Misfolding the Way to Disease

Amyloid diseases such as Alzheimer's may result when proteins fold up incorrectly, causing them to aggregate and deposit abnormally in or around cells

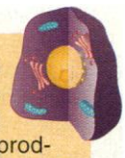
Anyone who likes to prowl through junk and antique stores knows that it's possible to find treasures among other people's discards. Now protein chemists are finding that the same thing can be true in their own line of work. Decades ago they noticed that proteins in solution have a dismaying tendency to form insoluble aggregates, hampering efforts to study them. To biochemists, these aggregates were "the gunk at the bottom of the test tube, the stuff everybody wanted to throw away," says Ron Wetzel, a protein chemist, formerly with SmithKline Beecham Pharmaceuticals. But now that gunk is beginning to take on new meaning.

Over the past few years, studies of how proteins fold have led researchers to realize that the way protein clumps form in the test tube is remarkably similar to how proteins form the so-called "amyloid" deposits that are the pathological hallmarks of some dozen different diseases—the best known of which is the common memory disorder Alzheimer's. Although every protein is made as a long string of amino acids, these linear molecules normally fold up into specific three-dimensional shapes, determined by their amino acid sequences.

But the new work suggests that both test tube protein aggregation and amyloid diseases result when normal protein folding goes awry, allowing incompletely folded protein molecules to grab onto each other and self-assemble into insoluble fibril aggregates. In the lab, those aggregates are mainly nuisances, but in living tissue, their buildup, which can be promoted by mutations that either alter how the protein folds initially or destabilize its final structure, can eventually prove fatal.

This new understanding also points to possible strategies for treating amyloid diseases. Over the years, researchers have learned how to inhibit protein aggregation in the test tube, and the same techniques might be put to use in developing therapies for amyloid diseases, including, conceivably, Alzheimer's. The protein aggregates, says protein biochemist Jonathan King, of the Massachusetts Institute of Technology (MIT), "are not some irrelevant state of polypeptide [protein] chains, not an accident of a graduate student's technique, but the beginning of understanding and treatment of these diseases."

One source of this new respect for protein aggregates has come, somewhat surprisingly, not from the protein chemists or the amyloid disease specialists themselves, but from the biotech industry. Since the 1960s, protein chemists have held a fundamental tenet: the idea that one sequence means one structure. Indeed, the late Christian Anfinsen won the 1972 Nobel Prize in chemistry for his 1960 work at the National Institutes of Health (NIH) indicating that the final three-dimensional form a protein takes in solution is determined solely by its amino acid sequence.



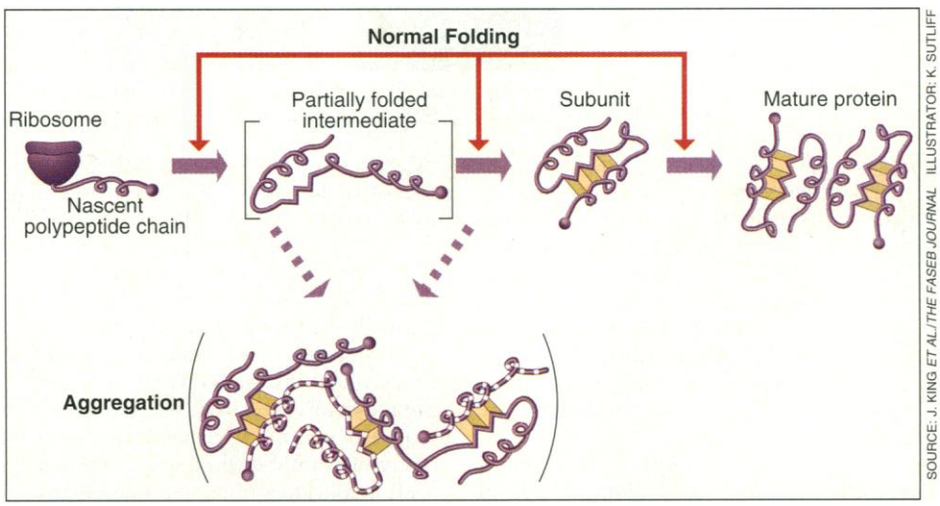
New genes often seem to steal all the glory in biology these days, but researchers haven't been neglecting the ultimate products of most genes: the cell's proteins. One area of rapid progress is the study of how proteins fold and move to their final destinations inside or outside the cell—a topic known as protein kinesis. This special issue on the subject includes this News report on how protein folding can go wrong, plus an Editorial on page 1477 and five Articles starting on page 1513.

But in the late 1970s, researchers using recombinant DNA techniques to grow valuable proteins, such as human growth factor or insulin, in bacteria found that getting proteins to fold properly in this new environment is just not that simple. Often, instead of folding into the normal structure, the foreign proteins made in bacterial cells clumped together into insoluble "inclusion bodies"—aggregates of protein molecules tangled in an amorphous and apparently hopelessly confusing mass. As King puts it, "They end up forming something like scrambled eggs inside the bacterial cell." And as proteins don't have their normal activities unless they are folded correctly, biotech workers wanted to find out just why they formed inclusion bodies in hopes that the clumping could be prevented.

Pointing the way

Although unnoticed at the time, a few clues to what was happening already existed in the literature. In the 1970s, for instance, Michel Goldberg of the Pasteur Institute in Paris was studying a protein known as chymotrypsinogen, which is the inactive precursor form of the common protein-digesting enzyme chymotrypsin. Goldberg and his collaborators wanted to find out how chymotrypsinogen refolds after they induced it to unwind from its natural three-dimensional structure—or "denatured" it, in the protein chemists' jargon. But instead of folding properly, Goldberg says, the denatured chymotrypsinogen molecules often aggregated into clumps.

Further studies indicated the protein did in fact begin to refold properly, but if the partially folded intermediates came into contact, they stuck to one another rather than



Fold them or hold them. If a partially folded protein is out of its normal milieu, or has a mutation, it may form self-aggregates rather than a correctly folded product.

finishing the folding process. Goldberg and his colleagues found, for instance, that they could increase the yield of properly folded protein simply by lowering the concentration of the protein in solution, thereby decreasing the chances that partially folded molecules would come into contact. "This suggested to us," says Goldberg, "that the formation of aggregates was ... not the result of the formation of badly folded protein." And the Goldberg team also provided an early indication that protein aggregation is specific. Their experiments with another protein, the bacterial enzyme tryptophanase, showed that the partially unfolded enzyme associates only with itself, not with other proteins.

But while Goldberg and his collaborators published two papers on their findings, one in 1974 and another in 1978, "nobody paid any attention," he recalls. Within the past several years, however, researchers have begun building on Goldberg's work. One key line of recent work suggests that the specificity of protein aggregation depends on interactions between particular amino acids in the protein molecules.

Some of this evidence came from experiments in which researchers showed that even very small changes in a protein's amino acid sequence could either foster aggregation or prevent it. In 1981, for instance, while studying the assembly of the coat proteins of bacterial viruses, King and his colleagues at MIT discovered temperature-sensitive folding mutations. A viral coat protein with one of these mutations, which changes only a single amino acid, was perfectly folded at lower temperatures. But at higher temperatures, which cause proteins to unwind, the mutant proteins, but not the normal ones, self-assembled into aggregates. This was strong evidence, says King, that aggregation can be preprogrammed in the protein sequence, just as proper folding is, and that specific sections of the coat protein's amino acid chain must be interacting to form the aggregates.

And in the mid-1980s, David Brems and his colleagues at Upjohn Corp. in Kalamazoo, Michigan, found that equally small changes can also protect against aggregation. In test tube studies, they showed that replacing a single amino acid in bovine growth hormone completely prevented its aggregation without affecting its correct folding. Results such as these showed, says MIT biochemist Peter Lansbury, that protein aggregation is not "just a nonspecific mess," as people once thought.

At the same time, the realization that these interactions can take place during normal protein folding also tied in with work indicating that cells have evolved machinery to help prevent newly synthesized protein molecules from sticking to one another before they attain their final three-dimensional structures. Work over the past several years has shown, for example, that cells contain proteins called "chaperonins" that bind to newly formed protein chains and help guide them through the folding process. Chaperonins "prevent the folding intermediates from getting into this state from which there's no recovery," says King. "But when some mammalian proteins are made in bacterial cells, their folding intermediates apparently clump because bacteria don't have the right chaperonins to protect them."

The amyloid connection

While Brems, King, and others were demonstrating the specificity of the protein aggregation routes, other researchers, including Wetzel at SmithKline and Jeffery Kelly at Texas A&M University, began applying the new information to try to understand what causes the formation of the abnormal protein deposits in amyloid disease. "It seemed unusual that people weren't considering amyloid diseases in this context," says Kelly, "since amyloid fibrils seemed to be composed of protein, clearly in a different conformation [three-dimensional structure] than the normally soluble and functional form."

Tracing the folding pathways. From left to right are protein chemists Jeffery Kelly, Ron Wetzel, and Jonathan King.

Part of the problem may have been that the insoluble deposits couldn't be studied by ordinary protein analytic techniques, which require that a protein either be in solution or in crystal form. So while specialists in amyloid diseases knew that mutations can predispose to amyloid deposition, they knew little about how those alterations bring about the protein aggregation.

And as King points out, what the neurobiologists and others studying the amyloid diseases read in the literature was unlikely to help them much, given that the aggregates were considered until relatively recently to be—at best—worthless junk, and papers that said otherwise were too few in number to be noticed readily by researchers who were not specialists in protein chemistry. "The average neurologist who got interested in proteins, studying amyloid or Alzheimer's filaments, would start reading about protein folding, but would get only the orthodox view of protein folding that there were no aggregate states," King says.

For his experiments, Kelly chose to work with one of the best studied amyloid-forming proteins, transthyretin. When altered by any of 50 different mutations, this protein, which normally occurs in the blood plasma, deposits in the heart, lungs, and gut, causing a lethal disease called familial amyloidotic polyneuropathy (FAP) whose victims eventually die from organ dysfunction. As shown in recent x-ray crystallography studies by two teams, one led by Colin Blake of Cambridge University in the United Kingdom and the other by Jean Hamilton of Indiana University School of Medicine in Indianapolis, these mutations don't alter the normal folding of the protein. But Kelly's work indicates that they do destabilize the protein's structure, facilitating the formation of partially folded intermediates that readily aggregate with one another.

As Kelly describes it, the original protein is a tetramer, containing four identical protein chains. The Texas A&M team found that in the test tube, these monomers separate from one another in acid conditions. They then partially unfold, exposing certain amino acid residues, which are normally buried deep in the protein but are now free to bind to similar chains on other monomers, forming aggregates that look very much like transthyretin amyloid deposits.

The transthyretin mutations lead to amyloid formation by promoting this disassociation and unfolding, Kelly says. He and his team found that proteins with the mutations disassociate much more readily into the single chains having the partially unfolded structure than do the normal version. "And then," Kelly says, "that guy [the monomer] self-assembles into amyloid."

Something similar also happens in the two amyloid diseases that Wetzel studies, known as light chain–related amyloidosis and light chain deposition disease. Both involve the lighter of the two proteins that form antibodies, and Wetzel and his colleagues showed that the mutations causing these diseases, which again alter just one amino acid in the protein, destabilize its folded structure and thus make it more susceptible to the structural rearrangement required for aggregation.

While the light chain diseases and FAP are relatively rare diseases—affecting one in every 10,000 to 100,000 people—there's evidence that aggregation of partially folded

proteins may also be at work in the most common amyloid disorder: Alzheimer's disease, which afflicts some 4 million people in the United States alone. The route leading to formation of the Alzheimer's deposits may be somewhat different from that in FAP, however.

The brains of people with the memory disorder are studded with abnormal structures called plaques, which have cores of amyloid protein deposits. Neurobiologists have argued for years about whether the deposits, which consist mostly of fibrils of a small protein called β amyloid, cause the neuronal degeneration of Alzheimer's or are merely a secondary result of the nerve cell breakdown. But one line of evidence suggesting that β amyloid deposition might be primary came about 5 years ago.

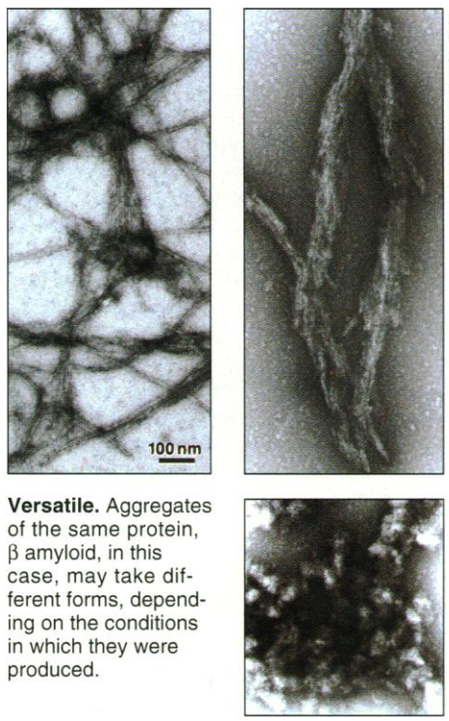
β Amyloid, which contains about 40 amino acids, has to be cut out of a much larger protein known as the amyloid precursor protein (APP). And in 1991, a research team led by neurogeneticist John Hardy, who was then at St. Mary's Medical School in London, found that a hereditary form of early-onset Alzheimer's is caused by mutation in APP. But while that pointed to the protein's importance for the development of Alzheimer's, it didn't reveal what it might do to cause the brain degeneration.

Since then, however, Dennis Selkoe of Harvard Medical School and Stephen Younkin of the Mayo Clinic in Jacksonville, Florida, have shown that APP is cleaved to form β amyloid in everyone, not just people with Alzheimer's. Moreover, the mutation identified by Hardy, as well as other, more recently identified APP mutations, serve to increase β amyloid production. Selkoe suggests that this excess β amyloid production may lead to fibril formation because having higher concentrations of the protein will increase the likelihood that any partially folded intermediates will be able to grab onto each other and aggregate, just as high chymotrypsinogen concentrations facilitated clumping of that protein in Goldberg's experiments.

At the root of prion diseases?

Abnormal protein folding may even account for a group of mysterious infectious diseases, which include scrapie in sheep, mad cow disease, and Creutzfeldt-Jakob disease in humans. For years, virologists thought that these conditions, which are all characterized by amyloid deposition in the brain and a resulting dementia, were caused by slow-acting viruses. They were unable to identify a viral pathogen, however, and many researchers have come to accept an explanation advanced by Stanley Prusiner of the University of California, San Francisco (UCSF). This holds that the infectious particles, which Prusiner named prions, are not viruses, but purely proteins, lacking any nucleic acid.

What makes researchers speculate that the protein aggregation paradigm holds for prions is the fact that prion proteins apparently come in two forms, one that folds normally and another that aggregates into amyloidlike deposits in the brain. Researchers have speculated that when this abnormal protein, which in some cases is produced by a mutated prion protein gene, comes in contact with normal prion proteins, it can induce them into the abnormal form as well. That could explain how injecting the abnormal protein into the brain could induce amyloid deposits and brain degeneration in susceptible animals. "There's a normal conformation, the one you and I have," says UCSF biochemist Fred Cohen, who works with Prusiner, "and an abnormal [one], which has a tendency to aggregate and create problems."



Versatile. Aggregates of the same protein, β amyloid, in this case, may take different forms, depending on the conditions in which they were produced.

But so far the prion hypothesis has run aground on one major obstacle: There are different strains of the agents that faithfully cause the disease, with somewhat different characteristics. Some, for example, have much longer incubation times than others. These strains breed true from generation to generation and animal to animal, an ability that's hard to explain if the infectious agents don't have nucleic acid genomes that could maintain and transmit their genetic characteristics.

Prusiner and other researchers have suggested that the strain differences could have something to do with different conformations of the prion proteins, and last summer, Lansbury and Byron Caughey of NIH's Rocky Mountain Laboratories in Hamilton, Montana, provided evidence in favor of that idea. They took purified prion proteins from the brains of hamsters infected with either of two different prion strains and then mixed them with the normal healthy prion protein in a test tube. An enzymatic assay indicated that the normal protein acquired the three-dimensional structure of whichever abnormal protein it had contacted. "We were astonished to see that there seemed to be a very faithful strain-specific conversion reaction in the cell-free system," Caughey says. That indicates that strain characteristics could be faithfully propagated just by the proteins and might therefore help remove a major obstacle to the prion theory.

Given the growing evidence that abnormal protein folding leads to amyloid diseases, researchers have begun to turn their attention to devising potential treatments that work by preventing protein misfolding. Indeed, showing that such treatments work would be "the ultimate judgment" that β amyloid deposition causes Alzheimer's, says Selkoe, who favors the idea. As he points out, "If one can inhibit amyloid production and deposition and change the symptoms of disease, then people will say the data are overwhelming."

The obvious approach to preventing protein misfolding, says Kelly, is to design a small molecule or protein that will bind specifically to the intermediates that make amyloid and prevent them from aggregating. Indeed, biotech firms and other companies are trying to devise just such drugs, but aren't making the results public yet for proprietary reasons. "The bulk of that work," Kelly says, "is tied up in the patent literature right now. Companies are definitely pursuing it, but they haven't been publishing much." If the approach should work, however, then the protein chemists and neurologists would have found that the junk at the bottom of the test tube contains riches, after all.

–Gary Taubes

Additional Reading

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