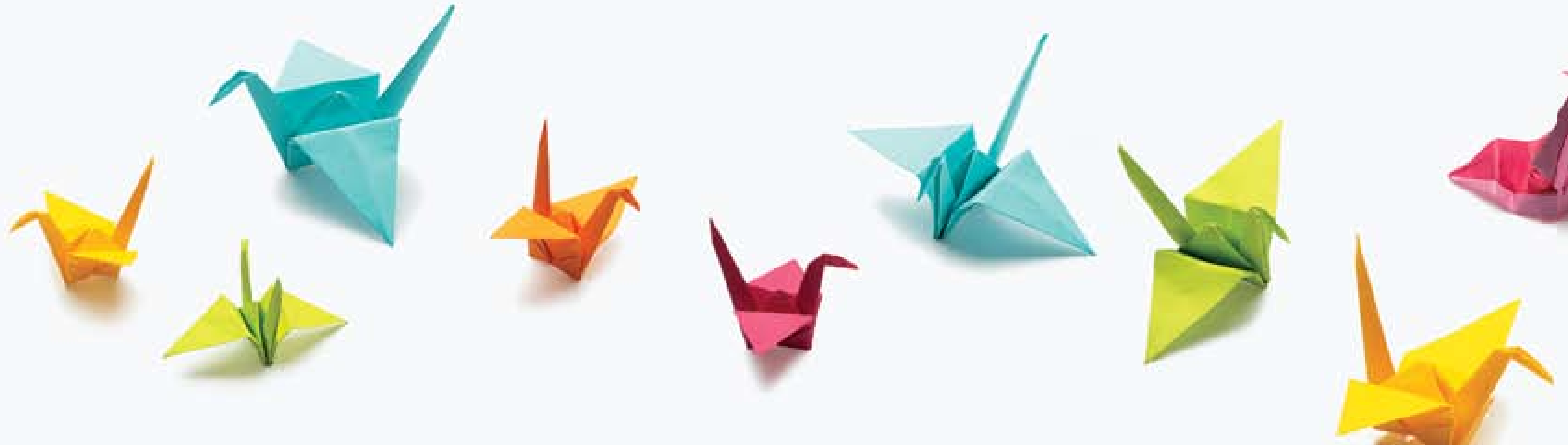


WHEN A PROTEIN MISFOLDS:
It's unable to perform crucial work within the cell // or it bands together with other such proteins to cause havoc // and sets in motion a wide variety of disorders.

Origami Medicine

BY LAUREN WARE // PHOTOGRAPHS BY DENISE BOSCO

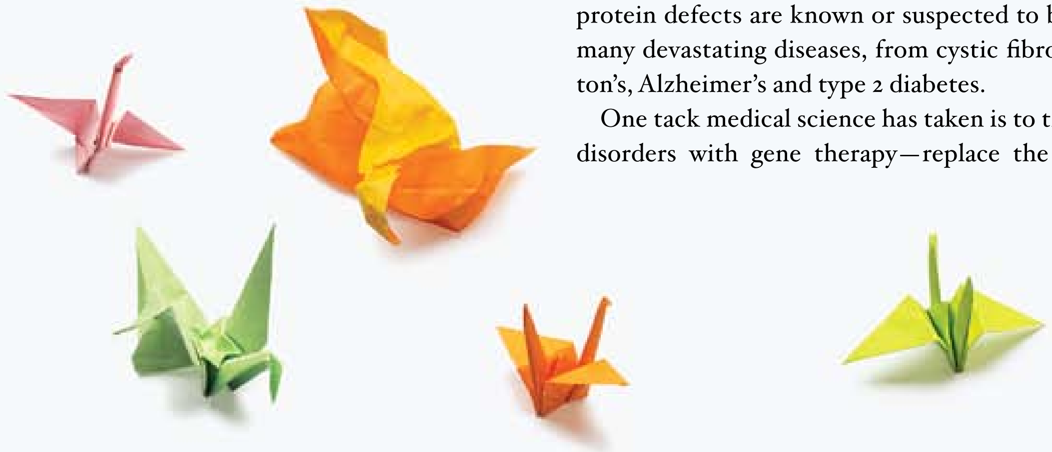
The proteostasis network has always existed, but the name is new, and only recently emerging is the notion that the network is as essential to life as a beating heart or DNA. Proteostasis is what the cell aspires to: a state of equilibrium in which the many proteins inside it coexist and interact. When the network is perfectly balanced, it's a hallmark of a healthy cell—and of any healthy organism, from the primitive worm *Caenorhabditis elegans* to the gloriously complex human. But when it's unbalanced—because of defective proteins, aging, physiological stress or other factors—disease results.

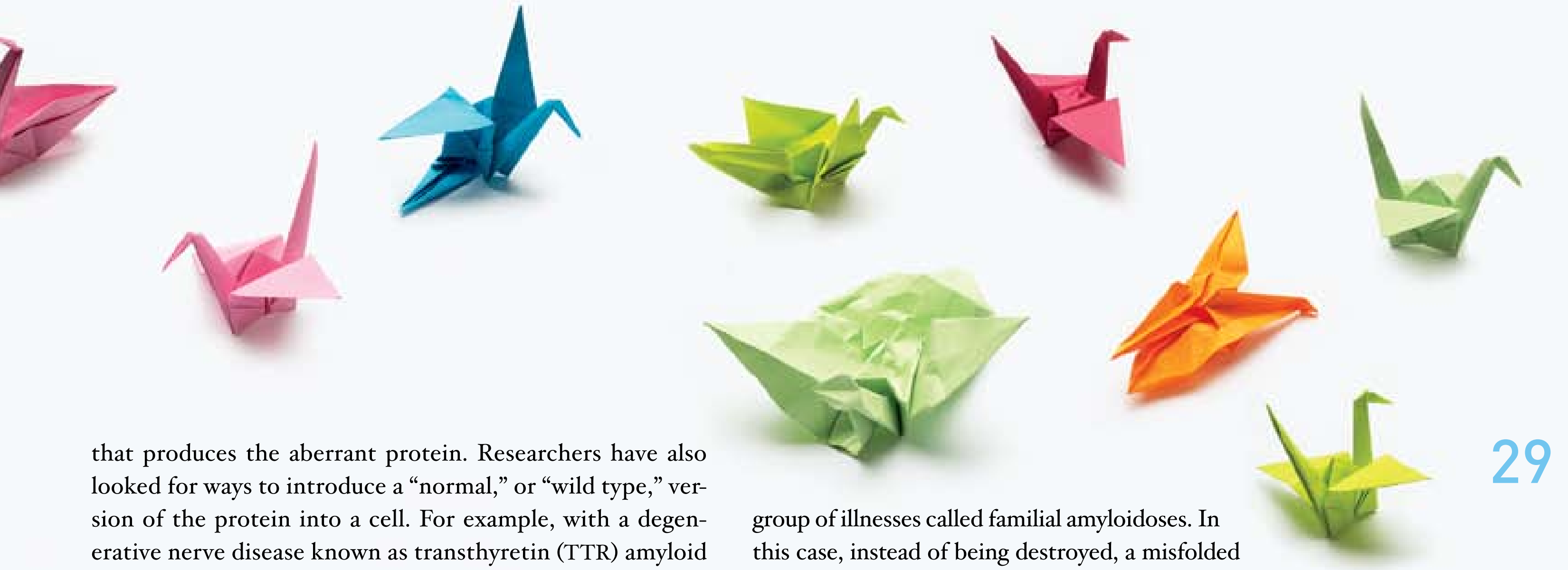
Despite its name, proteostasis is anything but static. There's constant activity, as every day the cells of the human body pump out thousands of proteins that carry out a remarkable range of functions, from preventing lung tissue damage to transporting crucial ions across membranes and stabilizing nerve cells. To achieve these functions, proteins must fold into specific three-dimensional shapes, find their way to their destinations and, if defective or no longer needed, degrade to make way for newly synthesized replacements. A network of more than 1,000 enzymes, molecular chaperones and other components controls these processes. Numerous signaling molecules work together in pathways that respond to cues from the cell or its environment to regulate the number of chaperones, enzymes and components.

William Balch, professor of cell biology at the Scripps Research Institute in La Jolla, Calif., whose work focuses on the pathways of proteostasis, describes the process of folding, transporting and chaperoning a protein: "It's like raising a kid. After he's born, you don't just put him out on the street. You work with him. The cell is working all the time with the proteins that it makes on a minute-by-minute basis." Each cell must ensure that proteins are made to proper specifications, maintained in the three-dimensional shape that allows them to work properly, and launched into adulthood in a way that supports the cell's overall health.

Though many scientists are now focused on deciphering the details of the system, progress has been frustratingly slow, and the more researchers learn, the more complexities they unearth. Discovering the processes and interactions involved in just one pathway may take years. But there's an urgency underlying this work, because folding errors and other protein defects are known or suspected to be implicated in many devastating diseases, from cystic fibrosis to Huntington's, Alzheimer's and type 2 diabetes.

One tack medical science has taken is to try to treat these disorders with gene therapy—replace the defective gene





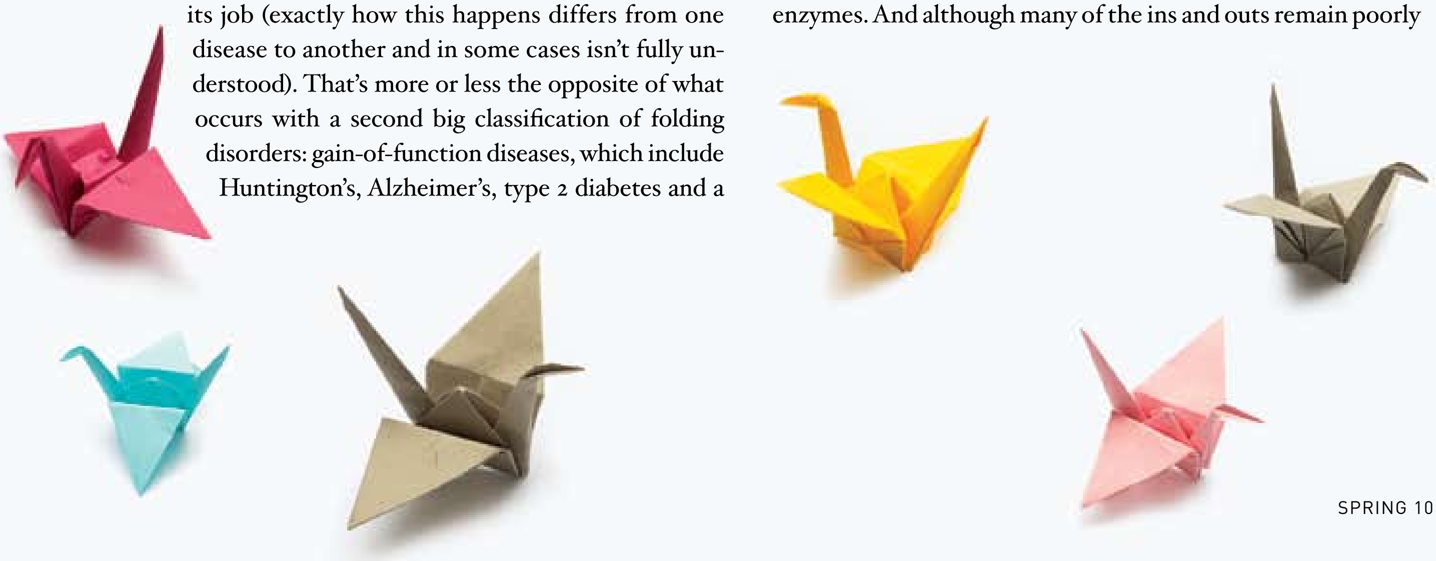
that produces the aberrant protein. Researchers have also looked for ways to introduce a "normal," or "wild type," version of the protein into a cell. For example, with a degenerative nerve disease known as transthyretin (TTR) amyloid polyneuropathy, caused by a misfolded protein produced by the liver, a transplanted liver cures the condition by making perfect, wild-type TTR that doesn't cause problems. But in most cases, these methods haven't proved effective, so the treatment of misfolding disorders has usually been relegated to relieving symptoms.

Recently, however, researchers have begun exploring ways that science can influence parts of the process, tweaking the diseased system with drugs designed to bring proteostasis back into balance. In some cases, they've been able to make partial molecular fixes that help a misfolded protein achieve its original purpose. Sometimes, it turns out, perfection isn't needed, and "good enough" solutions, hurried from concept to drug discovery through a process of high-technology trial and error, may get the job done—often years in advance of more complete or elegant therapies.

Many genetic disorders stem from mutations in DNA that produce a protein with a slightly different sequence of amino acids than normal, which causes the protein to misfold. In one main category of these illnesses—loss-of-function diseases such as cystic fibrosis, Gaucher's disease and related lysosomal storage diseases—the errant protein is targeted for early destruction and is never able to do its job (exactly how this happens differs from one disease to another and in some cases isn't fully understood). That's more or less the opposite of what occurs with a second big classification of folding disorders: gain-of-function diseases, which include Huntington's, Alzheimer's, type 2 diabetes and a group of illnesses called familial amyloidoses. In this case, instead of being destroyed, a misfolded protein breaks down and is put back together in an aggregate form that causes toxic damage—it does things that were never intended to occur (here, too, specific mechanisms are still being studied).

The symptoms of a particular disease depend on the type of tissue that holds the misfolded protein. In the most common mutation that causes cystic fibrosis, ΔF508, a protein called the cystic fibrosis transmembrane regulator, or CFTR, misfolds and is destroyed, and the lack of CFTR causes thick, sticky mucus, which builds up in the lungs and the pancreas. In Huntington's disease, the misfolded huntingtin protein breaks down, then re-forms into an aggregate in the brain that causes neurological damage. In TTR amyloid polyneuropathy, the liver makes the protein TTR, which misfolds and ends up in peripheral nerve tissue as the aggregate TTR amyloid, which results in loss of sensation, muscle weakness and autonomic nerve problems that may affect the gastrointestinal and urinary tracts, among other systems.

During protein folding, ribosomes—small cellular structures in the cytoplasm—translate genetic information into long strings of amino acids known as polypeptides. From these chains, the protein folds itself into an intermediate structure and then into its final three-dimensional form. Proteins constantly fold and refold as they interact with other proteins and enzymes. And although many of the ins and outs remain poorly



understood, research has shone light on the roles of chaperones and signaling pathways, which moderate this dynamic process. Chaperones are molecules that promote proper folding by binding to misfolded or aggregated proteins and providing a second chance for them to fold correctly. For their part, signaling pathways respond to cues from the environment to regulate not just how proteins fold but also how they're made, moved, aggregated and degraded.

One of the best understood pathways is the heat shock response. Richard Morimoto, a professor of molecular biology at Northwestern University, began working with heat shock genes more than 30 years ago, just after their discovery, and he and other researchers found that cells have a molecular thermometer that can turn genes on and off—thus increasing or decreasing production of the proteins associated with those genes. When a cell's temperature was raised, the cell began to synthesize great numbers of heat shock proteins, or HSPs. This same pathway turns out to exist in all organisms, from yeast to humans. A substance called heat shock factor, or HSF, controls the response.

Extrapolating from what they have learned about this and other pathways and chaperones, researchers have been pushing to find treatments for proteostasis disorders. They're studying small molecules that can bind to and stabilize misfolded proteins, such as CFTR, the protein thought to cause many symptoms of cystic fibrosis. In its most common mutation, ΔF508, CFTR is missing one amino acid, phenylalanine, which causes it to fold improperly. The endoplasmic reticulum—a tubular transport network that winds from the membrane of the cell nucleus to the outer cell membrane—then destroys the mutant CFTR before it can reach the cell membrane. When CFTR, which lets chloride into and out of the cell, is lacking, the amount of water in tissues is altered. In the lungs, for example, a layer of mucus depends on water to keep it diluted and not sticky.

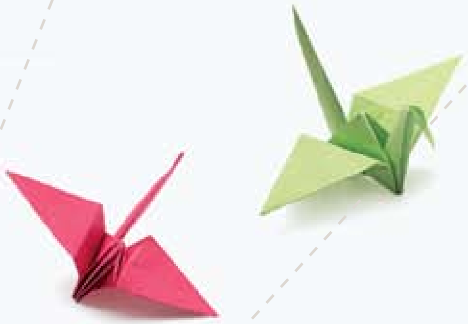
In an alternative scenario, a small molecule known as a pharmacologic chaperone might bind to CFTR while in the endoplasmic reticulum and somehow partially repair the misfolded protein, possibly by making it "look" like normal CFTR. Even though such a repaired protein isn't identical to its perfectly folded counterparts, it avoids destruction and seems to function effectively. And if enough ΔF508 CFTR makes it to the cell membrane, the symptoms of CF could be much milder or even disappear entirely.

For gain-of-function diseases, in which misfolded proteins aren't destroyed but rather break down and are reassembled into toxic aggregates, a type of chaperone that researchers call a kinetic stabilizer could bind to and stabilize a protein before the damage begins, preserving it in its functional state.

Yet another approach is to use something called a proteostasis regulator to influence a signaling pathway and, by increasing its ability to work with the mutated proteins, protect proteins from early degradation and allow them to reach their destination. "We don't have to fight the system," Balch emphasizes. "We can work with it." This concept of "using biology to correct biology"—a phrase several researchers employ—could allow cell problems to be corrected at a very early stage, by nudging the pathways that target mutated proteins for destruction or reaggregation so that they are preserved and remain functional.

A recent paper by Balch and his colleagues shows remarkable potential for this approach. The researchers used a compound that inhibits a specific part of the proteostasis system, the HDAC signaling pathway. They knew that CFTR folding requires a particular heat shock protein, Hsp90, whose production is influenced by HDACs, and that by manipulating the Hsp90 system in a certain way, they could rescue the mutated CFTR. This led them to speculate that a drug that blocked the action of the HDAC pathway might also save the mutated CFTR protein from destruction and get it to the cell membrane. They tried various HDAC inhibitors and found success with suberoylanilide hydroxamic acid (SAHA), already in use as a chemotherapy drug.

"Lo and behold," Balch says, "we saw the most striking rescue ever of CFTR activity on the cell surface." In cultured human lung cells, SAHA seemed to restore the protein's function,



raising it to 28% of the level that an undamaged, wild-type CFTR would provide. "You saw a remarkable degree of stability," he says. "The proteins degraded very slowly, compared with extremely rapid decay in a cell that had not been treated with an HDAC inhibitor." The next step, Balch adds, is to "dig in and really understand how" an HDAC inhibitor achieves that desirable result.

Yet the lack of deeper understanding hasn't prevented drugs that affect the proteostasis network from proceeding to clinical trials. When researchers know what they want a pharmaceutical agent to do—in this case, to restore the function of the CFTR protein—but are unclear about some of the mechanisms and pathways underlying the problem, they can use a process called high-throughput screening. This screening employs automated, computerized systems to rapidly test hundreds of thousands of molecules to begin the search for an effective drug. Though the great majority of compounds tested won't be effective, that doesn't really matter, because it's relatively fast and easy to run through whole libraries of chemicals.

About a dozen years ago, the Cystic Fibrosis Foundation approached Aurora Biosciences, a company experienced in high-throughput methods, and asked it to develop a program to screen for compounds to treat CF. At the time, the idea of doing something to get the CFTR protein to the cell surface—rather than trying to correct the underlying gene, which hadn't worked—was relatively new. After Aurora was acquired by Vertex Pharmaceuticals in 2001, scientists at Vertex used high-throughput screening and eventually found a compound the company calls VX-770, a "potentiator" molecule that increases the activity of CFTR once it reaches the cell membrane and helps it function more effectively. But this compound is thought to work only if CFTR lies on the cell surface (and some gene mutations that cause CF do allow some CFTR to reach the surface, though it doesn't function properly once there). So Vertex scientists simultaneously searched for a second kind of compound—a "corrector"—that would restore ΔF508 CFTR to its normal shape, allowing it to get to the surface. They found it and named it VX-809.

"We don't have to fight the system," Balch emphasizes. "We can work with it." This concept of "using biology to correct biology" could allow cell problems to be corrected early.

Eric Olson, project leader for cystic fibrosis at Vertex, says the company developed both compounds in part because research had shown that when the temperature of cells was reduced, ΔF508 CFTR could get to the cell surface, but once there it seemed to function less efficiently than wild-type CFTR. Further research has revised that view, showing that ΔF508 CFTR does work correctly if it can reach the cell membrane. But Vertex's potentiator drug VX-770, which fared well in 2008 Phase II clinical trials, can boost the effectiveness of the protein further. So even if the corrector doesn't fully fix the mutated protein and restore a normal quantity to the cell surface, the potentiator can help the CFTR that does arrive to transport more chloride ions.

In February, Vertex released the results of a clinical trial of its corrector compound, VX-809. While the drug didn't improve lung function, it did have an impact on patients' sweat chloride tests, a marker of how effectively skin cells are transporting chloride, and thus an indication of how much CFTR protein is getting to the cell surface and functioning properly. According to Olson, the company plans to conduct a clinical

trial using both VX-809 and the potentiator, VX-770. Judging from in vitro studies that used a combination of VX-809 and VX-770, he thinks the combined therapy could help reduce CF symptoms in patients with the ΔF508 mutation.

Other potential therapies for gain-of-function disorders are also emerging. Jeffery Kelly, chair of the department of molecular and experimental medicine and professor of chemistry at the Skaggs Institute for Chemical Biology at Scripps, and his colleagues have discovered a kinetic stabilizer for the protein implicated in TTR amyloid polyneuropathy, keeping it functional and preventing it from unfolding and aggregating into its toxic form. FoldRx Therapeutics, a company Kelly founded, has developed a drug, tafamadis meglumine (FX-1006A), now in clinical trials, and preliminary results show that it significantly slows progression of the disease. FoldRx is also researching treatments for Parkinson's, Huntington's and a form of amyotrophic lateral sclerosis using the same strategy of stabilizing defective proteins.

Many gain-of-function disorders, including Parkinson's and Alzheimer's, are considered diseases of aging; work with the *C. elegans* worm, which researchers often use as a model for how human diseases develop, suggests that as cells age, misfolding may become increasingly commonplace. In studying Huntington's disease in *C. elegans*, Morimoto and his colleagues discovered that as the worm

Folding Fixers //

Misshapen proteins are implicated in a wide array of disorders. Here are just a few, and how drugs might restore function to errant proteins.

	DISEASE	HOW MISFOLDING MAY BE INVOLVED	POTENTIAL INTERVENTION
LOSS-OF-FUNCTION DISEASES Occur when a misfolded protein can't achieve its intended purpose, either because it's not functioning properly or because it's targeted for early destruction. A genetic mutation is typically the cause.	**CYSTIC FIBROSIS**	In the most common mutation, ΔF508, a missing amino acid causes the CFTR protein to fold improperly, leading to its destruction. Without CFTR, ion transport across the cell membrane is impaired, affecting the amount of water in epithelial tissues and clogging the lungs and pancreatic ducts with mucus.	Pharmacologic chaperones (molecules that partially repair a misfolded protein, ushering it out of the cell); proteostasis regulators (molecules that stimulate chaperone production and/or affect signaling pathways, increasing the probability of proper protein folding)
	LYSOSOMAL STORAGE DISEASES (Gaucher's disease, Fabry disease and more than 40 others)	A mutation in a lysosomal enzyme causes it to misfold and be destroyed. Lysosomal enzymes break down discarded components in the cell for reuse; without them, these components build up and cause tissue damage. The specific symptoms depend on which enzyme has mutated and was destroyed.	Pharmacologic chaperones; proteostasis regulators
GAIN-OF-FUNCTION DISEASES Occur when a misfolded protein broken down by the cell is reassembled into an aggregate that causes cellular damage. These diseases are often associated with aging, though genetic mutation may also be at fault.	**HUNTINGTON'S DISEASE**	A mutation inserts extra glutamine, an amino acid, in the huntingtin protein. The extra glutamine causes the misformed protein to stick to other proteins, damaging brain cells.	Kinetic stabilizers (molecules that bind to the misfolded protein, preventing it from breaking apart); proteostasis regulators
	PARKINSON'S DISEASE	A protein in the brain, called α-synuclein, misfolds, breaks apart and re-forms into aggregates that poison neurons.	Kinetic stabilizers; proteostasis regulators
	ALZHEIMER'S DISEASE	Amyloid precursor protein is broken down into smaller proteins that misfold and stick together in clumps called oligomers, which eventually form the plaques in the brain that are characteristic of the disease.	Kinetic stabilizers; proteostasis regulators
	TTR AMYLOID POLYNEUROPATHY	Mutated TTR becomes unstable and dissociates into smaller components that partially unfold and then stick together, forming tangles of protein called amyloids. The amyloids cause damage to nerve tissues.	Kinetic stabilizers; proteostasis regulators

aged, the huntingtin protein did, in fact, become more likely to misfold, aggregate and become toxic. One of the researchers, Jim Morley, decided to experiment with a version of the worm that carries a life-span-extending mutation as well as the genes that cause Huntington's. In those worms, in which aging was long delayed, the huntingtin protein didn't misfold. "That was an aha! moment," Morimoto says, "because by enhancing life span, we had suppressed protein misfolding."

As Morimoto and his team looked more closely at the proteostasis system, they found that as a cell ages, proteins become very unstable, and misfolding and aggregation grow rampant. Morimoto says it's beginning to appear that the same components of the network that malfunction in Huntington's, Alzheimer's and other age-related, gain-of-function disorders are also implicated in the aging process itself.

Yet while research aimed at restoring cells' protein machinery to health and preventing age-related deterioration is gaining momentum, there are some concerns. One is that increasing the proteostatic capability of a cell might activate latent viruses by allowing the cell to begin producing the enormous amounts of proteins they need for replication. Another essential question is how much protein in a particular disease must be restored or prevented from becoming toxic before the symptoms of a disorder will disappear. Researchers surmise that 100% is not needed because carriers of one copy of the cystic fibrosis mutation, who have one good gene and one that produces mutated protein, have only half the normal CFTR function but show no signs of the disease. In other folding disorders, the calculation isn't always as straightforward. It remains to be seen how much protein will suppress disease symptoms, and it will take more trials like those at Vertex to find out how much restoration of protein is adequate for a particular protein misfolding disorder.

As more of these questions are answered, however, new drugs for misfolding disorders are likely to be developed, and some of those may even prove effective against multiple diseases. If they involve the same molecular pathway, even seemingly unrelated disorders may respond to the same drug. "It could be that half a dozen molecules would treat 50 to 60 diseases," Kelly says. Because it would be relatively easy to test a compound that shows promise against one disease on several others, the time it takes to find effective treatments could be shortened.

At the same time, combination therapies involving multiple drugs may be required to eradicate the symptoms of misfolding disorders. "You might need two or three drugs to create the best situation for the cell," says Pamela Zeitlin, professor of pediatrics at the Johns Hopkins University School of Medicine and a CF researcher and clinician who studies proteostasis. Some researchers now think it may be possible to find widely acting proteostasis regulators, and such drugs might boost the effectiveness of therapies that work on a specific pathway. Or, similar to the Vertex strategy with its corrector and potentiator compounds, two compounds focusing on a single problem may work better than just one.

Regardless of which drugs eventually pass muster in clinical trials, researchers are optimistic that by focusing on proteostasis, they're getting closer to finding treatments that do more than put a patch on symptoms for disorders caused by misfolded proteins. They're looking toward a future in which new pharmacologic therapies may fix these disorders at their root. ■

DOSSIER

1. **"Biological and Chemical Approaches to Diseases of Proteostasis Deficiency," by Evan T. Powers et al., *Annual Review of Biochemistry*, 2009.** An in-depth, highly technical look at the way defective protein maintenance is implicated in diseases, as well as how small molecules can enhance proteostasis.
2. **"Adapting Proteostasis for Disease Intervention," by William E. Balch et al., *Science*, Feb. 15, 2008.** A succinct explanation of how proteostasis offers insights into research for treatment of loss-of-function and gain-of-function misfolding disorders.
3. **"Researching the Surface in CF," by Lev Osherovich, *SciBX: Science-Business eXchange*, Jan. 7, 2010.** An overview of potential avenues for correcting the CFTR protein, including Vertex's compounds and William Balch's work with HDAC inhibitors.