Know When to Fold 'Em

When the cell's protein-folding machinery is stressed, timing is everything in the decision to keep going or die.

ON THE MISSION BAY CAMPUS OF THE UNIVERSITY OF CALIFORNIA, SAN Francisco (UCSF), Peter Walter's corner office is distinctive for its tall, elegant cactus-like plants—and its poetry-quoting African grey parrot. After months of effort, Walter has trained the parrot, named Beaker, to badger lab members on what's most important: "We need more data." ¶ That's a common refrain from research leaders, but "in this case, Peter's got a parrot to say it," says postdoctoral researcher Jonathan Lin, laughing.

In keeping with Beaker's command, Walter's team has collected convincing data to explain exactly how the endoplasmic reticulum (ER), a maze-like compartment that fans out around the nucleus inside the cell, can determine whether a cell lives or dies. The ER serves as a cellular factory where newly synthesized proteins are folded into their proper structure. A protein must be folded correctly to do its job.

"Only the perfectly made proteins pass quality control," says Walter, an HHMI investigator. Proteins that fail to attain the right conformation are degraded before they can cause cellular dysfunction and disease.

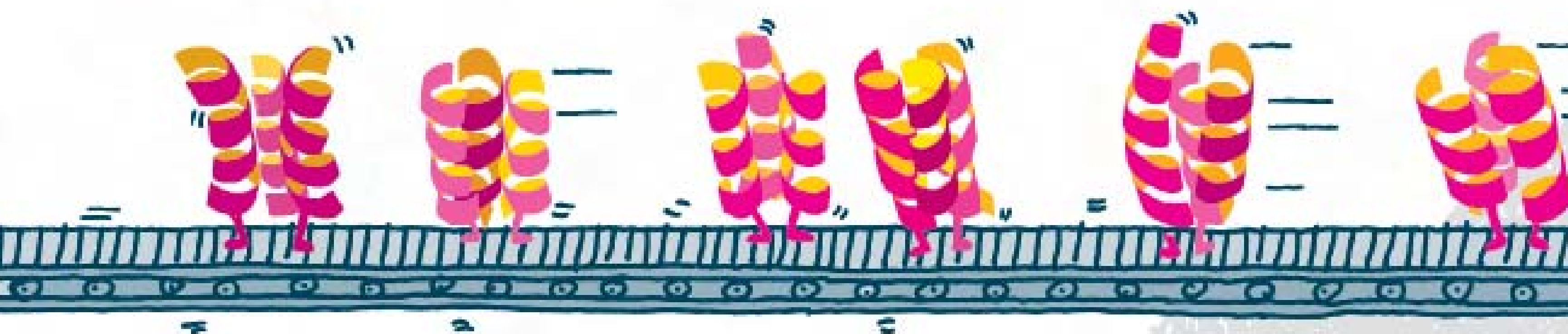
If the ER machinery is insufficient or defective, however, unfolded proteins pile up. Fifteen years ago, in studies of yeast, Walter and his colleagues discovered that the ER copes with the stress of such overload by triggering a set of biochemical reactions, known as the unfolded protein response, or UPR. Later work by various research groups uncovered a similar mechanism in mammalian cells, including human cells: three enzymes, molecular sensors called IRE1, ATF6, and PERK, detect the glut of unfolded proteins. They then activate various genes that expand the ER and step up its folding capacity, reduce the synthesis of new proteins, and crank up the protein degradation process.

Those protective measures bring the system back into balance. "It's a feedback loop that adjusts supply to demand," Walter says. Yet, paradoxically, if the ER cannot regain equilibrium, the UPR prompts the cell to commit suicide. In a study published in *Science* last November, Lin, Walter, HHMI investigator Kevan Shokat, and colleagues explored how the same signaling pathways could cause such diametrically opposite fates.

The team exposed cultured human cells to drugs that prevent proteins from folding and eventually cause cell death. Over 24 to 30 hours, the researchers measured the activity generated by the UPR. Initially, all three pathways rapidly turned on, but results unexpectedly showed IRE1 shutting off after about 8 hours, around the time when cells began to deteriorate. ATF6 activity followed a similar pattern. By contrast, responses triggered by PERK—including production of

A GOOD FIT

IT'S A TALE OF GREAT CHEMISTRY between two HHMI investigators at the University of San Francisco: around 2000, Peter Walter talked to Kevan Shokat about devising a drug that would work in yeast to selectively suppress IRE1, which belongs to a vast family of enzymes called kinases. Shokat already had a designer compound in hand that blocked other kinases after he modified them to respond to it. Taking the same approach, he used genetic tinkering to slightly widen IRE1's active site, the pocket where energy-molecule ATP normally plugs in and activates the enzyme. Those changes permitted Shokat's drug to fit only into that mutated pocket, blocking ATP binding. Shokat expected this chemical-genetics strategy to shut off the enzyme, but the reverse happened. "We've done this with 100 kinases and IRE1 is the only one where the drug actually turned *on* the function of the kinase," he says. "So that was an absolute surprise." A surprise that proved helpful when Walter needed a persistent, rather than a suppressed, IRE1.



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a protein that promotes cell suicide—stayed on the whole time.

The drop-off in IRE1 appeared to be "a switch between the pro-survival versus the pro-death phases of the UPR," says Lin. To test that hypothesis, he and Walter wanted to see what would happen if IRE1 did not power down. Fortunately, UCSF colleague Shokat gave them a "wonderful trick" to do just that, says Walter. Shokat used genetic methods in yeast to alter IRE1's structure so that the sensor could be selectively turned on by a designer drug (see sidebar).

Lin and Walter repeated their cell culture experiments, this time using human cells engineered with the mutant version of IRE1. Adding Shokat's drug artificially stimulated and sustained IRE1 levels in the cells—and substantially fewer of them died, confirming the researchers' theory that the enzyme was pivotal for cell survival.

Going a step further, the researchers examined developing eye cells in rats with retinitis pigmentosa. This inherited form of blindness results from degeneration of retinal cells that make misfolded light-sensing proteins. Those experiments revealed a downturn in IRE1 signaling, typical of cell suicide.

The study raises fresh questions: could future drugs be designed to enhance the UPR's protective responses or stave off overzealous cell suicide that occurs in this and other diseases—such as diabetes and Alzheimer's disease—in which cells die from protein-folding glitches? Lin is exploring that possibility in the blind rats.

Walter is investigating the other side of the coin. Could inhibiting the UPR's protective side within cancer cells, which must crank out many proteins to sustain rapid growth, put an end to a tumor's growth? Beaker the parrot's likely response is: "We need more data." ■ –INGFEI CHEN