Proteins are the building blocks of life. Every cell, tissue, and organ in the body is made by and from proteins. Most of the time, they do their jobs with amazing reliability. But what happens when something goes wrong?

When Good Proteins Go Bad

BY SALLY POBOJEWSKI
Genes may get the glory, but in the day-to-day life of cells, it’s proteins that do most of the work. While genes remain safe and sheltered inside the cell’s nucleus, proteins are out there on the front lines doing everything it takes to keep cells alive, healthy and functioning normally.

There’s a lot of turnover in the protein business. Human cells must synthesize thousands of new proteins every minute to replace old ones that wear out and are recycled back into their component parts.

The first step in protein synthesis takes place in a part of the cell called the ribosome. Following genetic instructions, the ribosome selects specific amino acids from a pool of 20 possible variations and strings them together to make a long chain. Then, this chain of amino acids is folded into one specific three-dimensional shape.

For many proteins, folding takes place inside a cellular compartment called the endoplasmic reticulum. Other proteins are produced and folded at different locations within the cell. No matter where it takes place, the details of exactly how this feat of cellular origami occurs are still unclear. And, as with any complex process, there are lots of things that can go wrong along the way.

Proteins can be fussy. If they get too hot, they fall apart. If it’s too acidic, they stop working. They require helper molecules called chaperones to push and pull them into the correct shape. If their chaperone-of-choice isn’t available, they won’t fold.

A cell has several ways of dealing with a protein-folding problem. It can shut down protein production, giving it time to catch up with a folding backlog. It can make more chaperones to guide proteins through the folding process. It can digest unfolded proteins and recycle the component parts. But one way or another, the cell has to do something quickly because if nothing works, it’s going to die.

Folding errors can result in the absence of a protein that’s required for normal cell function, a misfolded protein that cannot do its job, or — most dangerous of all — a clump of sticky abnormal protein called amyloid. Regardless of the type of protein abnormality, the effect on the cell can be catastrophic. Improperly folded proteins are never a good thing, and aggregations of misfolded protein can be toxic.

“Protein folding is the most error-prone step in gene translation,” says Randal Kaufman, Ph.D., the Warner-Lambert/Parke-Davis Professor of Medicine who has studied protein folding since the 1980s. “The more difficult the protein is to fold, the greater the association with disease.”

Researchers are just beginning to understand the connections between abnormal protein folding and human disease. The growing list of what are now called protein misfolding diseases includes Alzheimer’s disease and other dementias, atherosclerosis, cancer, congenital hypothyroidism, cystic fibrosis, diabetes, fatty liver disease, hemophilia, polycystic kidney disease, Parkinson’s disease and retinitis pigmentosa.

Protein folding is an area of strong research focus at the U-M. Teams of scientists and clinicians from many disciplines are working together to study the effects of abnormal protein folding in different types of cells and different diseases. But sorting out all the factors involved is not easy.

It’s one thing to study protein folding in a test tube, but determining exactly how folding defects contribute to disease in a cell or a living organism is far more difficult, according to Kaufman. “It’s like looking at an automobile crash and trying to figure out what went wrong,” he says.
Death by Toxic Protein

Unlike many cells, neurons in the brain are not expendable. If you kill a muscle cell, the body just grows a new one. If you kill a neuron, it is not easily replaced and any memory stored in that neuron is gone forever. This makes the brain particularly vulnerable to the lethal effects of toxic proteins.

There are many types of neurodegenerative disease, each characterized by toxic deposits of a different abnormal protein in the brain. People with Alzheimer’s disease have accumulations of two different proteins — beta-amyloid outside neurons, and tau inside — the so-called plaques and tangles of Alzheimer’s. In Parkinson’s disease, a protein called alpha-synuclein forms aggregates called Lewy bodies. Huntington’s disease and eight other hereditary polyglutamine disorders each involve a different abnormal protein.

Regardless of the specific protein involved, the outcome is the same. Slowly and inexorably, neurons die and the brain is destroyed. It’s unclear whether deposits of toxic protein actually cause the disease or are just by-products of the disease process. There is still no definite answer to one basic question: What kills the neurons?

“The one commonality in all these neurodegenerative diseases is that they involve protein folding defects,” says Jason Gestwicki, Ph.D., a research assistant professor in pathology and in the U-M Life Sciences Institute, who studies proteins involved in neurodegenerative diseases. “We believe something is wrong with the protein quality-control machinery that should be preventing abnormal proteins from accumulating in neurons.” Gestwicki and his colleagues believe that understanding how cells deal with abnormal proteins could lead to new, more effective therapies for human neurodegenerative diseases.

Genetic mutations also are involved in neurodegenerative diseases, but they are more important in some diseases than others. “Huntington’s disease and the other polyglutamine disorders are always directly caused by a genetic mutation,” explains Paulson, “but less than five percent of Alzheimer’s disease is directly due to gene defects. The biggest risk factor for Alzheimer’s disease is aging. The risk goes up exponentially as we get older.”

Gestwicki believes that “drug-like small molecules” targeted at a specific component of the protein quality control process could be a solution for protein-folding diseases. One promising candidate is Hsp70, or heat shock protein 70. Found in every cell of every organism, Hsp70 is a chaperone molecule that helps cells deal with high temperatures, lack of nutrients or oxidative damage — conditions that make cells more likely to misfold proteins and form toxic protein deposits. One of the ways cells under stress protect themselves is to make more Hsp70.

Gestwicki has discovered several small molecules that increase the activity of Hsp70 in cells. He’s testing these molecules in animals and, if results are positive, hopes to obtain approval from the Food and Drug Administration to start a small clinical trial in elderly patients with dementia.

Gestwicki thinks a deficit of Hsp70 in neurons could explain why neurodegenerative diseases are much more common in older people. “When cells from young people are exposed to higher temperatures, they make more Hsp70,” he says. “But in the elderly, this protective mechanism doesn’t work well. Someone in their 80s makes only about half as much Hsp70. It’s part of a general aging-related suppression of the protein quality-control machinery that could explain the increased risk of neurodegenerative diseases with age.”
While Gestwicki’s lab focuses on finding therapies to boost the effectiveness of the cell’s quality-control machinery, Paulson’s research team is searching for ways to prevent production of abnormal protein in the first place. Currently, he’s exploring a technique called RNA-interference that uses short segments of genetic material to turn off genes associated with toxic protein aggregates in neurons. Paulson’s lab also studies chaperone proteins and how they interact with enzymes that tag abnormal proteins for degradation in cells.

“I believe in taking a broad view of potential therapies,” says Paulson. “We won’t know for years which of these experimental treatments will turn out to be an effective therapy, so it’s important not to put all our eggs in one basket.

“If you look at HIV treatment, it’s a cocktail of medications,” Paulson adds. “Cancer therapy, when it’s successful, usually involves a cocktail of medications. I believe the final powerful therapeutic entity for Alzheimer’s and other neurodegenerative diseases will involve a cocktail approach.”

Understanding what goes wrong during proinsulin folding in beta cells is crucial to preventing diabetes, says Arvan, the William K. and Delores S. Brehm Professor of Diabetes Research in the Division of Metabolism, Endocrinology & Diabetes (MEND). Just like brain neurons, the capacity of beta cells to regenerate is very limited. If a chunk of misfolded protein gets stuck in the endoplasmic reticulum and kills a beta cell, the cell and the insulin it produces are not easily replaced.

“The number of beta cells you have dictates your maximal insulin production capacity,” says Arvan. “Once you reach the point where you have only a few remaining beta cells, the game is over.”

There used to be a clear distinction between type 1 and type 2 diabetes, explains Arvan. Type 1 was believed to be an autoimmune disease where the immune system attacked and destroyed the pancreatic beta cells of children. Type 2 was considered a lifestyle disease of overweight, sedentary adults whose cells gradually lost the ability to respond to insulin’s signal. Now, these distinctions are “not quite as crisp” as they used to be, according to Arvan.

Not all juvenile onset diabetes is autoimmune in nature, explains Arvan. Neonatal diabetes that develops in the first six months of life is largely non-immune. One of the newly discovered causes is derived from mutations in the human gene for insulin. There are several other recently discovered

Saving the Beta Cell

Peter Arvan, M.D., Ph.D., and his colleagues in the Brehm Center for Diabetes Research believe research on protein folding could help them find a way to prevent diabetes — the insulin-deficiency disease that affects 24 million American children and adults.

Insulin is an essential protein that regulates how cells store and use glucose. Without enough insulin, blood sugar levels skyrocket causing extensive damage to cells and organs. Researchers know that diabetes is caused by the malfunction and death of specialized beta cells in the pancreas that produce insulin. But in spite of decades of research, there is still no definitive answer to the most basic question about diabetes: What kills the beta cell?

Pancreatic beta cells make insulin from a precursor molecule called proinsulin, which is folded into its proper shape in the beta cell’s endoplasmic reticulum. Arvan and his research team are part of a small-but-growing group of scientists who think the answer to the beta-cell murder mystery will be found in the endoplasmic reticulum and abnormal proteins that accumulate there.
genes that affect the performance of pancreatic beta cells and can predispose people to type 1 diabetes. “Just having the mutation does not guarantee that you will get the disease,” Arvan says, “but it greatly increases your risk.”

The situation is even murkier for type 2 diabetes, says Israel Hodish, M.D., Ph.D., an assistant professor in MEND who works with Arvan. “Many people are obese and have a sedentary lifestyle, but they don’t all develop diabetes,” he says. “Type 2 diabetes is one of the most prevalent diseases in the world and still we don’t know the basis of the disease.”

Now, thanks to a transgenic mouse that makes green insulin, Arvan’s team can see when beta cells stop working. Hodish created the mouse by introducing a human insulin gene called a transgene tagged with a green fluorescent protein marker. Using a special microscope, scientists can see the green glowing proinsulin protein within the beta cell’s endoplasmic reticulum and determine whether it continued the folding process required to become insulin.

Researchers found that when the human transgene was normal, the beta cell secreted normal insulin. But if the human transgene contained one of the mutations that cause misfolded proinsulin and neonatal diabetes in human infants, the beta cell showed signs of being in trouble.

“We could see that some of the beta cells were insulin-deficient,” says Hodish. “Some beta cells were sicker than others, because they accumulated more misfolded proinsulin inside the endoplasmic reticulum. The mouse still had several good copies of its insulin gene and some functioning beta cells, so it could make enough insulin to prevent diabetes, but for how long? This could be a basis for type 2 diabetes or contribute to why people develop type 2 later in life.”

“The question raised by this transgenic mouse is what percent of abnormal folding is required before you get sick?” asks Arvan. “We all misfold proteins all the time, so it cannot be the case that if one molecule is misfolded, we get diabetes. There must be some sort of threshold. The big question now is, what is the threshold? How much proinsulin has to be bad before you have problems?”

Arvan noted that all but one normal copy of the insulin gene could be knocked-out or removed in a mouse and that mouse still would not get diabetes. “When mice get diabetes, it’s not because they lost a good copy of the gene,” he says. “It’s because they inherited a bad copy with a mutation that is toxic to the beta cell.”

Ming Liu, M.D., Ph.D., assistant professor in MEND and part of Arvan’s Brehm Center research team, recently published research results demonstrating how this gain-of-toxic-function mutation kills beta cells. He calls it the bystander effect.

“We found that misfolded proinsulin attacks normal bystander proinsulin in the endoplasmic reticulum,” says Liu. “Then, the normal protein folds into a ‘mess’ that can contain a combination of misfolded proinsulin derived from both mutant and normal genes. Becoming part of this ‘mess’ blocks the bystander proinsulin within the endoplasmic reticulum and prevents the beta cell from producing insulin.”

Suddenly, the good protein becomes a bad protein. It propagates like a wave and more proteins get recruited. There are many toxic events associated with diabetes, but researchers believe this one can be a root cause of the disease.

The image of malevolent abnormal proteins attacking healthy proteins and dragging them into some sort of toxic conglomeration is unsettling, but not unique to beta cells and diabetes. The same thing happens in human neurological disorders like Creutzfeldt-Jakob disease, in which small infectious proteins called prions destroy neurons in the brain.

Could this toxic bystander effect be responsible for the death of neurons in Alzheimer’s, Parkinson’s and other neurodegenerative diseases? U-M scientists who study protein misfolding say it’s too soon to know for sure, but it shows why it’s important to continue research on the basic mechanisms involved in protein quality control.

“This is a fundamentally fascinating biological problem that involves a remarkably wide range of human diseases,” says Henry Paulson. “Research to identify the underlying mechanisms could lead us to new, entirely unexpected routes of therapy and help us understand important connections between diseases that previously were unclear.”