People & Ideas

Feroz Papa: Saving cells from themselves

Papa studies adaptive and maladaptive responses to ER stress.

In humans, IRE1 is central to one of three pathways that sense the presence of misfolded proteins in the ER, initiating early steps of the unfolded protein response (UPR). ER stress induces IRE1 multimerization and activation of its cytoplasmic RNase domain, which then cleaves the RNA encoding the transcription factor XBP1. This cleavage step allows XBP1's translation into protein so that it can then activate transcription of genes that help the ER adapt to the presence of unfolded proteins. But when this response is overwhelmed, IRE1 activity somehow helps tip cells into apoptosis.

Feroz Papa is investigating why and how IRE1 goes from being an adaptive aide (1) to a suicide enabler (2, 3). He reasons that understanding the mechanisms that regulate IRE1 activity (1, 4) should enable the design of drugs (5) that short-circuit its maladaptive functions and give stressed cells a new lease on life. This could be important in treating the many human diseases that are driven by ER stress, such as diabetes mellitus. We called him at his office at the University of California, San Francisco (UCSF), to learn more.

PHOTO COURTESY OF FEROZ PAPA

Feroz Papa

THE SCIENCE BUG

Where did you spend your childhood?

I was born in Madras, India, which is now known as Chennai. My parents and I emigrated to Chicago when I was about three years old, but I returned to India and spent much of my early childhood living with my grandmother in Madras. I came back to the US when I was nine and have lived here ever since.

What subjects interested you in college?

My first college major was electrical engineering, but I'm not exactly sure why I chose that. I wasn't particularly interested in it. My real passion around that time was classical guitar, which I'd picked up in high school. I guess I was most interested in being a classical guitarist, but I knew that I'd started way too late to be good enough to do it for a living.

As a freshman at the University of Illinois I took a chemistry class with a fantastic teacher by the name of Steven Zumdahl. He had a program where students could apply to work in a research lab of their choice over the summers. Well, I was having so much fun at school that I decided I didn't want to come back home between my first and second years and that I'd rather stay in Champaign over the summer. So I applied for Dr. Zumdahl's program and got a spot working in George Ordal's lab over the summer.

I remember thinking that I wouldn't be doing anything of consequence; maybe washing some dishes or something. But George put me on a real project trying to find genes necessary for bacterial chemotaxis. It was during that summer, working in George's lab, that the science bug bit me.

> "I became interested in diseases of the secretory pathway, particularly diabetes mellitus."

Why did you decide to obtain an MD and a PhD?

I decided very early on that I would combine graduate school with a medical degree because I reasoned that one could learn a lot of human biology in medical school. I decided to do the combined program at the University of Chicago. So I first had two years of medical school, then took my board exams, and then I had to pick a mentor and go into a lab.

For a brief time I was in Vikas Sukhatme's lab, until he moved his lab to Beth Israel in Boston and I didn't follow him there. Meanwhile, I was a TA in a class that Mark Hochstrasser was teaching, and we started talking and really hit it off. I was fascinated by what he was studying: ubiquitin-dependent proteolysis in *Saccharomyces cerevisiae*. I started working on a project he gave me about a deubiquitinating enzyme called DOA4, and it just took off. Within about a year I had my first publication, an article in *Nature*. I guess at the time I may have thought this was the way it would always be. [Laughs] But it never works out that way.

A MEDICAL INTEREST

You returned to your medical training…

I agonized about whether I even wanted to do that, to tell you the truth, because it was such a big change to dive back into medical school culture. But then I decided to do it and go all out. I did a residency in internal medicine, which is what most appealed to me, because it covered the greatest spectrum of diseases and pathologies but allowed you to specialize later on. I applied and matched at UCSF. UCSF had a terrific program that supported a few years of a postdoctoral fellowship after a successful residency. After my residency I also did a clinical fellowship in endocrinology, where I became interested in diseases of the secretory pathway, particularly diabetes mellitus. That's why I chose a postdoctoral position involving the early secretory pathway.

And that was with Peter Walter, on IRE1 signaling…

Some really big papers had come out of Peter's lab on the key players in the unfolded protein response in yeast. They'd shown that IRE1 somehow sensed unfolded proteins inside the ER and that it somehow relayed a signal through its cytoplasmic kinase domain to activate its RNase domain, but the

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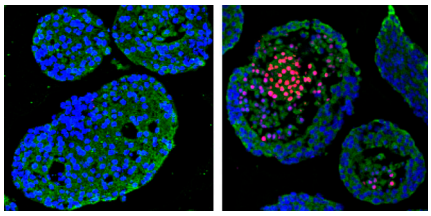

IMAGE COURTESY OF RAJARSHI GHOSH

Compared with control cells (left), mouse pancreatic islets undergoing ER stress (right) degenerate, lose insulin (green), and contain apoptotic cells (red).

details were murky. Initially, I was trying to find a way to conditionally inhibit IRE1's kinase activity with a small molecule. Using a rational protein engineering approach, I made a small space-creating mutation that is filled up with a cell-permeable designer kinase inhibitor. But this strategy actually yielded the opposite of what it was intended to reveal. When we used our inhibitor on the mutant protein, it turned the RNase on allosterically and was able to activate the entire unfolded protein response in yeast.

How did you react to that?

I didn't believe it at first. I spent a couple of months trying to refute it, thinking I'd messed up somehow and maybe there was some trivial explanation for this result. But every experiment I did confirmed that the protein is just wired in this crazy way, where the kinase is a conformational switch that allosterically activates the RNase. By the time I'd finished doing all these experiments, I realized I had most of the data I needed for a paper.

STRESSED OUT ER

When you started your own lab, you stayed at UCSF and also stuck with the problem of IRE1 signaling…

I had offers in other places, including a particularly attractive one at Stanford. But I'd made a lot of good contacts here, and I work closely with Scott Oakes, who was joining the university around the same time. So it made sense for me to stay.

And yes, I stayed with IRE1, although now we mainly study the mammalian orthologue. The wiring of the kinase and the RNase is basically the same in mammalian cells, but there is also strong evidence that ER stress is involved in many mammalian diseases, such as diabetes mellitus. I've become very interested in the idea that, if we could control the unfolded protein response with a drug, we could perhaps control its physiological outputs in a way that could enhance cell survival. Could we preemptively prime the ER to make cells perform better and survive better under ER stress?

Do you still work in yeast?

I still have some projects going in yeast. For example, we've used yeast to develop a reporter whose redox state can be read out as a proxy measure of ER stress. Yeast is very close to my heart as a model organism. It's nice to still have it in the lab, even though we mostly work in mammalian cells and mice these days.

> "I never forgot what I wanted to do when I was a postdoc."

IRE1 can have both adaptive and maladaptive responses to ER stress…

The parsimonious explanation for that is that, when stress remains high, the cell fails to close a negative feedback loop. IRE1 remains phosphorylated, or maybe its level of phosphorylation gets higher and higher, reaching some threshold beyond which the activity of the RNase climbs so high that it now becomes promiscuous. It relaxes its specificity towards the XBP1 transcript and now starts cleaving other messenger RNAs that are in close proximity to the ER membrane—in a process first described by Julie Hollien and Jonathan Weissman. Slowly over time, this depletes the ER of important factors that are needed for folding proteins, and the ER then becomes subject to functional and maybe even structural collapse. Our chemical genetic systems show that this is one outcome of extreme ER stress.

Another outcome of this nonspecific cleavage is that IRE1 starts cleaving RNAs that directly or indirectly contribute to inflammation and cell death. We've shown that, in cases of extreme ER stress, IRE1 starts cleaving a microRNA that targets the RNA for thioredoxin-interacting protein (TXNIP). This stabilizes TXNIP RNA, and TXNIP protein then promotes release of an inflammatory cytokine, causing inflammation and pyroptotic cell death. This pathway could be very important in diabetes, where islet cell ER stress and inflammation work together to advance disease. We've been working a lot on TXNIP and the consequences of its action.

My lab is also focusing on trying to cut off all these signals at the source, IRE1, through small molecule development. I never forgot what I wanted to do when I was a postdoc, which was to inhibit the protein with a small molecule. We already know there are kinase inhibitors that can allosterically activate IRE1's RNase, called type I kinase inhibitors, but I've been working with Dustin Maly, who's in Seattle, and Bradley Backes at UCSF to design kinase inhibitors that can allosterically inhibit the RNase as well. We've already characterized, optimized, and published about one group of inhibitors with this activity, which we call type II kinase inhibitors.

Do you still practice medicine?

I don't do much clinical work nowadays because I'm so busy with my lab. But I would love to someday be involved in a clinical trial in which I could contribute both the scientific concepts we have learned in my lab and my skills as a physician.

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Papa relaxing with his dog, "Pepper!".