

ASCB AWARD ESSAY

Walking Along the Serendipitous Path of Discovery

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Deciphering of the molecular mechanism of the “unfolded protein response” (UPR) provides a wonderful example of how serendipity can shape scientific discovery. Secretory and membrane proteins begin their journey to the cell surface in the endoplasmic reticulum (ER). Before leaving the organelle, proteins are quality-controlled, and only properly folded proteins are transported onwards. The UPR detects an insufficiency in the protein-folding capacity in the ER and in the ways of a finely tuned homeostat adjusts organelle abundance according to need. If the protein-folding defect in the ER cannot be corrected, the UPR switches from a cell-protective to a cell-destructive mode and activates apoptosis in metazoan cells. Such life or death decisions position the UPR in the center of numerous pathologies, including viral infection, protein-folding diseases, diabetes, and cancer. The UPR proved to be a rich field for serendipitous discovery because the molecular machines that transmit information about insufficient protein folding and activate appropriate gene expression programs function in unusual, unprecedented ways. A key regulatory switch in the UPR, for example, is a cytoplasmic, nonconventional mRNA splicing reaction, initiated by a bifunctional transmembrane kinase/endoribonuclease.

Students entering graduate school often ask for the “recipe for success.” Yet looking back at the events that shaped my own career, both personal and scientific, I continue to be amazed by the convoluted paths, which are neither linear nor predictable. This is a difficult message to transmit to those embarking on a research career, and it invariably prompts the next question: “So you say that our success in science will be determined by dumb luck?” Not so—it’s *serendipity*, a word that much better describes our path to discovery. It is not uncommon that initially disappointing turns lead to unexpected outcomes that then miraculously align to appear as a progression of incredibly good fortune.

The word serendipity was coined in 1754 by Horace Walpole, Earl of Orford, who in a letter to a friend recounted the fairy tale of the “The Three Princes of Serendip.” As the story tells, serendipity is a very particular kind of luck. In the country of Serendippo, in the Far East, a great king sends his three sons on a journey to experience the world. One day, while walking down a deserted road, they meet a camel driver, who laments that he has lost his camel. The princes wonder if he is referring to the lame camel that is missing a tooth, is blind in its right eye, and is carrying honey on its right and butter on its left side. The camel driver promptly has them arrested, because—obviously—they know so much about the camel that they must have stolen it.

But the camel is quickly found, and it turns out that the princes had not even seen the animal. Rather, they had concluded all of their knowledge from observations they made while traveling down the road. The tracks showed the

prints of only three feet, the fourth being dragged, indicating that the animal was lame. The grass had been eaten on one side of the road where it was less lush than on the other, and the princes deduced that the camel must be blind to the other side. Because there were lumps of chewed grass on the road, presumably they had fallen through the gap left by a missing tooth. Ants had been attracted to melted butter on one side of the road and flies to spilled honey on the other. Serendipity thus defines a process of “discovering something by accident and cleverness while investigating something quite different,” often leading to unexpected insights. The word thus marvelously portrays the detective work, the deciphering of clues derived from indirect observation, that defines our everyday pursuits in experimental science.

Some anecdotes that accent my laboratory’s studies on the unfolded protein response (UPR) powerfully illustrate the concept. Unraveling the molecular details of this intracellular signaling pathway—conserved in all eukaryotic cells—has occupied our efforts and imagination for the last 15 years.¹ Proteins that enter the endoplasmic reticulum (ER) have to fold in this compartment into their ultimate functional state. Antibody molecules, for example, are assembled in the ER from light and heavy chains, sugars are added and disulfide bonds are formed. All of these essential processes are helped by resident proteins in the ER, such as molecular chaperones and protein modifying enzymes.

When a cell has insufficient ER, and therefore senses an overload of proteins entering a compartment that is too

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¹ I wish to acknowledge implicitly the many other labs that have contributed significantly to our understanding of the UPR, including the co-discovery of the ER kinase Ire1 by Katsutoshi Mori, Mary-Jane Gething, and Joe Sambrook. For further reading on the subject, see Ron and Walter (2007); Lin et al. (2007); and Bernalles et al. (2006).

small to function normally, protein folding is compromised and unfolded proteins accumulate. This imbalance is detected, and a signal is sent to the nucleus where numerous appropriate genes are switched on to produce more ER. In this way, the UPR allows the cell to adjust the amount of ER according to need. It is one of the many homeostatic mechanisms by which cells keep their various constituent parts in proper balance.

We started investigating the UPR by asking a very simple question: how does the nucleus know what's happening in the lumen of the ER? There is at least one membrane barrier that separates the two compartments, and somehow the information has to be transmitted across it. Inspired by the late Ira Herskowitz, two graduate students in my lab, Jeff Cox and Carolyn Shamu, set out on a pioneering genetic adventure. Jeff and Carolyn isolated yeast mutants with defective communication between the ER and the nucleus.

We cloned the genes and ended up with a transmembrane kinase. This was an exciting result, because in animal cells, transmembrane kinases form a huge family and have been studied extensively. We know that they are signal transduction devices that lie in the plasma membrane and monitor changes in the environment. Our transmembrane kinase does not sit on the cell surface, but spends its life in the ER membrane, where it senses the protein-folding status in the lumen and then transmits that information across the membrane. We also identified a transcription regulator that up-regulates those genes that are necessary to make more ER.

So within a short period, we had constructed a plausible hypothesis of how the UPR works. Because all kinases talk to other molecules by transferring phosphate groups to them, we postulated that our ER-resident transmembrane kinase would do the same and ultimately activate the transcription regulator in an analogous manner.

A perfectly reasonable hypothesis, yet nothing could be further from the truth. While wandering down a logical path of testing our hypotheses, Jeff Cox observed signs by the side of the road, which he was not in quest of. In particular, he made the serendipitous observation that when the transmembrane kinase was activated, the mRNA that encodes the transcription factor changed in size; it got smaller! That made no sense whatsoever, and I recall that my first reaction was one of disbelief. Jeff would not have been the first student in my lab who showed me a sample of degraded RNA. But Jeff persisted, and he proved to me and the world that activation of the UPR specifically triggers the change in the mRNA. He discovered that the mRNA becomes spliced: a small piece of the mRNA is excised, and the ends of the fragments generated are then stitched together again. And because the piece of the mRNA that is removed blocks its translation, only the spliced mRNA can produce the transcription factor. In this way, the splicing reaction is a switch that turns the UPR on or keeps it off.

This was an exciting discovery because there is no other example of an mRNA splicing reaction used in a signaling pathway. But even more exciting, this splicing reaction follows none of the rules of normal mRNA splicing, which occurs in the nucleus and uses small RNAs and dozens of different proteins. By contrast, the UPR splices the transcription factor's mRNA in the cytoplasm on the surface of the ER, and the reaction is carried out by two enzymes only. It turns out that one of the two enzymes is our transmembrane kinase, which surprisingly harbors not just one, but two distinct enzymatic activities. In addition to being a classic kinase with all of the molecular bells and whistles one would expect to transfer phosphate groups, it also is a highly specific endoribonuclease, which cleaves the tran-

scription factor's mRNA precisely at both splice junctions to remove the intron.

The UPR requires only one additional enzyme, an RNA ligase that we also had identified genetically, to put the appropriate pieces back together, completing the splicing reaction. Ironically, until Jeff discovered the mRNA splicing reaction, we had completely ignored the ligase, because it made no sense whatsoever why an RNA ligase would be involved in signaling mediated by a protein kinase. But suddenly, it all came together in unprecedented and completely unpredictable ways.

The UPR is a marvelous example of how Mother Nature experiments with the molecular modules that She has at Her disposal. Evolution tinkers with their structures and rearranges them in seemingly endless combinations and unexpected ways. It is up to us to interpret the clues properly, and, most importantly, we can only succeed if we free ourselves from conventional thinking. Can a kinase also be a nuclease? Why not? As Pasteur observed, chance favors the prepared mind.

With that recipe in mind, the UPR story continues to yield new discoveries. We learned, for example, that the UPR kinase itself is the only client to which it transfers phosphates. Kinase molecules are brought together when unfolded proteins bind to them in the lumen of the ER, and the kinase becomes phosphorylated by its juxtaposed neighbors. With our long-term collaborator Bob Stroud, we determined the crystal structures of relevant domains and have developed hypotheses of how unfolded proteins could bind and stitch the transmembrane kinases together into elaborate oligomeric assemblies. We now think that the primary function of phosphorylation is to stabilize such oligomeric assemblies. Only in its oligomeric state is the RNase turned on so that it can carry out the splicing reaction, forming little splicing factories on the ER membrane. We can directly watch these molecular arrangements in the fluorescence microscopes using the powerful fluorescent protein tags for which last year's Nobel Prize in Chemistry was awarded. Yet as always, we stand ready to change our views by the next unexpected result that serendipity may bring our way.

Evolution conserved many of the bizarre turns and twists that we discovered in yeast. In cells from multicellular organisms, however, the UPR has an important additional feature. As I mentioned before, the UPR is triggered by insufficient protein folding capacity in the ER. The cell then attempts to fix the problem by making more ER. Physiologically, this is a very important process, because the vast majority of the protein molecules by which cells communicate are made in the ER, including cell surface signal receptors and secretory hormones that carry information throughout the organism.

If these crucial communication devices malfunction, chaos ensues. Cells would receive garbled information and perhaps be misinformed about their location in our bodies or what they are supposed to do. As a result, they may act in an uncontrolled way, become selfish, migrate at will, and grow and divide in the wrong places—a condition that we know as cancer. The UPR in multicellular organisms has a safety mechanism built in: if protein folding in the ER remains problematic and cannot be mitigated by the UPR, cells will induce a suicide program. Rather than becoming a rogue cell that potentially endangers the whole organism, such cells kill themselves. The UPR therefore makes life/death decisions in mammalian cells, a fact that places the pathway in the center of many different human diseases.

Viruses, for example, use the UPR to make more membrane to enclose themselves. If we could manipulate the

pathway without harming the host organism, we might be able to develop novel, broad-spectrum antiviral drugs. Diabetes results in death of the insulin-producing beta cells in the pancreas. It is thought that cell death is triggered by the UPR because cells cannot cope with the increasing demand to fold more insulin in the ER. Abundant evidence suggests that many cancers have the UPR turned on to sustain their rapid growth, especially if they are derived from cells that are programmed to secrete large amounts of protein. Our ability to manipulate the pathway may thus prove useful for novel approaches to cancer therapy.

Personally, I would consider it a crowning highlight of my career if some aspects of the basic knowledge that we have accumulated over the years are translated into a tangible benefit for mankind. Yet importantly, none of these tremendous opportunities were obvious when we started on our journey; they only emerged gradually as we playfully and fervently followed the turns of our meandering and serendipitous path.

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