



NEUROSCIENCE

SEEDS OF DEMENTIA

A chain reaction of toxic proteins may help explain Alzheimer's, Parkinson's and other killers—an insight that could lead to desperately needed new treatment options

By Lary C. Walker and Mathias Jucker

UNDER A MICROSCOPE, A PATHOLOGIST SEARCHING through the damaged nerve cells in a brain tissue sample from a patient who has died of Alzheimer's disease can make out strange clumps of material. They consist of proteins that clearly do not belong there. Where did they come from, and why are there so many of them? And most important, what do they have to do with this devastating and incurable disorder? The search for answers has

turned up a startling discovery: the clumped proteins in Alzheimer's and other major neurodegenerative diseases behave very much like prions, the toxic proteins that destroy the brain in mad cow disease.

Prions are misshapen yet durable versions of proteins normally present in nerve cells that cause like proteins to misfold and clump together, starting a chain reaction that eventually consumes entire brain regions. In the past 10 years scientists have learned that such a process may be at work not only in mad cow and other exotic diseases but also in major neurodegenerative disorders, including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis (also known as ALS or Lou Gehrig's disease) and the concussion-related dementia of football players and boxers.

IN BRIEF

A Nobel Prize-winning discovery found that mad cow and related infectious diseases occur when aberrant proteins—prions—wreak havoc by causing normal versions of those proteins to become malformed.

Prionlike disease processes also appear to be at work in major neurodegenerative disorders, including Alzheimer's, Parkinson's and Lou Gehrig's, although they are not transmitted from person to person.

How proteins contort into a form that causes others to undergo a similar transformation may lead to new approaches to preventing and treating some of the world's leading neurological illnesses.

Alzheimer's and Parkinson's, all evidence suggests, are not contagious like mad cow or, for that matter, the flu. Rather the significance of these recent findings is that they provide scientists with a prime suspect for a slew of devastating brain disorders—a signpost that points toward a pathway for eventual treatments. Drugs developed for Alzheimer's might be used directly—or else inspire new pharmacology—for Parkinson's, traumatic brain injury or other terrifying conditions that rob an individual of a basic sense of self—good news for tens of millions worldwide who suffer from neurodegenerative disorders.

The new thinking owes a debt to research that first led to the discovery of prions. It began in the early 18th century, with reports of a curious, fatal disease of sheep called scrapie, so named because affected animals compulsively rubbed the wool from their skin. Later, as scientists began to investigate the disease, they noticed under the microscope that the nervous system was shot through with holes. In the 1930s French and British researchers determined that scrapie could be transmitted from one sheep to another, but the infectious agent was elusive and behaved strangely: the incubation time between exposure and symptoms was much longer than for conventional disease-causing agents such as bacteria or viruses, and the immune response that usually kicks in to eliminate such invaders seemed to be absent.

Those oddities were a hint that the usual suspects were not the cause, but for about 20 years after these reports scrapie remained just an obscure veterinary malady. In the 1950s, though, William Hadlow, then at the British Agricultural Research Council Field Station at Compton, noted conspicuous similarities in brain pathology between scrapie and a mystifying human disease called kuru. Kuru is a progressive neurodegenerative disease, mainly confined to the Fore people of Papua New Guinea, in which a steady decline in coordination and mental function invariably ends in death. The disease among the Fore was ultimately found to result from the ritual cannibalism of tribe members who had died of the disease, which implied that some infectious agent was at fault and somehow reached the brain from elsewhere in the body.

In the 1960s D. Carleton Gajdusek of the U.S. National Institutes of Health and his colleagues confirmed that the disease was transmissible, showing that kuru could be conveyed by the direct injection of brain material from victims of the disease into the brains of nonhuman primates. Gajdusek's team also recognized key parallels in brain pathology between kuru and another neurodegenerative brain disorder: Creutzfeldt-Jakob disease (CJD), a rapidly progressive type of dementia that occurs in roughly one in a million people worldwide. Gajdusek went on to demonstrate that CJD is transmissible to primates in the same way as kuru, although CJD most often arises in people spontaneously.

In the 1980s Stanley B. Prusiner of the University of California, San Francisco, identified the agent responsible for scrapie and related disorders, which are known collectively as spongiform encephalopathies for the way they cause the brain to take on the appearance of Swiss cheese [see “The Prion Diseases,” by Stanley B. Prusiner; *SCIENTIFIC AMERICAN*, January 1995]. In a beautiful series of experiments, he and his co-workers amassed persuasive evidence that the infectious agent consists solely of a misfolded version of an otherwise innocuous protein called PrP. Prusiner also coined the term “prion” (pronounced “pree-on”) at that time, for “proteinaceous infectious particle,” to distinguish protein

Lary C. Walker is a research professor at the Yerkes National Primate Research Center and associate professor of neurology at Emory University.

Mathias Jucker is a research professor at the Hertie Institute for Clinical Brain Research at the University of Tübingen in Germany and at the German Center for Neurodegenerative Diseases, also in Tübingen. The authors have collaborated extensively over the past two decades on research into brain aging and Alzheimer's disease.



agents that spread disease on their own from viruses, bacteria, fungi and other known pathogens. (Today the term is expanding to include other proteins that impose their shape on like proteins and does not necessarily imply infectiousness.) Prusiner's ideas sparked a huge controversy when he proposed that a protein could transmit disease, but in 1997 his efforts were rewarded when he won the Nobel Prize for this work.

Recently in-depth research into Alzheimer's and other neurodegenerative conditions indicates that these disorders, though lacking the infectiousness of classic prion diseases, may arise and amplify in the brain in a similar way; that is, by a process we call pathogenic protein seeding. Like the prions responsible for scrapie and its kin, the proteinaceous seeds can be released, taken up and transported by cells, which may explain how disease spreads from one place to another. These commonalities suggest that the prion paradigm could soon unify our thinking about how seemingly diverse diseases arise and wreak havoc.

IS MISFOLDING BEHIND ALZHEIMER'S?

THE FIRST HINT of this connection came as far back as the 1960s, when researchers struggling to grasp the mysteries of prion diseases began to notice some suggestive similarities to the brain changes that occur in other neurodegenerative disorders, especially Alzheimer's. The most common cause of dementia in aging humans, Alzheimer's appears stealthily and progresses relentlessly over the course of many years, robbing the victim of memory, personality and, ultimately, life itself. The incidence of Alzheimer's doubles every five years after the age of 65 until, by 85 years, nearly one in three adults is afflicted.

Researchers of the time also understood that protein clumping was involved. In 1906 Alois Alzheimer, after whom the disease is named, associated dementia with two peculiar microscopic abnormalities in the brain: senile plaques (now known to be clumps of a misfolded protein fragment named amyloid-beta, or Aβ), located outside of cells, and neurofibrillary tangles (filaments composed of aggregations of a protein called tau), located inside the cell. When these clumps are highly magnified with an electron microscope, the proteins can be seen to form long fibers made up of Aβ or tau. In addition, the proteins form smaller assemblies known as oligomers and protofibrils that can also interfere with the normal function of neurons.

In the late 1960s Gajdusek's team set out to test the hypothesis that Alzheimer's, like scrapie, kuru and Creutzfeldt-Jakob, might be transmissible—so they injected processed brain matter from Alzheimer's patients into the brains of nonhuman primates. Independently, a team led by Rosalind Ridley and Harry Baker, then at the Clinical Research Center in Harrow, England, later under-

A Molecular Forced March That Destroys the Brain

Proteins that contort into aberrant shapes—and then initiate a chain reaction that causes other proteins to do the same—underlie a number of neurodegenerative diseases, including Alzheimer's. In Alzheimer's, a misfolded A β protein acts as a "seed" instigating a process that eventually leads to both small and large clumps of proteins that damage and ultimately kill nerve cells.

Domino Effect of Misfolding Proteins

A β can fold into a misshapen form that causes nearby A β molecules to assume the wrong shape and to also clump together. Proteins may later break off from the aggregate and seed the beginnings of the same process elsewhere.

Seeds

Small A β aggregates (oligomers and protofibrils)

Synapse

Damage to Neurons

Small aggregates of A β , called oligomers and protofibrils, occupy the connection points, or synapses, between brain cells and may disrupt transmission of chemical signals between neurons. Senile plaques, larger A β aggregates, surround cells, causing additional damage.

Neuron

Plaque

Swollen nerve cell extension

Spread through the Brain

The inexorable progression of A β deposits engulfs most areas of the cerebral cortex, the brain's outer layer (left), before moving to other brain regions (center) and finally reaching the lower brain stem and cerebellum in the organ's deepest reaches (right).

SOURCE: "PHASES OF A β DEPOSITION IN THE HUMAN BRAIN AND ITS RELEVANCE FOR THE DEVELOPMENT OF AD," BY DIETMAR R. THAL ET AL., IN *NEUROLOGY*, VOL. 58, NO. 12, JUNE 25, 2002 (brain series)

took similar experiments. The results of the Gajdusek studies were indeterminate, and neither group reported that it had triggered fully developed Alzheimer's. The British researchers, however, found a hint of an effect: after an incubation period of at least five years, A β plaques were more abundant in marmosets that had received the processed Alzheimer's brain matter than in a comparison group of control marmosets.

At this point, our research groups considered initiating studies to see if misfolded A β —in the form of small aggregates—acted as a seed that set off a chain reaction of protein misfolding and clumping that eventually led to the type of protein deposits that overwhelm the brain in Alzheimer's. But we were discouraged by the five years or so it took to incubate seeded plaque formation in monkeys.

Our outlook changed considerably in the mid-1990s with the advent of "transgenic" mice that were genetically engineered to produce the precursor protein from which the human A β fragment is generated—APP (for amyloid precursor protein). Together with a talented group of colleagues and students, we began a series of experiments exploring the A β -seed hypothesis in these mice. The transgenic animals do not embody all features of Alzheimer's (which appears to be unique to humans), but they offer considerable advantages for our experiments: they are small, easy to maintain and short-lived, and each transgenic mouse spontaneously develops A β brain deposits at a relatively consistent age.

In our studies, we concentrated on A β rather than tau because even though plaques and tangles both contribute to the neurodegeneration that causes dementia in Alzheimer's, much of the evidence implies that misfolded A β is a key catalyst for the disease's development. Indeed, many of the risk factors for Alzheimer's influence cellular processes involved with the production, folding, aggregation or removal of A β . Genetic mutations that cause disease onset at a very early age alter APP or the enzymes that splice A β from that precursor [see "Shutting Down Alzheimer's," by Michael S. Wolfe; *SCIENTIFIC AMERICAN*, May 2006]. Scientists also now know that the brain begins to show signs of Alzheimer's a decade or more before the symptoms appear—and that the abnormal clumping of proteins occurs very early in the disease process [see "Alzheimer's: Forestalling the Darkness," by Gary Stix; *SCIENTIFIC AMERICAN*, June 2010]. Aware that the accumulation of misfolded A β is pivotal to the development of Alzheimer's, we wanted to know what first spurs protein aggregation in the brain.

During our first experiments, we set out to determine whether extracts of brain tissue from patients who had died of Alzheimer's would initiate A β aggregation in the brains of APP-transgenic mice. In other words, could we induce and propagate A β aggregation in the same way that prions trigger PrP aggregation in the spongiform encephalopathies? Using methods developed for the study of those prions, we first took small brain samples from Alzheimer's patients or from control patients who had died of causes other than Alzheimer's. We ground up the tissue and spun the samples briefly in a centri-

fuge to remove the larger debris. Then we injected a tiny amount of the extract into the brains of young transgenic mice.

The results were positive. Three to five months later, before the mice would normally start generating their own A β plaques, substantial aggregated A β appeared in the brains of mice that received the Alzheimer's brain extracts. The degree of A β plaque formation was proportional to the amount of A β in the donor brain extract and to how long it had to incubate—patterns you would expect to see if the extracts caused the plaques. Most crucially, donor brains lacking aggregated A β did not seed plaque formation in the transgenic mice.

DEFINING THE A β SEED

ALTHOUGH THESE EXPERIMENTS showed that A β deposition could be initiated by Alzheimer's brain extracts, they did not definitively indicate that A β in the extracts accounted for the plaques. That uncertainty compelled us to address several additional questions. First, we asked whether the A β deposits that we saw in the mice were merely the material that was injected. Here the answer was

no: one week later no evidence could be found of aggregated A β in the brain. Rather plaques became apparent only after a lag of a month or more.

Second, we considered the possibility that plaque formation was stimulated by some component of the human brain extract besides the A β , perhaps a human virus. We ruled out this prospect by confirming that brain extracts from aged but pathogen-free APP-transgenic mice can seed as effectively as human brain extracts, as long as the samples contain ample aggregated A β . In addition, because extracts from non-Alzheimer's brains did not cause A β

clumping, we could eliminate the possibility that the plaques were just a response to brain injury incurred during the process of delivering the extract.

Although the evidence now strongly pointed to A β as the culprit, we wanted more direct proof. Our third step was to selectively remove A β from the brain extracts using antibodies that specifically mop up A β . This simple procedure abolished the ability of the Alzheimer's brain samples to induce plaque formation. Finally, when we used a strong acid to make the misfolded proteins unfold, the brain extracts failed to induce plaque formation. We thus confirmed that the shape of the protein governs its ability to induce the misfolding and aggregation of other A β molecules.

We were now reasonably certain that misfolded A β was the active seeding agent in the brain samples, but a key piece of the puzzle remained elusive. If aggregated A β alone is the seed, it should be possible to induce plaques using A β that is synthesized and made to clump in a test tube in the absence of the many other substances in the brain. We knew that seeding with synthetic proteins might be challenging because studies with prions had shown that laboratory material can differ in subtle but apparently important ways from that taken directly from the brain.

With this caveat in mind, we injected various forms of synthetic, aggregated A β into APP-transgenic mice, then waited out the usual incubation period of three to five months. The results were

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disappointing; no obvious initiation of plaque formation was apparent in this time frame. Recently, however, Prusiner, Jan Stöhr, Kurt Giles and their collaborators at U.C.S.F. injected synthetic A β fibers into the brains of APP-transgenic mice. After a prolonged incubation period of more than six months, the mice showed clear evidence of seeded A β deposition in the brain. Although the synthetic seeds proved less potent than naturally generated A β seeds, the findings provide a persuasive demonstration that pure, aggregated A β alone, in the absence of other factors, is able to stimulate the formation of A β deposits in the brain.

In more recent experiments, we have begun to investigate the features of A β seeds that enable them to promote protein clumping in the brain. Because most of the A β protein in the seeding extracts is contained in the long, insoluble fibers, we expected that these fibers would be the most effective seeds. The results surprised us. By spinning the brain extracts at high speed in a centrifuge, we divided the A β -rich brain extracts into two components: an insoluble pellet containing mostly A β fibers at the bottom of the centrifuge tube and a clear liquid above the pellet containing very small, soluble forms of the A β protein. As anticipated, the vast majority of the A β settled into the pellet, which, when broken up and injected into the brains of transgenic mice, induced A β aggregation as effectively as did the whole-brain extract. Unexpectedly, though, the soluble portion also strongly induced A β aggregation and plaque formation, despite containing less than one one-thousandth as much A β as the pellet fraction. What is more, the soluble seeds were readily destroyed by an enzyme, proteinase K, whereas the insoluble seeds were not.

There is good news and bad news in the variable size and fragility of A β seeds. The bad news is that small, soluble assemblies, which can move through the brain with greater ease than the larger fibers, are particularly potent seeds. On the other hand, their sensitivity to proteinase K hints that soluble seeds might be especially amenable to treatments designed to eliminate them from the brain. Also, being soluble, the small seeds might be readily detectable in bodily fluids and so might serve as molecular sentinels for the early diagnosis of Alzheimer's, possibly well before the onset of dementia. Because protein seeding appears to begin at the very earliest stages of the disease, having a way to detect and neutralize those seeds could go a long way toward preventing brain damage and dementia.

BEYOND ALZHEIMER'S

NATURE SELDOM MISSES an opportunity to exploit a mechanism for multiple purposes, and seeded protein aggregation is no exception. It turns up not only in disease but also in beneficial processes. In the 1990s, for instance, Reed Wickner of the NIH proposed that some fungal proteins use this strategy to aid in cell survival, a postulate that now has been confirmed in numerous labs. Moreover, Susan Lindquist of the Massachusetts Institute of Technology and Eric R. Kandel of Columbia University have championed the intriguing hypothesis that the prionlike propagation of specific proteins helps to stabilize brain circuits, thereby acting to preserve long-term memories.

So far, however, the lion's share of the research points to a role for seeded protein aggregation in disease. Proteins whose seeded aggregation has been implicated in brain disorders include α -synuclein (in Parkinson's), superoxide dismutase-1 (in ALS), TDP-43 (in ALS and frontotemporal dementia), huntingtin

(in Huntington's disease) and tau (in a number of neurodegenerative diseases). Many other neurodegenerative diseases involve protein aggregation, and it will be important to see whether the seeding principle applies to these as well.

In a new development, investigators have discovered that some proteins involved in the regulation of gene function include a prionlike domain—that is, a stretch of amino acids that enables a protein to induce its same structure in like molecules. By their nature, these proteins tend to aggregate, a proclivity that can be augmented by certain mutations. A research team led by J. Paul Taylor of St. Jude Children's Research Hospital in Memphis and James Shorter of the University of Pennsylvania has reported that mutations in the prionlike domains of nucleic acid-binding proteins called hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy, a complex malady affecting the nervous system, muscle and bone. Moreover, seeded aggregation has been demonstrated experimentally for other proteins that cause conditions outside the nervous system, such as certain amyloidoses—and the spectrum of disorders involving prionlike propagation of proteins may continue to grow.

If therapies are to emerge from our growing understanding of the seeding concept, we must establish how misfolded proteins injure cells and tissues; such information could help block damage even if halting unwanted protein aggregation itself proves difficult. Research shows that aggregated proteins can disable cells in many ways, ranging from toxic interactions of the aggregates with a cell's components to preventing normal proteins from reaching the sites where they usually function. At the same time, we must understand more fully how pathogenic proteins arise and break down and the conditions under which they misfold and form seeds. Further insights into the progression of disease will also certainly come from clarifying how cells take up, transport and release protein seeds. Finally, a critical open question is why growing old so strongly increases risk for neurodegenerative diseases. Answers to these questions could suggest new ways to defang pathogenic proteins.

The weight of evidence increasingly favors the once unorthodox notion that a simple change in shape can transform a protein from friend to foe. In his Nobel Prize lecture describing the discovery of prions, Prusiner predicted that the basic process by which prions involved in mad cow and related illnesses impose their toxic features on normal proteins would be found to operate in other degenerative diseases. The past decade has witnessed the experimental confirmation of this prediction. Indeed, prionlike seeded protein aggregation may explain the origin of some of the most feared diseases of old age—and provide a compelling conceptual framework that may one day translate into treatments that alter the relentless progression of neurodegenerative illnesses. ■

MORE TO EXPLORE

Pathogenic Protein Seeding in Alzheimer Disease and Other Neurodegenerative Disorders. Mathias Jucker and Lary C. Walker in *Annals of Neurology*, Vol. 70, No. 4, pages 532–540; October, 2011.

Prion-Like Spread of Protein Aggregates in Neurodegeneration. Magdalini Polymenidou and Don W. Cleveland in *Journal of Experimental Medicine*, Vol. 209, No. 5, pages 889–893; May 7, 2012.

SCIENTIFIC AMERICAN ONLINE

To watch a slide show of a cascading chain of toxic proteins, go to ScientificAmerican.com/may2013/prions