Lasker Award Winner **Peter Walter**

Peter Walter, Professor at the University of California, San Francisco (UCSF), and Kazutoshi Mori, Professor at Kyoto University, share the 2014 Lasker Basic Medical Research Award for their work that resulted in identification of key components of the unfolded protein response (UPR). Walter identified the IRE1 component of the UPR in 1993, shortly after starting his laboratory at UCSF.

What prompted you to study the unfolded protein response (UPR) in general?

Basic curiosity. At the time we entered the field, it was clear that protein misfolding inside the endoplasmic reticulum (ER) lumen elicited a transcriptional response. Thus we asked a very simple question: how does the gene expression program in the cell nucleus know what is going on inside the ER? It was clear from first principles that a signaling pathway must exist which senses conditions inside the ER, transmits the information across at least one membrane and ultimately drives a transcriptional response. We then set out to find components that would map onto this pathway.

What particular biological question were you seeking to answer that led you to Ire1, and were a lot of people seeking to answer the same question?

Our overarching goal was to learn more about intracellular communication. While walking down the hallway as a graduate student in the cell biology department at Rockefeller University, I passed daily by some of the most wonderful electron micrographs of George Palade. I was always fascinated by the question of how cells can be so different from one another. How does a muscle cell know how many mitochondria it needs? How does a secretory cell know when it has enough ER? All cells must regulate the abundance of their components and organelles according to need, and such regulation must be critical as cells become specialized during development. We realized that this was a wide-open question with very few people working on it. Looking at ER-nuclear communication seemed to be a good and tangible way to glean new insights into the molecular machineries that carry out such regulation.

How did your experimental approach differ from that taken by others?

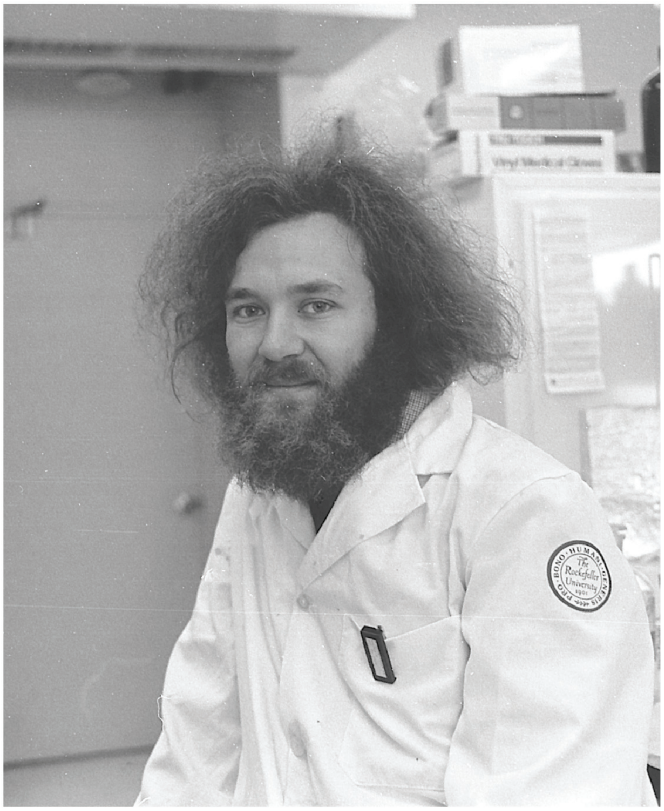
I started my studies as an organic chemist and then did my PhD work as a biochemist and cell biologist working in Günter Blobel's lab on the signal recognition particle. After I moved to UCSF [the University of California, San Francisco], I was immersed in an institutional culture in which the "awesome power of yeast genetics" was central. The late Ira Herskowitz was a strong promoter of applying genetic approaches to virtually every problem. Ira became a good friend and career-shaping mentor, and I became fascinated by the idea of experimenting with what genetics could do for us. We started timidly by playing with the signal recognition particle in yeast but the biochemistry always remained ahead, and we never really got much traction to make major new discoveries. For the UPR, by contrast, genetics in the hands of two wonderful adventurous graduate students, Jeffery Cox and Caroline Shamu, fulfilled its promise and opened the door to an astounding new field. Kazutoshi Mori was also seduced by yeast and similarly approached the UPR from a genetic angle. In retrospect, it is wonderful to see how our initially parallel, in fact competing, approaches evolved into two continuously intertwined paths that alternately confirmed and complemented each other and moved the field at an amazing pace.

Ire1 turned out to be a type I ER transmembrane protein. Is that what you expected it would be?

We had no idea what to expect, which is one of the beautiful aspects of genetic methods. You throw a wide net, unbiased, and then find what you screened for. Our screen could have just as easily identified the Hac1 transcription factor or tRNA ligase, which we discovered in later screens. These components would have been difficult to place. Indeed, as we learned later, Kazu Mori's screen also produced the tRNA ligase. In the absence of any knowledge that there is an RNA-processing step in the pathway, these investigators could not make any sense out of it, and it was not published. But as good luck would have it, with Ire1, we and Kazu independently found a protein with features that immediately suggested what its role might be in communicating a signal from the ER lumen. As [Ire1 is] an ER-resident transmembrane protein with a substantial lumenal domain and cytosolic kinase domain, predicted from its sequence, we drew conceptual parallels between Ire1 and growth factor receptors in the plasma membrane of mammalian cells, suggesting that Ire1, likewise, acts as a signal transduction device that effects communication across a membrane.

Once you identified Ire1, you must have generated hypotheses about how it was functioning in the UPR. Were your initial hypotheses correct?

Our first hypothesis was guided by the principle of Occam's razor, postulating the simplest plausible model consistent with established principles. For Ire1 this meant that we thought of how its kinase domain would be used to pass a signal on by transferring phosphate from ATP to some other component. Our first paper rested on the homology of Ire1's kinase domain to CDC48, as did Kazu Mori's first paper on Ire1. In the end, this view turned out to be wrong, but this realization required insights that we just did not yet have at the time. It was only after we discovered the



Peter as a graduate student.

unusual properties by which Ire1 regulates the transcription factor Hac1 that we were forced to break the stranglehold of preconceived models. I am still baffled by the abundance of unexpected phenomena that define Ire1 signaling. We and other investigators pieced the pathway together over the many years that followed. First, we found that Hac1 is not made in cells unless Ire1 is activated by unfolded proteins in the ER. Second, the *HAC1* mRNA is spliced in an unprecedented way. The reaction happens in the cytosol on the surface of the ER and is mediated by two enzymes only, Ire1 and tRNA ligase, with no involvement of the classical spliceosome. Third, Ire1 does not need to transfer any phosphates; it uses its kinase domain in a novel way as a conformational module that links Ire1 oligomerization to its activation. Fourth, the salient features of this unusual signaling machine are conserved from the budding yeast *Saccharomyces cerevisiae* to mammalian cells. And fifth, that despite this incredible conservation, another yeast, the distantly related fission yeast *Schizosaccharomyces pombe*, uses a completely different mechanism by which Ire1 does not splice *any* mRNA to induce a transcriptional program to increase the ER's protein folding capacity, but rather degrades select mRNAs on the ER surface to decrease the protein folding load in the organelle's lumen. Our current state of knowledge is a result of persistent detective work taking more than 20 years. The pathway shows how Mother Nature haphazardly redeploys modules proven to be useful in elegant, yet often new and completely unpredictable, ways.

How important was the crystal structure of the Ire1 lumenal domain, which you solved with Robert Stroud in 2005, in advancing study of the UPR?

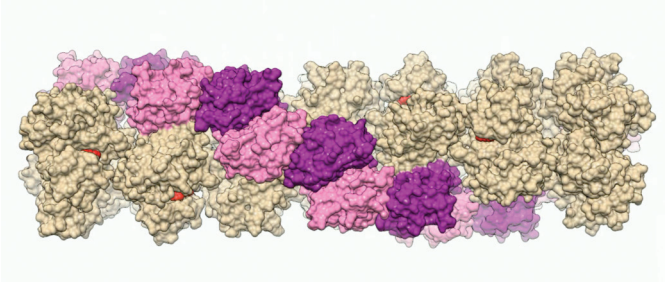
Our structure of the Ire1 lumenal domain was another 'ah-ha' moment. Structural biology is such a powerful way of looking at biological events because it can guide our thinking very profoundly. When I joined the faculty at UCSF in 1983, I participated in Robert Stroud's crystallography graduate class. We soon established a lasting collaboration that over the years punctuated our work with tangible images of what our favorite molecules look like in their three-dimensional beauty. The structure of the Ire1 lumenal domain was particularly revealing. Crystallizing as a two-fold symmetric dimer, two Ire1 lumenal domains join such that they form a deep groove that architecturally resembles that found in MHC [major histocompatibility complex] molecules, which bind peptides to display on the cell surface for surveillance by the immune system. This intriguing analogy suggested to us that Ire1 may bind directly to stretches of unfolded proteins. Being flexible and loosely structured, unfolded proteins can reach the depth of this groove, where they then act as agonists to activate Ire1 oligomerization and signaling. We have since collected numerous pieces of supportive evidence for this idea, which ultimately suggests that a protein, even before it has been locked into a folded structure, can be an active participant in a signaling pathway.

How did the community react to you initial conclusion about Ire1? Was there resistance or disagreement?

As long as Ire1 was viewed as a 'generic' transmembrane kinase, it was perfectly accepted. Even the legendary 'Reviewer #3' had no major objections to our first papers. As soon as things became less orthodox and we had to break current paradigms, things became more contentious. I recall vividly when my graduate student Carmela Sidrauski returned from a meeting of the RNA Society, where she first presented her discovery of a role for tRNA ligase in the UPR. This community was quite hesitant to consider the idea that tRNA ligase, which at the time was thought to function exclusively in the nucleus and on pre-tRNA only, might also participate in a cytosolic mRNA splicing event. Carmela had isolated an allele of tRNA ligase that was defective in the UPR and *HAC1* mRNA splicing yet left tRNA splicing perfectly intact. Still, Carmela was told that her results *must* arise from some indirect pleiotropic effect of a yet undetected defect in pre-tRNA splicing. It was a challenging experience for a graduate student, yet in profound ways it helped shape Carmela to become the exceptional and self-confident scientist she is today.

How are the various UPR pathways altered in disease? Are efforts underway to target these pathways for therapeutic purposes?

The UPR has been linked to numerous diseases, and we have great hope that the fundamental research on its mechanism will be foundational for therapeutic intervention. By controlling the protein folding capacity of the ER, the UPR ascertains that the fidelity of membrane proteins displayed on the cell's surface or proteins secreted from cells is maintained at high standards. If this goal cannot be reached, that is, if unfolded protein stress in the ER remains unmitigated, the cell commits to apoptosis. Thus, rather than displaying misfolded and potentially malfunctioning proteins, leading to a rogue cell no longer able to send or interpret signals properly, the cell sacrifices itself to protect the organism. It is this life-or-death decision made by the UPR that puts the response in the center of



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Crystal structure of active, oligomeric yeast Ire1 kinase (gold) and RNase (purple) domains. The kinase active (ATP-binding) site is filled with a small molecule Ire1 activator (red). Seven homodimers form one turn of the helical oligomer.

many diseases. Thus, while we think of the UPR in health as a finely tuned homeostat that adjusts protein folding capacity in the ER to the needs of the cell, it emerges in disease as either an executor that kills cells that we would rather have stay alive or, conversely, as a protector that keeps cells alive that we would rather have die. Examples of the first are protein folding diseases, such as retinitis pigmentosa or type 2 diabetes, in which retinal cells or pancreatic beta cells die because they cannot handle the unmitigated accumulation of misfolded mutant rhodopsin or the vast load of proinsulin. Here, preventing cell death might be of therapeutic value. Conversely, many cancers, especially those derived from secretory tissues, use the cytoprotective arm of the UPR to promote their growth, as do enveloped viruses that co-opt the pathway to their own good. In these conditions, sensitizing the UPR to promote cell death might prove helpful. To explore whether there is a therapeutic window in which UPR manipulation can do more good that harm, we and other groups have developed potent small-molecule tool compounds to modulate the individual UPR branches pharmacologically. We are now employing these compounds in various disease models.

At the time you discovered Ire1, did you have any idea that there were two more integral ER membrane proteins that mediated other aspects of the UPR (ATF6 and PERK)?

We did not have any idea of additional UPR branches, but it came as no surprise that metazoan cells evolved further bells and whistles adapting the bare-bones pathway we found in yeast to the more complex physiology of multicellular organisms. Even today, Ire1 remains the only known ER unfolded protein sensor in yeast. Rather than immediately venturing into more complex systems, we decided to focus on mechanism and study yeast Ire1 in considerable depth. This decision paid off profoundly; for example, our insights into the unusual use of Ire1's kinase domain and all of the initial enzymological and structural work resulted from studies of yeast Ire1. In the meantime, David Ron and Ron Wek discovered PERK and Kazutoshi Mori discovered ATF6 working in parallel UPR signaling branches. Their findings advanced our understanding of the UPR from that of a linear pathway into that of a complex signaling network, in which the target genes downstream of Ire1 in yeast have been distributed between three variously interconnected branches. Moreover, we discovered that the regulation of Ire1 itself became more complex as cells evolved, acquiring a timer in the form of an additional 'attenuated' state assumed by Ire1 after prolonged, unmitigated ER stress. We now think that the decision point that governs whether the UPR remains cytoprotective, trying to bring the system back into homeostasis, or engages the suicide machinery, lies in the relative timing of the three UPR branches.

What do you think are the most important as-yet-unanswered questions about the UPR, and do you think that new technologies will help us answer them?

I have an endless list of unanswered questions, many of which concern the detailed mechanisms by which IRE1, ATF6 and PERK exert their regulatory functions. In the end, we would like to understand these molecules and the pathways in which they act as macromolecular machines with all of their moving parts defined, working in a signaling network in fully described and predictable ways. But, to quote Albert Einstein: "As the circle of light increases, so does the circumference of darkness around it. With every single, small advance, many more questions arise. Tangible, important questions include the role of oligomerization and phosphorylation at specific sites in juggling the splicing versus mRNA degradation functions of IRE1, the mechanism of UPR activation by changes in membrane lipid compositions, the code for unfolded protein recognition by each of the three sensors, the mode by which ATF6 activates, the molecular difference in UPR regulation in different cell types, and so on. Most importantly, we want to know how different cells weigh UPR inputs to arrive at a life/death decision: 'can I fix the problem and reestablish homeostasis, or do I need to kill myself?' The more we can learn in molecular detail how these decisions are made, the greater our chance will be that we can develop ways to manipulate them for therapeutic benefits.

Peter and Kazu Mori at 2005 Wiley Prize celebration. It was the first award they received together.

You and Mori, with whom you share this prize, have worked in parallel but independent pathways to unravel the molecular basis of the UPR. As this is not the first prize that you have won together, have you gotten to know each other over the years?

Kazu and I have developed a very nice and open relationship over the years in which we both feel comfortable to share ideas and reagents. We have met each other's families and connected well on a personal level. Unfortunately, we have not seen each other all that often, and when we did, our schedules were usually so packed that there was little time to socialize in a deeper way. But, it now seems that Kazu and I will spend much of this September together, first in New York and then in Hong Kong. I very much look forward to it.

I hear that you're a sculptor. Do you ever sculpt unfolded proteins? Or just properly folded ones?

One of the most impacting decisions in my career was choosing my partner, Patricia Caldera, to whom I have been happily married for 29 years. Patricia loves to cook, and her spaghetti very much resembles unfolded proteins—very occasionally sculpted as irreversible aggregates. But, as in cells, most are degraded quickly. I prefer to concentrate on more folded structures made from wood and metal. They are less sticky and last longer.