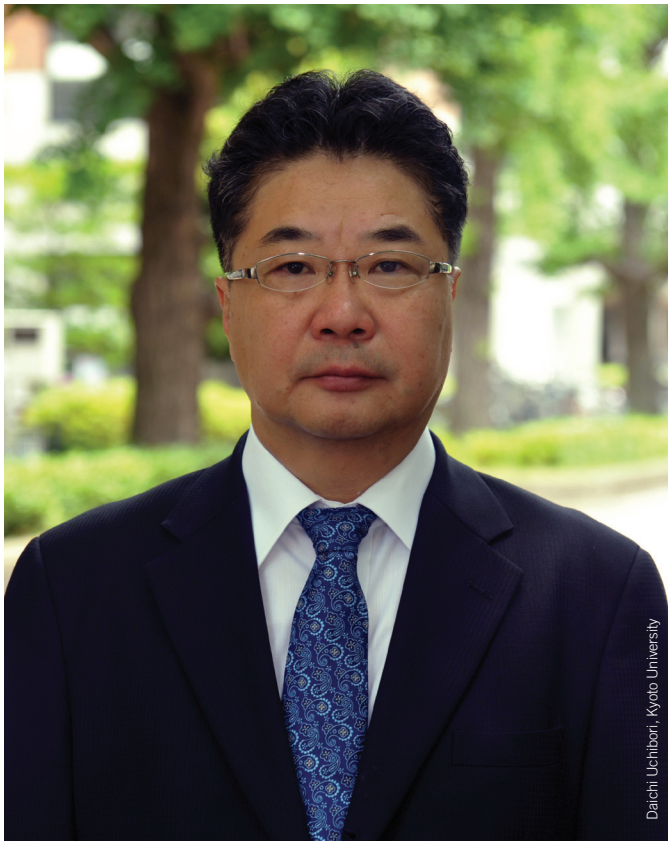

Lasker Award Winner **Kazutoshi Mori**

Kazutoshi Mori, Professor at Kyoto University, shares the 2014 Lasker Basic Medical Research Award with Peter Walter, Professor at the University of California, San Francisco. Walter and Mori are honored for their work identifying core components of the unfolded protein response (UPR). Mori made his first major discovery in this area — identification of the IRE1 component of the UPR—in 1993, while he was a postdoctoral fellow at the University of Texas Southwestern Medical Center.

What prompted you to come to the United States—to UT Southwestern—for your postdoctoral fellowship?

I first studied biochemistry in the lab of Dr. Ikuo Yamashina at the Graduate School of Pharmaceutical Sciences of Kyoto University. After graduating in 1985, I was fortunate to obtain a permanent position as an instructor in the lab of Dr. Kyozo Hayashi at a local university in Japan. Dr. Hayashi tasked me with biochemically investigating a factor secreted by cancer cells. I worked hard and published eight papers in the four years from 1985 to 1989. But I did not think this project was promising for my future. I wanted to do something more interesting and important, and also wanted to learn molecular biology. So I decided to quit that job and go to the USA. I sent application letters to several professors. Very fortunately, Mary-Jane Gething and Joe Sambrook accepted me. Joe is one of the three authors of the book *Molecular Cloning*, which is considered the bible of molecular biology. I became a postdoc in their lab at the University of Texas Southwestern Medical Center at Dallas in April of 1989, and it was there that I encountered the UPR. This happy meeting was to change my entire life.

What particular biological question were you seeking to answer that led you to IRE1?

It was well known that secretory proteins are synthesized on ribosomes bound to the endoplasmic reticulum (ER) and then secreted through the Golgi apparatus. The ER is considered to be an open corridor for the movement of newly synthesized secretory proteins. In 1988, Mary-Jane and Joe showed that glucose starvation causes protein unfolding/misfolding in the ER that triggers transcriptional induction of BiP/GRP78, a molecular chaperone in the ER. The next year they showed that this system is conserved in yeast *Saccharomyces cerevisiae*. So, essentially all eukaryotic cells cope with the accumulation of unfolded/misfolded proteins in the ER by inducing molecular chaperones in the ER. This nice homeostatic response is called the UPR. As the UPR is a transcriptional program coupled with intracellular signaling from the ER to the nucleus, it requires at least three components. First, a protein must somehow detect the presence of unfolded/misfolded proteins in the ER and transmit this ER stress signal. Second, a protein must activate transcription of the BiP gene in the nucleus. Third, the event in the ER must be connected with the event in the nucleus. However, we did not know anything about these components at that time.

What experimental approach did you take, and was it challenging?

I first characterized the promoter region of the yeast BiP gene, a target of the UPR, and identified a 22-base-pair *cis*-acting unfolded protein response element (UPRE), which is responsible for transcriptional induction of the yeast BiP gene in response to ER stress. I hooked the UPRE up to the *E. coli* β-galactosidase gene and incorporated this new reporter gene into yeast cells. My aim was to identify molecules involved in the UPR by carrying out yeast genetic screening. I had wanted to do yeast genetic screening for a long time, since I was a biochemist. In this experiment, not only yeast BiP but also this reporter would be induced when unfolded/misfolded proteins accumulate in the ER. So if yeast cells growing on plates containing X-gal were subjected to ER stress, wild-type yeast cells turned blue. However, mutant yeast cells defective in the UPR remained white. It was a simple blue-white selection in which hard work and good luck were needed. I mutated yeast genes randomly and screened 100,000 colonies. I succeeded in isolating three white mutant cells. Each yeast colony had a different mutation in IRE1. It took one year.

IRE1 turned out to be a type 1 ER transmembrane protein. Is that what you expected it would be, and was there precedent for this type of protein in this organelle?

As IRE1 is a type I transmembrane protein with a protein kinase domain in its cytoplasmic portion, we thought that it had a structure suitable for an ER stress sensor. For example, analogously to the many transmembrane-type protein kinases expressed on mammalian cell surfaces, we expected that IRE1 would somehow detect the presence of unfolded/misfolded proteins in the ER, become dimerized, trans-autophosphorylate and then transmit the signal.

Did others in the field initially resist your conclusions?

I believe people in the field welcomed the discovery of IRE1 made by Peter and I independently in 1993 because it opened up a new research field.

You returned to Japan after identifying IRE1, to set up your own lab. How did you decide what direction to take your research, and did anyone give you helpful advice?

After publication of the IRE1 paper in 1993, I came back to Japan and obtained a temporary position at the HSP (Heat Shock Protein) Research Institute. My new director Dr. Takashi Yura, who had just retired from Kyoto University, allowed me to continue working on the UPR. The next target was the transcription factor specific to the yeast UPR. Yeast researchers usually carry out multicopy suppressor screening to obtain the next gene of interest after isolation of yeast mutant cells; Peter did so. But Takashi advised me not to employ multicopy suppressor screening because IRE1 is a kinase, and there may be a kinase cascade downstream of IRE1. In other words, in this scenario if you do a multicopy suppressor screen you may end up obtaining many kinases, but not the transcription factor you want. Takashi told me, "Because the HSP Research Institute focuses on transcription, we do not want kinases. You should think of a method by which you can obtain your transcription factor directly." It was a very difficult task because I did not want to purify the factor biochemically. After struggling for one-and-a-half years, I finally came up with the idea of one-hybrid screening. This was the most important moment in my career. It worked very well, and I identified the HAC1 gene. Again, Peter and I identified HAC1 independently in 1996.

What prompted you to search for ATF6, and how did your approach differ from the approach you took to identify IRE1?

The HSP Research Institute existed for only seven years (1993–2000); financial support for the institute was divided, with half provided by the government and half by four Japanese pharmaceutical companies. Because the goal of the HSP Research Institute was to develop drugs by exploiting the heat shock response and the UPR, I decided to extend my work to mammalian cells.

Many homeostatic GRP genes are simultaneously induced when the UPR is activated. To do so, their promoter regions must contain common *cis*-acting elements. However, no one had identified such elements in mammals, unlike in yeast. It had also been noted that the ER stress–responsive promoters of mammalian GRP genes contain multiple CCAAT motifs. Aligning these CCAATs and neighboring sequences, Hiderou Yoshida in my group, now a professor at Hyogo Prefectural University, noticed that the structural motif CCAAT–9 nucleotides–CCACG is present in all GRP promoters. We named this the ER stress response element (ERSE). Discovery of ERSE was a major breakthrough because with it we could start the identification of mammalian UPR-specific transcription factors.

Peter Walter (left) and Kazutoshi Mori (right) at a meeting at Cold Spring Harbor Laboratory in May 2002.

April 1989 party celebrating a farewell to Kenji Kohno (center) and a welcome of Kazutoshi Mori (right). Left, Mary-Jane Gething.

Because the one-hybrid screening I used to obtain HAC1 is a modified version of two-hybrid screening, we could use it for identification of transcription factors from any species. Hiderou carried out one-hybrid screening and pulled out two positive clones among six million candidates. One encoded ATF6 and the second encoded XBP1. These results were published in 1998.

What led you to understand the function of XBP1?

After Peter and I unraveled the basic mechanisms of the yeast UPR (ER stress sensor IRE1 and transcription factor HAC1, which are connected by IRE1-dependent unconventional splicing of HAC1 mRNA), many people looked for their mammalian homologs. In 1998, Randy Kaufman and David Ron independently identified mammalian IRE1 proteins (now called IRE1α and IRE1β, respectively), but no one could identify a mammalian HAC1. In retrospect, after the completion of various genome projects, we realized that yeasts have HAC1 but metazoans do not. But we knew it must be there. Although it took some time, we discovered that the XBP1 that Hiderou obtained with one hybrid screening is the mammalian version of yeast HAC1.

What do you think are the most important as-yet-unanswered questions about the UPR?

The mammalian UPR consists of three pathways: IRE1, PERK and ATF6. Analyses of mice knocked out for components of each pathway showed the importance of UPR activation. PERK knockout mice suffer from diabetes because pancreatic β cells undergo apoptosis. IRE1 knockout causes embryonic lethality due to the failure of liver development. ATF6 knockout also causes embryonic lethality at a very early stage, which we found (using the medaka fish system) was due to the failure of notochord development. So, we can usually survive if we can activate the UPR, but we still do not know why knockout of the ubiquitously expressed ER stress sensors IRE1, PERK and ATF6 causes these different defects in different organs.

Furthermore, yeast cells have only IRE1 as an ER stress sensor but we humans have ten sensors. However, we do not actually know what

ER stress is. What kinds of proteins are unfolded/misfolded under what kinds of situations? We are seeking answers to such questions by investigating when and where the ten ER stress sensors are activated during normal development from zygote to adult using medaka fish. Then we will characterize ER stress in various disease models with the hope of using this information to cure disease.

How are IRE1 and ATF6 involved in human disease, and do you think that it will be possible to target them therapeutically?

The importance of the UPR is now recognized in a wide variety of fields. Researchers are trying to harness the UPR to help people with various diseases using their own particular strategies and methodologies. It is very difficult to summarize them. But I think we need more and more research because we still do not know what ER stress actually is: what kind of proteins are unfolded/misfolded in what kinds of situations. In addition, some cells, such as insulin-producing pancreatic β cells, are very sensitive to ER stress, but others, such as glucagon-producing pancreatic α cells, are resistant to ER stress. We have no idea of the basis for this difference. We need much more research to use the UPR to cure diseases.

Also, we now know cancer cells are very sensitive to ER stress because cancer cells need the UPR to survive under the very stressful situations of low oxygen and low nutrients. We will soon start the screening of chemicals able to kill cancer cells by inhibiting the UPR. Starting now, we will switch to both basic and applied research.

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

I met Peter for the first time at the American Society for Cell Biology meeting in San Francisco in December of 1996, when we identified HAC1 independently. Do you really want to know what happened there? I can say that severe competition between Peter and me moved the field forward quite rapidly. We respect each other and are good friends now.