The Scientist

Volume 22 | Issue 11 | Page 54

By Karen Hopkin

The Science of Stress

NIH's Gisela Storz has spent her career drilling down to the core of questions such as how bacteria respond to oxidants - work that has taken her in some unexpected directions.

© Jason Varney | Varneyphoto.com

Her initial model was deceptively simple. As a graduate student exploring *Escherichia coli's* response to oxidative stress, Gisela "Gigi" Storz proposed that a protein called OxyR interacts directly with potentially destructive oxidants, such as hydrogen peroxide, and then switches on the genes needed to neutralize the threat.

However, her colleagues were not impressed. "We thought her hypothesis was naïve," says James Imlay of the University of Illinois, who was a fellow student at the University of California, Berkeley at the time. "She was suggesting that this protein could directly sense hydrogen peroxide and then bind to DNA and act as a transcriptional regulator—that a single protein did the whole job. There was just no precedent." At a practice run of the talk that Storz was to present to her thesis committee as part of her preliminary exam, Imlay says, "we just tore her apart. In the time-honored, senior grad-student style, we criticized her up one side and down the other."

Related Articles

Making Pretty Pictures

2008 Lasker Awards announced

MicroRNAs: An emerging portrait

Of course, "Gigi passed her exam," he says. "And it turns out that every single prediction she made was correct."

Since her Berkeley days, Storz has continued to ask questions about OxyR. In the 1990s, as an independent investigator at the National Institutes of Health, she unraveled the detailed mechanism by which OxyR acts as a peroxide sensor and a transcriptional activator. At the same time, following up on another observation she made as a graduate student, Storz discovered a small RNA that controls gene expression, a finding that placed her at the forefront of the regulatory RNA field.

"She picks up a thread and follows it wherever it goes," says Imlay. "That's the way to do science. The way to be cutting-edge is not to look around and guess what might be the next big thing, but to stay with a problem and drill down deeper than anybody else does. Gigi has been at the forefront of all these fields by simply pursuing one question to the next."

Once upon OxyR

It all started with OxyR. Storz was recruited to the OxyR project in 1985, when her thesis advisor Bruce Ames got interested in studying how bacteria adapt to oxidative stress. "We knew that if you treated *E. coli* with low doses of oxidants, they became resistant to high doses of oxidants," says Storz. "And the question was: How did they induce these defenses?"

"Her findings have opened up a whole new way of thinking about the functioning of regulatory proteins." —Jon Beckwith

They set their sights on the cell's response to hydrogen peroxide, a chemical that can damage DNA. What they discovered is that the OxyR protein senses hydrogen peroxide by becoming oxidized by it. "So the protein is normally reduced in the cell," says Storz. In that state, it is not capable of triggering the whole oxidative stress-response program. "But when the cells are treated with hydrogen peroxide," she says, "OxyR becomes oxidized and it can then induce gene expression"—work that earned Storz her first *Science* paper (248:189-94, 1990).

"That was a real pioneering study," says former postdoc Michel Toledano of the

French Atomic Energy Commission (CEA) in Gif-sur-Yvette. Most cell biologists think of phosphorylation when asked how cells can regulate protein activity. "Gigi's work made people aware that protein oxidation could be another way to regulate protein function."

But Storz didn't leave the story there. After a brief postdoc at Harvard University in which she looked at the blue-light response in *Arabidopsis* with Fred Ausubel, Storz landed a position at NIH's National Institute of Child Health and Human Development in 1991. And she picked up where she left off with OxyR. As an independent investigator, Storz discovered that oxidation of OxyR leads to the formation of a disulfide bond between two cysteine residues in the protein—and that this reversible disulfide bond, and the large structural changes that accompany it, transform the protein into a transcriptional regulator (*Science* 279:1718-21, 1998; *Cell* 105:103-13, 2001).

"OxyR was the first transcription factor that was shown to be regulated by disulfide bond formation," says former postdoc Matthew Wood of the University of California, Davis. "Obviously disulfide bonds serve structural roles in proteins. But they were thought to be nothing more than that: Elements that maintain the tertiary structure of a protein so that it could function. What Gigi discovered was a transient disulfide bond that acts as a switch to activate or deactivate the protein."

"It was something that nobody had found before," says Jon Beckwith of Harvard Medical School. "A protein that switches back and forth between an active and an inactive state by forming disulfide bonds. Her findings have opened up a whole new way of thinking about the functioning of regulatory proteins."

The OxyR story also provides a satisfyingly complete picture of a bacterial regulatory network. "It's a gorgeous example, and one of the first examples, where we can track an environmental signal to what happens to a regulatory protein," says Susan Gottesman, chief of NIH's biochemical genetics section - and beyond, adds Leslie Poole of Wake Forest University School of Medicine. OxyR boosts the production of nine or ten proteins, including catalase and glutathione reductase—enzymes that eliminate hydrogen peroxide and help to reverse oxidative damage elsewhere in the cell. The protein also activates expression of glutaredoxin 1, an enzyme that reduces OxyR's disulfide bond and returns it to its inactive state. "It's a nice little loop that serves to shut the response back off," says Poole. "OxyR is fully activated by hydrogen peroxide within 30 seconds. And while it's oxidized it's activating transcription. But then it's slowly returned to its reduced state by glutaredoxin 1. That just makes so much sense in terms of the biology," says Poole. "And that's the Holy Grail. Figuring out right down to the molecule how a biological response works."

From stress to small

Her attention to the down-to-the-molecule detail has since taken Storz in an

interesting new direction—to the study of small, noncoding, regulatory RNAs. Again, the work traces back to her days at Berkeley, and to OxyR. "As a second year graduate student, I was carrying out a Northern blot analysis to look at the levels of the OxyR transcript," says Storz. Back then she wondered whether the amount of OxyR in the cell would increase in the face of oxidative stress. But the probe she used to look for OxyR mRNA was not entirely specific. So in addition to binding to the OxyR message, it consistently picked up the presence of another, smaller transcript. That transcript was only 109 nucleotides in length, says Storz, "but when cells were treated with peroxide, its expression was really strongly induced. The result was so striking, I was intrigued. What is this RNA doing?"

Storz poked at the problem over the years in her spare time as a grad student and postdoc, and then more pointedly when she got her own lab. She overexpressed the transcript, called OxyS. She knocked it out. She ran gels to identify proteins whose levels change when OxyS is abundant—or missing in action. And in 1997—12 years after she first spotted the little RNA on a Northern blot—Storz published a paper (*Cell*, 90:43-53, 1997) showing that OxyS base-pairs with mRNA molecules right next to the site where ribosomes normally bind. That interaction occludes the ribosome binding site and blocks translation of the message.

That kind of persistence is a hallmark of Storz's approach to science. "The project was so difficult, but Gigi wouldn't give up," says Toledano. "She was so stubborn. She just kept going, continually designing new experiments to look for the function of this small RNA. Because she knew this was important. She was onto regulatory RNAs before anyone else I know of." And her determination was matched by her meticulousness. "When Gigi does an experiment, it's perfect," he says. "I remember her protein gels soaking in the denaturation buffer. They were beautiful. And her results are always reliable. This is the paradox that characterizes her. She's rigorous with her experiments, but she's also adventurous in starting new projects. She always wants to study things that are unknown, that are completely new."

From small to really small

Storz has continued to pursue regulatory RNAs. In 2000, working with then-postdoc Karen Wasserman, she determined that an RNA called '6S' can control gene expression by binding to RNA polymerase. "This RNA had been around from the time when people first opened up cells. It was called '6S' because it sedimented at a particular size and little else was known about it," says Gottesman. When Storz and Wasserman determined that 6S regulates RNA polymerase, "that opened up a whole new world of what regulatory RNAs are capable of doing. It was a spectacular and totally unexpected finding."

In collaboration with Gottesman, Storz has continued to develop new approaches for discovering small, potentially regulatory RNAs on a genome-wide scale. In 2001, they discovered 17 new transcripts in *E. coli* that are likely to represent functional RNAs.

"Then we sat down and said, 'you pick one, I'll pick one' and we decided which ones we'd each like to pursue," says Gottesman. And their labs meet every two weeks to review progress and trade ideas, which Gottesman says "makes it a lot more fun."

In the meantime, Storz has set off in yet another new direction: identifying small proteins—really small proteins, 50 amino acids or less—that could have a regulatory function. "People didn't think about small RNAs until about 10 years ago," says Storz. "And small proteins constitute another group of molecules that people are ignoring or have missed." Again, the issue is an offshoot of her previous work. "When we characterized the small RNAs, we realized that a subset of them encodes these small proteins of 19 amino acids or 31 amino acids," she says. "So we've become interested in finding out: how many are there? And what are they doing?"

"It's a totally open-ended question," notes Gottesman. So far, Storz knows these small proteins are being translated, and that in some cases their production appears to be regulated. "So I think it's not just spurious random translation," she says. "I think they're likely to have real functions in the cell."

Time will tell, and Storz will likely be there when it happens. "There are some people who work on one promoter or one piece of DNA or one protein all their lives," says Gottesman. "Gigi is fearless about jumping into things where it's not clear what's to be found or how you'll get there. But she has a history of knowing what to follow up. I don't know how many things she hasn't followed. But the ones she has followed have ended up being beautiful stories."