences randomly, you would only rarely get one that can remain stable in the globular, soluble state," Dobson says.

Underlying that stability are a variety of evolved mechanisms. When proteins are first synthesized and start to fold, 'chaperone' proteins and related molecules are there to guard against amyloid formation. Other systems are in place to recognize, sequester and destroy amyloids when they do form.

The native folded state offers its own strong protection. Eisenberg's group examined more than 12,000 proteins whose folded, three-dimensional structures are already known. They found that 95% of the predicted amyloid-prone segments within them are buried within the structures of their host proteins, and that those that are exposed are too twisted and inflexible to zip up with partner segments[1]. "It seems that most proteins have evolved to fold in a way that effectively conceals their amyloid-prone segments," says Eisenberg. So it may have been unnecessary for evolution to get rid of the segments outright.

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Wear and tear

Yet all these safeguards amount to a defence line that will inevitably be breached. Some mutations and toxins, and the cellular wear and tear associated with ageing, can result in proteins that are less well folded and less protected by chaperoning and disposal mechanisms — and thus more liable to become amyloids. "The 40 or 50 amyloid-associated diseases we've found so far are probably only the ones in which our proteins are the most vulnerable," says Dobson. "If we were to live longer, we might have to contend with more of these conditions."

By the same token, even a subtle hindrance of amyloidogenesis with drugs might have a major effect on disease and even on ageing in general. "If we could just enhance the natural protective mechanisms that stabilize a protein," says Dobson, "we could take it back over to the side of the line where it's soluble and stable."

Amyloids may not be the prime causes of all the diseases in which they have been found, but, typically, some by-product of the amyloid process is suspected. In Alzheimer's disease, many scientists now believe that small and still-soluble forms of amyloid are the most toxic to brain cells. By contrast, the larger, insoluble fibrils "might even be protective to the extent that they sequester more toxic forms", says Dobson. The general hope is that by preventing or slowing the initial cascade of amyloid formation, the true 'toxic species' of amyloid will be stopped at its source.

One anti-amyloid strategy is to use small molecules as extra chaperones to lower the probability that a protein will expose its amyloidogenic segments. FoldRx, a biotech company based in Cambridge, Massachusetts and founded by Kelly and Susan Lindquist of the Massachusetts Institute of Technology in Cambridge, recently demonstrated this principle in a clinical trial against familial amyloid polyneuropathy, a fatal neurodegenerative disease.

Eisenberg says that this strategy is unlikely to work well against most amyloid-prone disease proteins, such as amyloid-β, because they are typically too small to stay tightly folded. "For those I think there would be no hope of stabilizing the native structure, because they don't have one," he says. Instead, his group is trying to develop compounds to 'cap' the steric zippers of amyloid fibrils, slowing down their formation in the hope that innate clearance mechanisms can then keep up.

A third strategy is to boost the activity of these clearance mechanisms — which, according to work by Kelly's lab, includes enzymes that specifically disaggregate amyloids[7]. "There's a group of 500–600 genes that protect us when we're young, even if we've been so unlucky as to inherit, for example, a predisposing Parkinson's or Alzheimer's mutation," he says. Finding ways to rejuvenate that system "is what almost our whole lab is working on these days," says Kelly. ■

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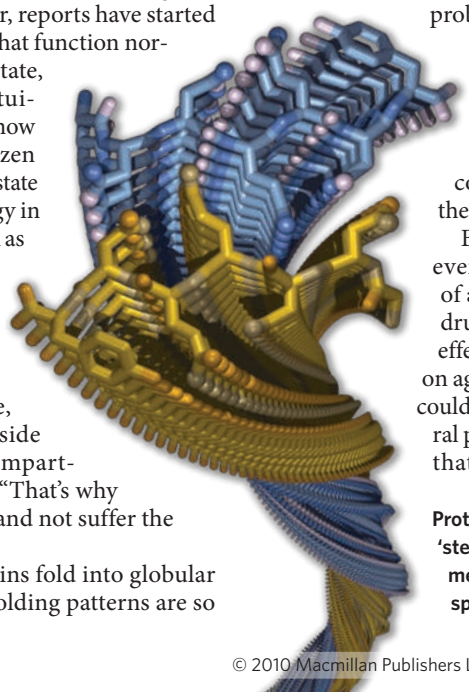

M. R. SAWAYA

Protein segments with a 'steric zipper' structure mesh tightly to form the spine of amyloid fibrils.

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