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Approximately one third of cellular proteins pass through the endoplasmic reticulum (ER), a netlike labyrinth of membrane-bound tubes and flattened sacs inside the cell. Work in the 1960s revealed that the ER sorts and transports proteins, and we now know that it allows cargo to pass only after applying stringent standards. In particular, proteins must assume correct three-dimensional shapes to perform their jobs, and the ER fosters this outcome. Furthermore, when unfolded proteins accumulate in this compartment, the cell bolsters the ER's folding capacity. This phenomenon forms the linchpin of the unfolded protein response (UPR).

The first clues about this system's existence emerged in the late 1970s, when researchers discovered that glucose starvation drives cells to boost production of particular proteins. Amy Lee (University of Southern California) reported in 1983 that the rise stems from an increase in the quantity of messenger RNA (mRNA) templates for these glucose-regulated proteins, or GRPs.

Three years later, Hugh Pelham (Medical Research Council, Cambridge) established that one of the GRPs, GRP78, resides in the ER and resembles a protein that prevents heat-damaged proteins from clumping. When glucose supplies drop, sugars that normally decorate some proteins are no longer available. Pelham proposed that the resulting sugar-deficient proteins stick together, perhaps because they misfold, and that GRP78, like its molecular relative, thwarts protein aggregation. Pelham also found that GRP78 was identical to another protein, BiP, that associates with partially assembled antibody molecules in the ER. In parallel, Mary-Jane Gething and Joseph Sambrook (University of Texas Southwestern Medical Center) showed that BiP attaches to misfolded forms of a different protein in the ER.

These findings hinted that BiP helps proteins fold; if true, manufacture of BiP in response to unfolded proteins would serve a clear benefit. The connection between glucose starvation and folding remained murky, however, and the model relied on that link. In 1988, Gething and Sambrook established that misfolded protein rather than sugar-adornment defects sends the alert to ramp up BiP output.

In 1989, the yeast BiP surfaced. Its quantities also climb in response to unfolded ER proteins. Mori joined Gething and Sambrook's lab as a postdoctoral fellow, and the

group identified a short series of DNA letters that abuts the BiP gene. This sequence spurs molecular machinery to copy, or transcribe, the BiP DNA into mRNA when unfolded proteins accumulate in the ER; the sequence, when placed next to a different gene, similarly turns on its transcription.

Cells must somehow monitor the abundance of unfolded proteins in the ER and transmit that information to the nucleus, which houses the genes. These events spark production of BiP and other proteins that promote folding, which reverse the problem. But no one knew how the nuclear equipment senses the ER environment.

The Complexity of Ire1

Independently, Mori, in Texas, and Walter, in San Francisco, placed the DNA stretch that Mori had uncovered next to a gene whose product makes a blue substance. When unfolded proteins accumulate in the ER and the engineered yeast cells send the usual signal to the nucleus, it stimulates not only typical UPR targets, but also the gene that turns the yeast blue. Yeast with defects in the UPR system would not change color, the researchers reasoned.

In 1993, the investigators used this scheme to isolate white yeast strains and thus tracked down the faulty genes, whose normal versions presumably contribute to the UPR. One encodes a protein called Ire1.

Sequence analysis of Ire1 suggested that it is a kinase -- an enzyme that adds phosphates to itself and/or other proteins. Additional work by Walter and Mori confirmed and extended this prediction. They found that Ire1 lies in the ER membrane with its kinase portion in the cytoplasm. In this orientation, the ER region could detect an unfolded protein signal and the other end could convey the message to cytoplasmic partners.

Mammalian kinases were well known to monitor environmental cues and, by adding phosphates to themselves or other molecules, trigger adaptive physiological changes. Perhaps, Mori and Walter reasoned, Ire1 would behave similarly.

To figure out how Ire1 delivers the unfolded-protein message, Walter and Mori (by then an independent investigator in Japan) set out to identify the presumptive courier that picks up its signal and carries it to the nucleus. They sought a protein that binds to the DNA sequences adjacent to UPR target genes and provokes

transcription. The investigators captured the component they sought, a protein that previously had been named Hac1.

Their results, reported in 1996, contradicted expectation. In the simplest scenario, the theoretical protein to which Ire1 affixes a phosphate would be ready for action upon stimulation. Hac1, however, is not ready for anything; rather, it is manufactured only after the UPR alarm rings.

A crucial clue to explain this result came from the observation that the Hac1-encoding mRNA shrinks when unfolded proteins accumulate. Instead of adding a phosphate to another protein, Ire1 prompts removal of a chunk of Hac1's mRNA (HAC1). Additional work by Walter, which was confirmed and extended by Mori, established that HAC1 precursor mRNA contains an internal stretch of 252 genetic letters that is eliminated to supply the blueprint for active Hac1.

A canonical molecular machine splices sequences from precursor mRNAs and operates in the nucleus. The plot thickened when Walter showed that this apparatus does not act on HAC1 mRNA. Instead, he found, the severed HAC1 mRNA is stitched together by a cytoplasmic enzyme that normally joins the two components of a different type of RNA, transfer RNA.

The search was now on for an enzyme that excises the middle piece of the HAC1 precursor mRNA. Inspired by a related protein's behavior, Walter showed that the cytoplasmic segment of Ire1, which contains the kinase and an additional stretch of protein, could cut HAC1 precursor RNA at the expected sites. Then he demonstrated that the splicing reaction could occur in the test tube with only two enzymes: Ire1 cleaves the HAC1 precursor mRNA at both splice junctions, and the transfer RNA ligase sews them together.

Mammalian Systems Unfold

As these details of the yeast UPR were materializing, researchers were struggling to gain traction in the mammalian system. In 1998, Mori unearthed a sequence that was common only to genes that fire up in response to unfolded ER proteins. This element rouses several UPR target genes, he found. Furthermore, a human protein called ATF6 binds to this DNA motif and activates adjacent genes.

Mori noticed that an overabundance of unfolded proteins incites conversion of full-length ATF6 to a smaller version; the large form dwells in the ER, whereas the trimmed one resides in the nucleus. This and other work suggested that excess unfolded proteins trigger release of a portion of ER membrane-bound ATF6. The liberated fragment travels to the nucleus and activates transcription of UPR target genes.

While Mori was discovering and elucidating ATF6's role in the UPR, David Ron (New York University School of Medicine) and Randal Kaufman (University of Michigan Medical Center) found mammalian versions of Ire1, which share fundamental functional features with their yeast cousin. Three years later, Mori and Ron identified the human and worm versions of yeast Hac1, a protein known as XBP1.

In the meantime, near the beginning of 1999, David Ron and Ron Wek (Indiana University School of Medicine) had independently uncovered a third arm of the UPR, which relies on a protein called PERK. Like Ire1 and ATF6, PERK also lies across the ER membrane. Furthermore, its ER domain resembles that of Ire1. On the cytoplasmic side, a protein kinase segment of PERK adds phosphates to a particular protein, which then impedes translation of mRNAs. As a result, fewer proteins enter the ER, thus lightening the folding load.

Strength in Numbers

In the last ten years, Walter, with UCSF colleague Robert Stroud, has peered more closely at Ire1 activation with X-ray crystallography. Previous work by Mori, Walter, and others, had suggested that UPR induction causes Ire1 molecules to snuggle up in the membrane. By studying yeast Ire1, Walter and Stroud provided an atomic-level rationale for those results and illuminated details of the reaction.

In addition to providing assistance during protein folding, BiP attaches to Ire1 on the side that lies within the ER; when BiP falls off, naked Ire1 molecules pair up and create grooves that bind the unfolded proteins, Walter and Stroud suggest. Multiple Ire1 duos then congregate to form higher order structures; such association rearranges their cytoplasmic segments, positioning them so they can grab and then snip the HAC1/XBP1 mRNA, according to the model.

Researchers are still uncovering layers in the UPR. For example, Ire1 governs ER membrane synthesis and a system that shuttles recalcitrant unfolded proteins from the ER to a cellular incinerator. Even so, the unfolded protein burden sometimes surpasses the cell's management capacity. That situation can trigger cell suicide, which obliterates unhealthy cells that might otherwise wreak havoc. Investigators are deciphering how the Ire1, ATF6, and PERK branches of the pathway help cells make life-and-death decisions. Many scientists are now pursuing ways to harness the UPR for medical advantage. Certain forms of some inherited diseases that cause elevated cholesterol levels, cystic fibrosis, and retinitis pigmentosa produce abnormal proteins that do not fold properly and overwhelm the UPR.

Walter and Mori have unraveled a process with numerous unusual features. Their work has unlocked a multi-layered, highly choreographed system that lies at the heart of normal cellular function.