PNAS PROFILE

Profile of Gisela Storz

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Gisela Storz has had a long and distinguished career as a microbiologist, but she says science did not capture her imagination as a child. As an undergraduate at the University of Colorado, Boulder, she realized how much she liked working in laboratories. "I was looking for a work–study job, and most of the jobs sounded really dull, with the exception of a job preparing reagents for a cell biology lab," says Storz. "I decided to try that job, and I found I really liked hanging out in labs," she says. Storz worked in a plant biology laboratory for the rest of her time in college.

Over the next 40 years or so, that sustained interest led Storz to elucidate how bacteria and yeast respond to oxidative stress and examine the regulatory role of small RNAs and small proteins. After her serendipitous discovery of one of the first small, regulatory RNAs, OxyS, she went on to discover and characterize several others. She discovered that some of these small RNAs also encode small proteins. Storz describes two such dual-function RNAs—AzuCR and Spot 42—in her Inaugural Article and an accompanying article (1, 2).

Storz is now a microbiologist at the Eunice Kennedy Shriver National Institute of Child Health and Human Development at the NIH and was elected to the National Academy of Sciences in 2012. In addition to her contributions to microbiology, she has tackled broad issues in science. "I feel very strongly that it is important to diversify science, especially at the higher levels and leadership levels and that the visibility of people of different backgrounds is important," she says. "I am devoting more and more time to that," she says.

Gisela Storz. Image courtesy of Ernie Branson, NIH.

Bacterial Transcription Factors

After Storz received a bachelor's degree in biochemistry in 1984, she decided to head west. "I went to the University of California, Berkeley for my PhD because I felt like there were a lot of options," she says. After two rotations in biochemical laboratories, it was her third rotation in a bacterial genetics laboratory that solidified her interest. "It was sort of a throw-away rotation, but I worked with Bruce Ames in bacterial genetics, and I really liked it, and so that's where I ended up," she says. "He was very hands off, so what it provided was the opportunity to pursue what I was interested in," she says.

During her doctorate with Ames, Storz discovered that the redox-sensitive transcription factor OxyR senses hydrogen peroxide stress in *Escherichia coli* oxidation responses (3). She also found that OxyR was regulated by reversible disulfide bond formation (4). However, it was the discovery of the OxyS small RNA that set the stage for the rest of her research career (3). "Just by serendipity I discovered a small regulatory RNA, which sort of set me on the trajectory of studying genes that have been ignored or that haven't been found," says Storz.

After Storz completed her doctorate in biochemistry in 1988, she spent a few years as a postdoctoral researcher with Sankar Adhya at the National Cancer Institute and with Frederick Ausubel at Harvard Medical School. "I did postdoctoral work on plants, but on the side and at night I kept playing with this OxyS RNA," says Storz.

In 1991, Storz joined the National Institute of Child Health and Human Development at the NIH and soon switched from plants to bacteria, in which she could continue studying OxyS. It turned out bacteria were a better fit for the questions she was interested in. "In graduate school, we were told to pursue a phenotype and understand that, but I've spent much of my career taking

This is a Profile of a member of the National Academy of Sciences to accompany the member's Inaugural Article, e2117930119, in volume 119, issue 10.

Published April 29, 2022.

genes that people don't know of and trying to figure out what their functions are," she says. "That's kind of [a] risky business, so it's been advantageous to do it in bacteria, where you can do experiments quickly," says Storz.

At the time, few small RNA molecules had been identified in bacteria, and little was known about their functions, but Storz was determined to find out.

From Small RNAs to Small Proteins

Over the ensuing decades at the NIH, Storz used a variety of methods—from genetics to bioinformatics and biochemistry—to determine the functions of small bacterial RNAs. "We realized that there are many more such RNAs than we initially appreciated," says Storz. "We try to … understand their functions," she says.

Storz has identified and deciphered the functions of several small regulatory RNAs. Additionally, she found that small RNAs are crucial to most regulatory circuits in bacteria and that the RNA chaperone Hfq is required to promote the base pairing of small regulatory RNAs and mRNA targets (4–6).

While characterizing small RNAs, Storz chanced upon a finding. "In the course of studying these regulatory RNAs, we realized there's a subset of these short transcripts that encode very small proteins." These proteins were not well studied because they did not turn up in many traditional biochemical assays and the genes encoding them were poorly annotated. "There's a reason they've been ignored, and they're very challenging to work with," says Storz.

Storz started looking for and characterizing these small proteins of less than 50 amino acids in *E. coli*. "With the small RNAs, we have a system down that has made studying them easier, but my lab has taken this new direction towards very small proteins, and it has been among the most challenging projects we've worked on," she says.

There are few tools available to detect or study the small proteins; even simple protein stains, such as Coomassie blue, may not work on them. "We often add tags to them to be able to stain them, but it's a challenge because the tag may be half the size of your protein or even five times the size of your protein," she says. "So, you just have to come up with workarounds, and I think sometimes people working on larger proteins don't appreciate how challenging it can be," says Storz.

In addition, the lack of annotation for the genes encoding the proteins makes them hard to identify in the genome. The small size of these proteins also makes them hard to identify by mass spectrometry and challenging to develop antibodies against. "There are definitely technical challenges, but I've also found it challenging to publish papers about some of the small proteins, as people haven't believed that they have functions," says Storz.

Undeterred by the challenges, Storz has not only elucidated some functions of the small proteins but has also described the interplay of the functions with those of small RNAs.

Finding Dual-Function RNAs

In her Inaugural Article and accompanying article (1, 2), Storz describes RNAs that have dual functions: they can act as small regulatory RNAs that base pair with target mRNAs, while encoding small regulatory proteins. "I am drawn to things that are a little bit different, and the idea that they have both base-pairing function and protein-coding function …was intriguing," says Storz.

Only a few such dual-function RNAs have been identified so far. Storz describes two such dual-function RNAs. One is a well-characterized base-pairing small RNA, Spot 42, which Storz found also encoded a 15-amino acid protein that appears to reinforce the activity of the small RNA.

The other RNA she describes regulates carbon use in *E. coli* and was originally identified as a noncoding RNA in the early 2000s. More recently, Storz realized, the RNA could also encode a short 28-amino acid protein, AzuC. "We detected it as a small protein, but then we realized that the phenotypes suggested it wasn't just acting as a protein and, in fact, it was also acting as an RNA. But to tease apart those separate functions took quite a while," she says. Interestingly, Storz found that the regulatory functions of the small RNA and the small protein competed with each other.

Storz showed that both Spot 42 and the AzuC RNAs act by base pairing with targets. "That was known already for a long time for Spot 42, but we also documented it for AzuC," says Storz. "But the biggest challenge in the small protein field is figuring out what the proteins do," she says.

Storz suggests that AzuC is an amphipathic helix present in the membrane as a way to recruit another protein to the membrane. "I think that could be a general role [that] small proteins have, and there's another example in *Bacillus subtilis*," she says. "These could be small widgets that bind to the membrane and then recruit other proteins."

Spot 42's function appears to be to bind to a transcription factor, cAMP receptor protein (CRP). "I suspect there might be quite a few of these really small proteins that bind to other transcription factors," she says. "The interesting thing is that CRP is one of the best-studied transcription factors and has been studied for decades, but nobody ever saw this small protein," says Storz.

Storz suggests that there may be many more such dual-function RNAs in bacteria and in eukaryotes. "I think one major takeaway is that these very small protein regulators exist and that there are probably many of them, and they're sometimes encoded by RNAs that themselves act as base-pairing RNAs," she says. The small RNAs and the small proteins they encode could have reinforcing or opposing functions. "So, there's different sorts of regulatory schemes you can come up with," says Storz.

Storz continues to hunt for small proteins in *E. coli* and is also pursuing a mechanistic understanding of the small proteins and small RNAs. The field is now growing rapidly, but Storz says she could not have imagined the discovery of the small proteins when she started studying *E. coli*. "When I started graduate school in the mid-80s, I thought everything was known about *E. coli* because it was so well studied," she says. "Yet, here we are, decades later, and we still don't even know all the genes."

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