Left to right: Benjamin Wolozin, MD, PhD, professor of pharmacology and neurology; John Berk, MD, associate professor of medicine at BUSM and clinical director of the Amyloidosis Center; David Harris, MD, PhD, professor and chair of biochemistry; Carmela Abraham, PhD, professor of biochemistry. Not pictured: David Seldin, MD, PhD, professor of medicine and microbiology and director of the Amyloidosis Center.
THE MISFOLDING MYSTERY

A formidable team of BUSM researchers is delving into the science of some of the body’s most devastating hidden enemies.

BY MARY HOPKINS
Alzheimer’s disease (AD) is the sixth leading cause of death in the United States, with five million Americans currently afflicted. It costs the nation $203 billion a year, an amount expected to increase to $1.2 trillion by 2050. Parkinson’s disease (PD) afflicts as many as one million people, costing approximately $25 billion in treatment, disability, and lost income from work. While amyotrophic lateral sclerosis (ALS), prion diseases, and the various amyloidoses are rarer, the course of these degenerative diseases still has much to teach us. What they and other devastating and costly diseases such as cancer and diabetes have in common is that there is a growing understanding of the role that misfolded proteins plays in their occurrence.

Humans are perfect examples of biological self-assembly, and proteins are the building blocks of this formation. They make us who we are. They are the engines of vital functions such as growing and differentiating cells, catalyzing metabolism, stimulating immune responses, and building muscle. They function inside and outside of cells carrying signals between them or protecting them.

Proteins are chains of amino acids whose sequences are determined by our genes. What ordains a protein’s function is how the amino acid chain folds into a three-dimensional structure. A properly folded protein is able to carry out its intended function.

BUSM researchers are studying how this process goes awry, leading to the misfolding and aggregation of proteins that then accumulate in tissues, resulting in a variety of pathological conditions. Teams of basic science and clinical investigators focus on broadening our understanding of this biochemical malfunction, creating a knowledge base for developing therapeutics to prevent or treat a variety of degenerative diseases like Alzheimer’s, Parkinson’s, ALS, or amyloidosis.

“Cells have normal mechanisms to cope with misfolded proteins,” says David Harris, MD, PhD, professor and chair of biochemistry. “Perhaps one-third of the proteins that a cell makes misfold, but there are adaptive mechanisms that cells employ to degrade the misfolded proteins or to refold them in a proper way.”

Harris explains that sometimes those adaptive mechanisms are overwhelmed and misfolded proteins start accumulating in cells either inside the cell or outside of it, inhibiting the normal function of the cell or becoming toxic to it. Protein misfolding can be caused by genetic mutations and environmental influences. Age is a common predisposing factor in that as cells age, they may not be as capable of dealing with the stresses that cause proteins to misfold.

“Some of the most common degenerative diseases like Alzheimer’s or the rarer ones like prion diseases, both of which I study, are diseases that result from misfolding of proteins in the central nervous system,” he says. “This has therapeutic consequences since, if these diseases
share a common underlying mechanism, then the compounds used to treat the diseases can be used for more than one disease. Or, at least some of the strategies used to treat one disease may be applicable to others.”

Harris has been studying prion diseases—which are unusual due to their infectiousness—for 20 years. “The whole prion field has attracted the attention of scientists for a long time because of the nature of the infectious agent,” says Harris. “With bacteria and viruses we understand how they propagate and grow, but the infectious compound that causes prion diseases is not a bacterium or a virus. It is a naked protein molecule that is actually an altered version of a normal protein that sits on the surface of cells and has a normal function in the body. During the course of infection, the normal protein is continuously converted into a form with an abnormal shape in a self-perpetuating process akin to ice crystal formation.”

Harris researches how this abnormal protein damages neurons and what forms of it are the most pathogenic. Although prion diseases are rare, the prion protein exists throughout nature. “There is evidence that other neurodegenerative diseases like Alzheimer’s that are not infectious propagate through the central nervous system by a prion-like or self-templating mechanism,” he says. “The Aβ peptide involved in Alzheimer’s disease misfolds, causing other Aβ peptides to misfold, thereby producing a self-perpetuating effect. This doesn’t mean that Alzheimer’s disease can be transmitted from one person to another, but it does mean that the abnormal proteins that aggregate in the brain can spread along neuroanatomical pathways in a mechanism that is similar to what prions do.” Plaques, the abnormal aggregation of misfolded proteins that build up between nerve cells in the brain, are made of the Aβ peptide. This buildup is believed to inhibit signaling between cells, resulting in memory loss and the other intellectual and physical declines associated with Alzheimer’s disease.

Benjamin Wolozin, MD, PhD, professor of pharmacology and neurology who

“Cells have normal mechanisms to cope with misfolded proteins. It is estimated that perhaps one-third of the proteins that a cell makes misfold, but there are adaptive mechanisms that cells employ to degrade the misfolded proteins or to refold them in a proper way.”

DAVID HARRIS, MD, PhD, professor and chair of biochemistry
“The essential point of the process is that stress granules form based on the same type of protein aggregation that occurs in disease. This process is not random aggregation; it is controlled and reversible. Once the stress goes away, the stress granules disperse and protein synthesis returns to normal. So, that means that an intracellular function we thought was unchangeable is totally changeable.”

Benjamin Wolozin, MD, PhD, professor of pharmacology and neurology

studies Parkinson’s, Alzheimer’s, and ALS, realized a different model for looking at abnormal protein activity in neurodegenerative diseases.

“What I am working on in one sense fits in with what everyone else is doing, but in another sense sets out an entirely new paradigm for understanding what is going on,” Wolozin says. “Proteins allow you to be yourself. What happens when you are injured? Your body recognizes the problem and adapts by temporarily shifting the kinds of proteins it makes from the specialized proteins that give rise to the amazing variety of biological activities occurring in normal body function, to proteins to deal with the injury. This shift in the types of proteins produced is accomplished by a class of proteins termed RNA-binding proteins. These RNA-binding proteins normally reside in the nucleus, which in response to stress flood out into the cytoplasm, where they bind up RNA, forming RNA-protein complexes that are termed stress granules.

“The essential point of the process is that stress granules form based on the same type of protein aggregation that occurs in disease. This process is not random aggregation; it is controlled and reversible. Once the stress goes away, the stress granules disperse and protein synthesis returns to normal. This means that an intracellular function we thought was unchangeable is totally changeable.”

According to Wolozin, the misfolding and aggregation is in equilibrium. Normally, the aggregation of proteins to form stress granules rapidly reverses after the stress is removed, but with chronic disease, whether caused by genetic mutations as in ALS or with Alzheimer’s, the dispersal part of the process, for different reasons, does not occur. “What happens in Alzheimer's is a pathological protein, tau, mis-localizes and accumulates,” says Wolozin. “In its benign state, tau actually helps axons extend. Under stress it redistributes, in part to stimulate stress granule formation. The RNA-binding proteins bind with the misfolded tau protein, forming stress granules in the neurons. We have shown that presence of tau in neurons is okay, and transient coupling with RNA-binding proteins to form stress
“When we look at one neurodegenerative disease after another it is all the same story,” Wolozin adds. “There is a common pathway. You normally sequester the RNA-binding proteins into stress granules for a short period of time, and then they disperse. However, if you bang your head a lot, as in sports-related concussions, or when there is a genetic mutation or a chronic disease like diabetes that reduces blood flow to the brain, you have chronic stress to the brain that causes these protein aggregates to form, persist, and get bigger; these persistent, pathological stress granules disrupt nerve function. If they don’t disaggregate, pathology and disease result. We are working on developing methods to reverse the growth of stress granules, and in fact have identified a nontoxic compound that reverses a type of pathological stress granule (TDP-43) found in ALS.”

Wolozin’s discovery of a single system of protein aggregation for an array of neurodegenerative diseases has important implications for developing therapeutics for these conditions. “The idea that the body needs protein aggregation and there is one system built around this that chronic disease and aging hijack, causing dysfunction, opens up new avenues for basic science and translational research discovery.”

Carmela Abraham, PhD, professor of biochemistry, and members of her laboratory conduct both basic science and translational research; the former to determine the function of certain proteins in the brain and the latter to produce compounds that can be developed as drugs to treat Alzheimer’s and other neurodegenerative diseases.

“When we look at the brains of patients who died of Alzheimer’s disease, we can observe under the microscope two hallmarks of the disease—plaques made of the protein Aß peptide and tangles made of the protein called tau. As a result of misfolding, these proteins aggregate and precipitate in the brain, and when they precipitate in the brain they become toxic.”
Clockwise from top:
NIA AD Pathology:
Pathological pathways in Alzheimer’s disease. Beta-amyloid is generated from cleavage of amyloid precursor protein (APP) by beta- and gamma-secretases. Secretion and aggregation of beta-amyloid causes degeneration of neurons, which is associated with misfolding of microtubule-associated protein tau. Diagram provided by the National Institute on Aging.

NIA Plaque Formation:
Cleaved beta-amyloid forms toxic oligomers, which are secreted. These oligomers aggregate further to form neuritic plaques, which can be observed pathologically. Diagram provided by the National Institute on Aging.

Plaques and Tangles:
Neuritic plaques and neurofibrillary tangles are pathological hallmarks of Alzheimer’s disease. Neuritic plaques accumulate outside of neurons, while neurofibrillary tangles accumulate inside neurons. Figure provided by Benjamin Wolozin.
Age-related neurodegeneration in Alzheimer’s and Parkinson’s diseases occurs in the autumn of life. The brain loses neurons just like the tree loses its leaves, but unlike the tree renewal we enjoy in the spring, nerve loss is irreversible.
Aβ peptide from forming. “We are working to develop compounds that prevent the formation of Aβ peptide that clogs the brains of people with Alzheimer’s.”

Abraham is also examining the role of the anti-aging protein Klotho in normal aging and disease. Her projects focus on identifying Klotho receptors in the brain and defining the signaling pathways by which Klotho exerts its protective effects on neurons and oligodendrocytes, the myelin-producing cells. She is also studying Klotho’s transcriptional regulation and has identified compounds to therapeutically exploit these protective effects.

“We found that Klotho expression is considerably decreased in the aged brains of monkeys, rats, and mice,” she says. “It is even more reduced in Alzheimer’s. The idea is to find out why it is reduced and how to bring it back to levels found at younger ages. In animal models, a higher level of Klotho resulted in healthier mice and an increased life span of up to 30 percent. This suggests to us Klotho is a protective protein that is good for all of us to have.”

Klotho circulates in the blood and cerebrospinal fluid and binds to an unknown receptor that Abraham is trying to find. “It initiates some biochemical pathways in the cell that make the cell protected from all sorts of insults,” she explains. “One of its protective properties is against oxidative stress, which is correlated to misfolded proteins.”

The Klotho research entailed high throughput screening of some 150,000 molecules to identify those that elevate Klotho to the levels determined to be protective. Abraham’s research with animal models has found that genetically engineered mice with Alzheimer’s who also have higher levels of Klotho do not have all of the symptoms of the disease, such as learning and memory deficits.

Her group also found that Klotho helps repair myelin, the insulating material around neuronal axons that allows for cell-to-cell communication. This is an important discovery for the potential treatment of multiple sclerosis in which an autoimmune attack destroys the myelin.

Abraham recently published a paper on genetic mutations that change the DNA of Klotho in some people, which has an effect on health and life span. People with two copies of the genetic mutation are adversely affected, whereas one copy actually has protective properties. “We are explaining the basis for this—which we believe may have something to do with protein folding and misfolding—but more work needs to be done to test this hypothesis,” she says.

Additionally, Klotho is reduced in all cancers. “We don’t study cancer, however, if we find molecules that are effective in increasing Klotho, we can look to produce drugs that may be effective in cancer treatment and prevention as well as in neurodegenerative disease,” she notes. “We will do this in collaboration with cancer researchers as we focus on what we know best, neuroscience.”

Amyloidosis is a term for diseases that have the extracellular deposition of insoluble fibrillar proteins in tissues and organs in common. Amyloids are protein aggregates created from misfolding of proteins and are associated with numerous diseases including Alzheimer’s, Parkinson’s, type 2 diabetes, prion diseases, and amyloidoses caused by a plethora of different proteins.

For more than 50 years, the BU Amyloidosis Center has been a world leader in the research and treatment of systemic types of amyloidosis in conjunction with Boston Medical Center, which has 600–700 amyloidosis patient visits each year. In addition, as an accredited diagnostic laboratory, the center receives samples from all over the world.

The most common form of amyloidosis in the US is AL or primary amyloidosis, which is an acquired plasma cell disorder where amyloid fibrils made up of immunoglobulin light chain proteins build up in organs of the body. Symptoms can occur in any organ of the body and include heart failure, protein in the urine or kidney failure, enlarged liver, neuropathy, or enlarged tongue.

Secondary, or AA amyloidosis, is caused by a chronic infection or an inflammatory disease such as tuberculosis, osteomyelitis, rheumatoid arthritis, familial Mediterranean fever, or inflammatory bowel disease. Infection or inflammation causes elevation of an acute phase protein, SAA, a portion of which deposits as amyloid fibrils. AA amyloidosis usually begins as disease in the kidneys, but other organs can also be affected. Medical or surgical treatment of the underlying chronic infection or inflammatory disease can slow down or stop the progression of this type of amyloid. A second clinical trial to evaluate a new treatment with a targeted inhibitor molecule, Kiacta, has just begun.

There are several types of inherited amyloidoses, the most common of which is caused by a mutation in the transthyretin (TTR) gene that produces abnormal transthyretin protein. The abnormal TTR protein deposits as amyloid fibrils. Symptoms of disease are usually neuropathy and cardiomyopathy and occur in mid-to late-life; untreated
patients die 10–15 years after disease onset. Treatment traditionally has been liver transplantation. In collaboration with George Murphy, PhD, of the BU Center for Regenerative Medicine and assistant professor of medicine, it has been possible to recapitulate this multi-organ disease. “George used the IPSC (induced pluripotent stem cell) technology to take cells derived from patients to make stem cells in the lab to generate cells similar to the patient’s liver cells that produce the misfolded proteins,” explains David Seldin, MD, PhD, professor of medicine and microbiology and director of the Amyloidosis Center. “He also was able to generate target tissue cells, neuronal and heart cells.”

In a recent study published in the *Journal of the American Medical Association* (JAMA) in December, led by John Berk, MD, associate professor of medicine at BUSM and clinical director of the Amyloidosis Center, researchers demonstrated that diflunisal, a generic anti-inflammatory drug, successfully reduced neurological decline and preserved the quality of life in patients with familial ATTR. Diflunisal is an inexpensive and safe medication marketed over the past 40 years for arthritis and pain.

“The proteins of origin for these degenerative diseases are very different, and the function or sequence of an antibody light chain or A-beta or TTR or prion protein doesn’t seem to be very similar, but the amyloid fibrils and structures they form are identical under the electron microscope, so there is something similar in the pathologic structure that the precursor proteins assume,” says Seldin. “In addition, the mechanisms of tissue damage are probably similar; that is an active area of research.”

Seldin stresses the importance of misfolded protein research. “You can view this body of research as the key to aging. If we could prevent proteins from misfolding in the brain, people might not lose their memory and develop dementia. In the rest of the body, we might keep organs functioning normally longer than they now do. Understanding and correcting misfolded proteins could be the key to helping people to live better and longer.”

“In one fell swoop, the findings from our NIH- and FDA-funded international trial provided the first highly effective, cheap, widely available, pill-based treatment to a population previously resigned to a slow death, while offering new purpose to an obsolete drug previously relegated to the shelf. The pill is by no means perfect, but in the world of lethal orphan diseases, we darn near hit a home run.”

JOHN BERK, MD, associate professor of medicine at BUSM and clinical director of the Amyloidosis Center