PHOTOGRAPHS BY RICHARD CARPENTER; SCULPTURES BY ELISABETH NEVINS

Within the folds, outside the box

Susan Lindquist uncovers the roles that misshapen proteins play across an astonishing sweep of phenomena

BY CAROL CRUZAN MORTON

Feverishly hot climates. Dizzying alcohol and sugar binges. Heavy metals. Toxic drugs. Genetic mutations. Over the years, yeast, fruit flies, mustard plants and mice have struggled through their own versions of an extreme reality TV show in the laboratory of Whitehead Member Susan Lindquist.

The challenges to these different critters have been limited only by the overactive imaginations of Lindquist and her colleagues. One recent episode involved forcing yeast cells to evolve resistance to the best drugs used in clinics to fight deadly fungal infections. In future installments, the yeast must first imitate and then overcome a protein problem underlying a horrific human neurodegenerative disease.

The amazing but true stories of how some of the yeast have endured, and even thrived, have advanced a strangely wide array of science: mad cow disease, neuroscience, nanotechnology, cancer, anti-fungal drug resistance and non-genetic evolution.

But these seemingly disparate discoveries share a common thread.

"The one universal theme in our lab is protein folding and how changes in protein folding drive many biological processes," explains Lindquist in her sixth-floor office overlooking MIT on a hazy day.

"People didn't realize how broad the protein folding problems are. A lot of things that started out as basic research into protein folding are now translating into a direct interest in human health and medicine."

Researchers in her lab have even found that proteins can trump DNA in passing along new traits into the genomes of future generations.

"You don't usually think about proteins this way," acknowledges James Shorter, a senior research associate. "Independent of the underlying DNA, protein folding can influence a wide variety of things, from evolution to disease progression and initiation. And it can act as a genetic element. Initially, this seemed just crazy, but it is true."

"When I was an assistant professor, I would sit down and design every experiment and write out the protocols," says Susan Lindquist. "I used to miss doing the bench work terribly. But now the exhilaration of working with so many creative people in so many different ways has got me hooked."

KATHLEEN DOOHER

PROTEIN PIROUETTES

Proteins start as linear strings of building blocks–assorted combinations of 20 amino acids specified by the genetic blueprint. Some strings are short. Some are long. All must fold into complex shapes to do their jobs. The shape of a protein gives it its function.

Less than a minute after they are formed, proteins loop, twist and scrunch themselves into neat preordained packages featuring lobes, spirals, pleats and hinges. The nascent protein attempts its precision maneuvers in a tightly packed cell, bumped and jostled by hundreds of other proteins hustling and bustling about the cell. It is as if everyone on a crowded bus started doing aerobics all at once, each to his or her own style, tune and timing.

Not surprisingly, nearly one-third of proteins cannot fold properly, Lindquist says. The problem could be the molecular equivalent of a knee to the gut, or it might have been a nick or ding in the original genetic information that miscued a crucial nook or cranny.

A distorted protein may be unable to carry out its crucial mission, or it may have transformed into something nasty. Either way, "it can be an absolute disaster," Lindquist says. "Misfolded proteins are responsible for many terrible illnesses of mankind. In cystic fibrosis, just one amino acid in several hundred is wrong. This means that this one protein can't quite fold up properly to get to the surface and function. Disaster." Another variation of misshapen proteins are amyloids, nearly indestructible amalgamations of proteins found in Alzheimer's, Huntington's and Parkinson's diseases.

Surprisingly, her lab is finding that in some circumstances, an alternative fold in a protein may underlie vital aspects of normal biology.

A MODEL MENAGERIE

Lindquist, who started as a fruit fly cell biologist, now tracks warped proteins and their consequences through model systems spanning millions of years of evolution.

She switched to yeast as her main model 22 years ago after attending the Cold Spring Harbor Yeast Genetics Course co-led by Whitehead Founding Member Gerald Fink. But the lab nimbly moves through an experimental menagerie that also includes plants, mice and human cancer cell lines. In collaborations with others, the team has added expertise in rats and sea slugs.

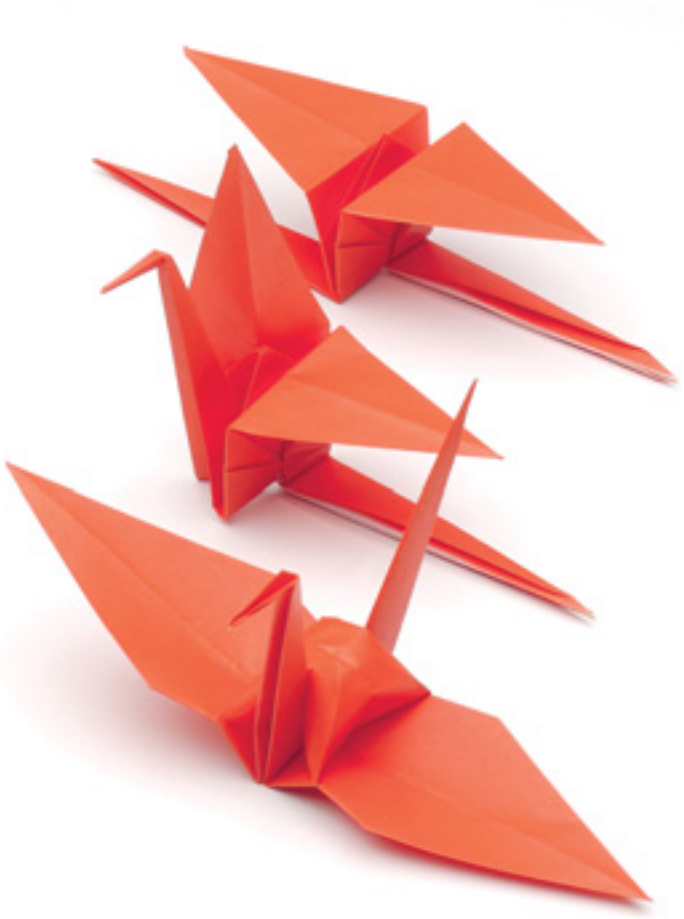
For example, in a sea slug's oversized neurons, the protein that sustains long-term memory at the junctions and synapses seems to work by shifting its shape into a prion, a configuration that bends other proteins to its same altered form.

This finding, reported two years ago in collaboration with neurobiologist Eric Kandel at Columbia University, complemented unexpected aspects of prion activity in yeast.

It contradicted a widely held belief that prion activity is inevitably toxic, a generalized assumption inspired by the well-publicized and frightening cases of mad cow disease transmitted to people.

And the slug protein work suggested an unexpected new mechanism for long-term memory in higher organisms as well.

"Protein folding is deeply rooted in biology," Lindquist says. "All organisms face the same problems and share the same solutions. Mother Nature has been coping with protein folding problems since the dawn of time. It makes sense that she would discover ways to turn it to her advantage."



"Mother Nature has been coping with protein folding problems since the dawn of time."

HEAT SHOCK AND AWE

Potentially devastating protein folding problems worsen when a cell or organism is stressed by hostile environmental factors, such as heat. In response, cells send in a rescue squad called heat shock proteins, also known as chaperones, to resuscitate or cart away proteins missing their full complement of nips and tucks.

Some of the emergency workers prevent unfolded proteins from aggregating. Some disaggregate dysfunctional clumps. Some hold proteins in partially folded states until they receive the right signal, perhaps from a hormone. Some act as trash collectors for the irredeemably malformed. Others refold wilted proteins and give them a second chance.

In a sense, heat shock proteins also have chaperoned Lindquist's career, beginning with her independent decision to study them in fruit fly tissue culture when she was a graduate student in the Harvard lab of Matthew Meselson. Then, she was more interested in the rapid change in gene expression patterns stimulated by environmental stress, which is anything but subtle. Indeed, it is a cellular shock and awe tactic, with genes churning out 50 to 10,000 times more heat shock proteins to try to save a cell from its environment.

At the University of Chicago, where she did her postdoctoral work and progressed to full professor and Howard Hughes Medical Institute investigator, Lindquist helped unmask the function of heat shock proteins in the protein folding response.

Back in her fruit fly days, Lindquist and her collaborators had figured out that one known heat shock protein, Hsp90, worked by helping proteins with minor mutations that would otherwise alter their form. Taking away Hsp90 unveiled complete sets of hidden mutations with new functions. If the new variations were advantageous, Lindquist's team showed, the breeding flies' offspring hung onto the

helpful mutations and the remodeled proteins, even without the assistance of Hsp90.

Three years ago, Lindquist's group announced that Hsp90 performs the same trick in the experimental plant thale cress, making it likely that other organisms can also save up genetic changes for a rainy day. The mechanism could help to explain some of the rapid diversification found in the fossil record.

On a darker note, the group also has found that Hsp90 enables mutations in human cancer cells to promote cancer growth. In animals, Hsp90 inhibitors can reverse oncogenic transformations and are now in clinical trials.

Most recently, postdoctoral researcher Leah Cowen has shown that Hsp90 allows yeast to rapidly evolve resistance to antifungal drugs (see "How pathogenic fungi evolve drug resistance," page 4). Once resistance has evolved, compromising Hsp90 functions with drugs or mutations can abolish it. Remarkably, yeast exposed to temperatures that simulated human fevers lost drug resistance, mimicking the effects of inhibiting Hsp90 function. This provides one of the first molecular explanations of a beneficial role for fevers.

PROTEIN BUSTERS

In yeast, Lindquist discovered another member of the heat shock family, Hsp104. This protein proved to be a powerful protein remodeling agent that saved yeast from sudden high-temperature heat shocks and all sorts of other stressful environmental conditions her lab could conjure.

And it seemed to be doing the impossible. Unlike Hsp90, which holds onto proteins and prevents them from misfolding, Hsp104 works to take apart proteins that have aggregated together. That finding reversed a common dogma that misfolded and aggregated proteins are irredeemable, Lindquist said. Instead, the chaperone rescues congealed proteins and restores them to their individual functions. "You can't unfry an egg, but you can uncoddle an egg," she says.

Strikingly, Hsp104 can also pass along and release hidden genetic variation. As part of normal yeast biology, Hsp104 remodels a protein named Sup35 into a prion named [PSI+]. Lindquist's team showed that Hsp104 was necessary to refold Sup35, but once transformed, [PSI+], a regulator of protein synthesis, is positively evangelical about converting other Sup35 proteins to the same altered shape. Hsp104 ensures that mother cells pass along the prion to daughter cells, whose proteins are thereby influenced to keep changing shape, too.

This goes on for generation after generation.

Why would cells have a protein that changes shape like this? [PSI+] removes the stop sign that normally appears when proteins are being synthesized: Ribosomes roll through their normal stopping point on an RNA strand and read into fresh genetic regions. Many proteins are outfitted with extra features, which may provide a survival advantage in a fluctuating environment and thus eventually become genetically fixed.

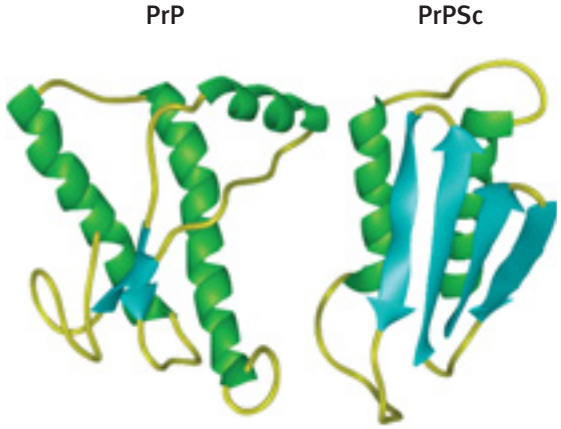
Perplexingly, the prions created by low levels of Hsp104 can be disaggregated by high levels of Hsp104. Last year, Shorter helped resolve this major conundrum, publishing in the journal *Science*. Shorter worked out the complicated and dramatically shifting biochemistry by mixing the two proteins Hsp104 and Sup35 in a test tube with various sources of energy.

"This is the first time that anyone has found anything that can catalytically take apart an amyloid fiber," Lindquist says. In the lab, protein amyloids, like those that clog up the brains of people who died from Alzheimer's disease, are impervious to just about anything, including extreme heat and cold and powerful detergents.

The yeast prion amyloid fibers are also remarkably resilient, able to withstand exposure to extended high temperatures, high and low salt, strong alkalis and acids, and 100 percent ethanol.

Before coming to Whitehead, in fact, Lindquist and her collaborators at the University of Chicago exploited the strength of these protein-protein connections to make nanoscale wires of gold and silver a thousandth the thickness of a human hair that successfully conducted electricity.

In the last few billion years, animal cells lost the ability to produce Hsp104. "You can imagine how it might be useful for diseases associated with protein aggregation," Shorter says. "If we understand how it works, we can apply it to other systems."



The brain of a victim killed by a prion disease, such as mad cow disease, typically is clogged with clumps of the prion protein PrP that has entered a rare, misfolded state called PrPSc. In 2002, Lindquist and Ohio State University's Jiyan Ma suggested a unifying theory that can help explain how these devastating diseases get started and how they kill.

COURTESY OF THE COHEN LAB/UCSF

THE MAD COW CONNECTION

The helpful yeast prion protein and the dangerous mammalian prion protein have virtually

nothing in common. But the ability of [PSI+] to self-replicate by changing the shape of other proteins is eerily similar to the way the infectious mad cow protein seems to corrupt a plentiful membrane protein in people's brains into an insidious shape that causes horrific disease. And the two proteins have one vaguely similar region.

Further experiments in yeast and mice along these lines led Lindquist to propose a new, unifying hypothesis to explain the origin of the human prion disease and the mechanism of its toxicity.

Bits of misfolded proteins processed by specialty organelles may accumulate in the main compartment of the cell, the cytosol, where they can be tagged for disposal by the cellular garbage service. The volume may cross a threshold, where the cell's quality control systems cannot remove the misfits fast enough. Even a barely detectable level of misshapen proteins can be toxic to a neuron.

"Seventy percent of what I'm now doing I couldn't have foreseen five years ago. You take unexpected twists and turns."

PROBING PARKINSON'S

Using the yeast as a living test tube, a team led by graduate student Tiago Outeiro has showed that overproduction of a human protein, alpha-synuclein, can convince neighboring proteins to abandon their normal shape and form protein clusters similar to those in Parkinson's disease. The afflicted yeast suffer from a similar range of symptoms and die.

"We have reason to believe it is a quality control problem," Lindquist says. "In some people, the protein misfolds at a higher rate, and that becomes a disaster in a hurry. In other people, as they age, the protein folding quality control system gets wimpy and can't keep up with the normal rate of misfolding."

Her team screened 116,000 chemical compounds to reverse the toxicity of alpha-synuclein overload in yeast. Among the 60 compounds, they found one that previously had been used as an antibiotic and is now in clinical trials for Alzheimer's disease. "That makes me think we've found something real," Lindquist says. "We hope we will be able to develop therapeutic strategies in yeast."

Postdoctoral fellow Aaron Gitler now is searching for the original defect that the Parkinson's protein triggers in yeast cells in hopes of identifying the underlying disease pathway and key drug targets.

OUT OF BOUNDARIES

Not surprisingly, Lindquist can't predict where this rich and deep collection of studies will lead her.

"I hope it won't be something I anticipate now," she says. "Seventy percent of what I'm now doing I couldn't have foreseen five years ago.

"It happens in other labs too. You take unexpected twists and turns not only from your own data but from responding to the scientific community at large."

Whatever the future brings, Lindquist is likely to be more closely involved in human diseases. Last year, she co-founded FoldRX Pharmaceuticals, which will develop drugs to treat diseases of protein misfolding. She also was elected to the board of directors for Johnson & Johnson.

"Susan has enormous creativity," says close friend Elaine Fuchs, a Howard Hughes investigator at Rockefeller University and a member of Whitehead's Scientific Advisory Board. "Her ability and vision to think about areas of science so broadly allow her to make connections that are quite extraordinary and lead to interesting science." Those significant connections extend beyond science, adds Fuchs, whose marriage resulted from Lindquist's penchant for matchmaking.

Lindquist puts it another way. "There's a great deal to be said for concentrating on one thing," she says. "I'm the exact opposite."