

# Bacterial Regulation: Past, Present, and Future

*Many scientists are lucky to have one fundamental insight to their name. As recently noted by the National Academy of Sciences when they awarded her the 2015 Selman A. Waksman Award in Microbiology, Susan Gottesman, Ph.D., Co-Chief of CCR's Laboratory of Molecular Biology, has made at least two seminal discoveries about post-transcriptional regulation in bacterial cells: 1) she uncovered the critical role of protein breakdown and the energy-dependent proteases that govern this process, and 2) she co-discovered small RNAs and established their role in bacterial cell regulation. Each of these discoveries has presaged their more complex counterparts in eukaryotic cells. Gottesman continues to study molecular regulation in bacteria both for the general principles to be learned and for their particular applications to host infection and immunity.*

As often happens in science, Susan Gottesman took the first step toward one of her most significant discoveries through serendipity... and the persistence of a talented team.

By the early 1990s, the project to study protein turnover in *Escherichia coli* (*E. coli*), which she began as a newly hired investigator at the NIH in 1976, had grown into a pioneering role for her laboratory in the field of regulation by protein degradation. One of the unstable proteins her laboratory was studying—RcsA—regulates capsule formation. Thus, it was not terribly surprising that a plasmid containing the *rcaA* gene caused overproduction of the capsule and a “mucoid” phenotype. What was surprising was that disruption of the protein-coding region of the gene did not affect the phenotype.

“That observation sat for a number of years. It was clear we didn’t understand what was going on,” said Gottesman. “Then, Darren Sledjeski, Ph.D., came to the laboratory and took another hard look. He realized that the phenotype was not due to

the protein, or even the *cis*-acting regulatory elements, but was due to another gene on the plasmid. It encoded a small regulatory RNA that wasn’t translated into protein.” They named the element *dsrA* for “downstream of *rcaA*.”

Nadim Majdalani, Ph.D., joined the group as a postdoctoral fellow in 1995. “When I came to the laboratory,

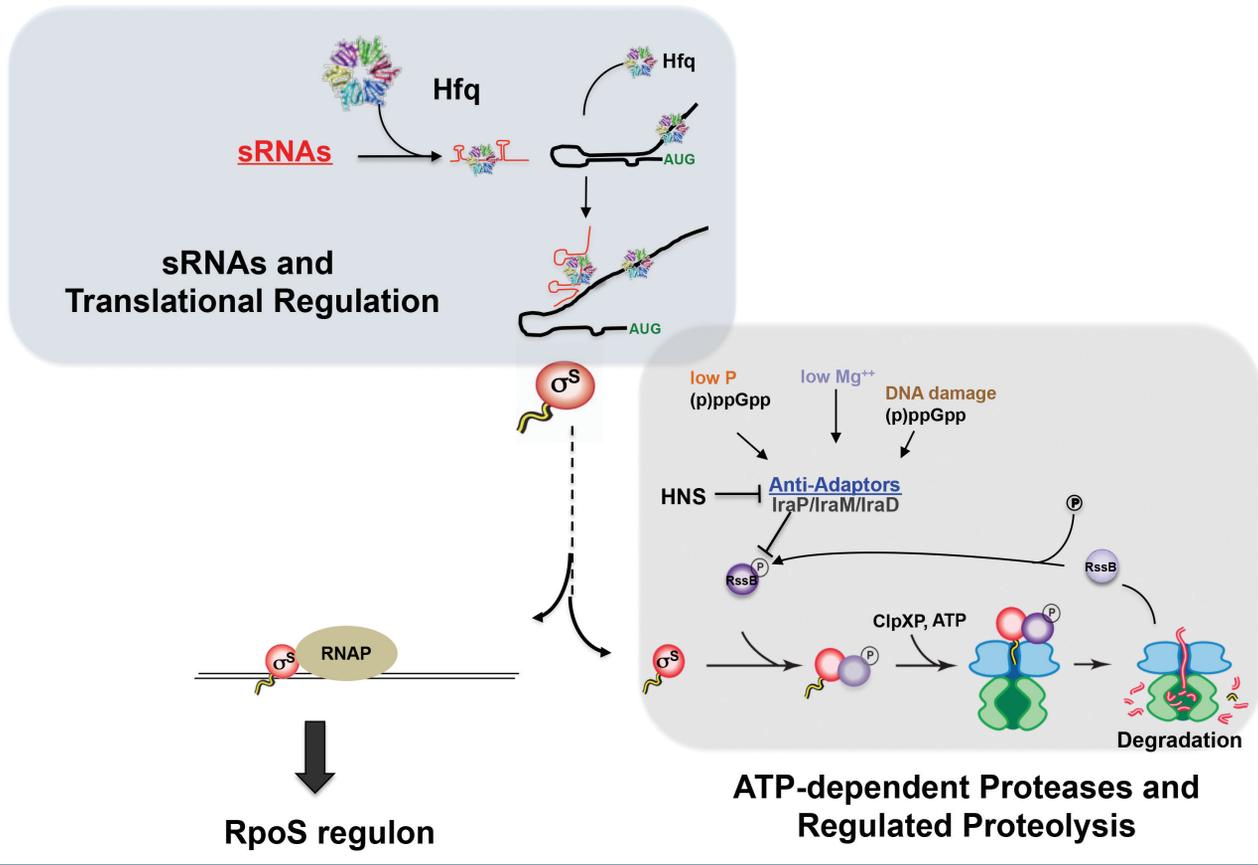
there was no such thing as a small RNA,” said Majdalani. He started working on the DsrA project to understand how it was creating the phenotype. Sledjeski and Majdalani’s work led to the discovery that it was both inhibiting the translation of a protein, H-NS, and positively regulating the expression of a transcription factor, RpoS.



(Photo: R. Buer)

Susan Gottesman, Ph.D.

## The Bacterial Response to Stress: Post-Transcriptional Regulation



“Here was one small RNA that could, on the one hand, repress and, on the other hand, enhance gene activation,” said Majdalani. He went on to demonstrate that DsrA was pairing with the mRNA encoding RpoS in such a way that it caused the release of an endogenous inhibitory loop within the mRNA and the resumption of translation.

The team published their findings on DsrA, a clear challenge to the existing dogma in molecular biology that RNA functioned as an intermediary between DNA and protein. But it was not clear how widespread this mechanism might be.

Large-scale, automated genomic screens were not available at the time, so Majdalani and Gottesman screened a library of genomic DNA for new small RNAs by creating summer projects for high school and

college students in the laboratory. Eventually, the work of multiple students led to the discovery of a second small RNA, RprA, which also regulated RpoS, but in response to different cellular stressors.

“The next big step in the evolution of the lab began with our collaboration with Gigi Storz,” said Gottesman.

### Building a New Paradigm

Gisella Storz, Ph.D., Senior Investigator in the National Institute of Child Health and Human Development’s Cell Biology and Metabolism Program, had come across her own small RNA, OxyS in 1985, the chance result of a flawed experiment to study the regulation of a transcription factor by oxidative stress. In 1997, she and her

postdoctoral fellow, Shoshy Altuvia, Ph.D., published the mechanism of its action, namely base pairing with mRNAs.

“We were both giving talks about our small RNAs in parallel and being asked how many there are, and how general a mechanism this might be,” said Gottesman. “It was relatively early in the bacterial genome sequencing era, but there was enough data to look for conserved regions that weren’t encoding proteins and ask if these could be small RNAs.”

By 2002, the collaboration spawned a surfeit of riches, approximately 30 small RNAs whose place in bacterial regulatory networks needed to be understood. As new members joined the lab, they could explore new regulatory territory.

A parallel story in eukaryotic



(Photo: R. Bucur)

Kumari Kavita, Ph.D., Daniel Schu, Ph.D., and Nadim Majdalani, Ph.D.

cells was beginning to emerge around the same time. In the late 1990s, gene silencing by small RNA molecules—a process termed RNA interference (RNAi)—was reported, as was the extreme conservation of microRNA sequences.

Meanwhile, Aixia Zhang, Ph.D., a postdoctoral fellow in the Storz lab, discovered that at least one small RNA relied on a small protein chaperone, Hfq, to facilitate base pairing with its mRNA target. Hfq turns out to be an essential chaperone for many of the small RNAs in *E. coli*. Whereas we now know that eukaryotic cells rely on the multiprotein RNA-induced silencing complex (RISC), prokaryotes rely on the single ring-protein chaperone Hfq to mediate

the regulatory interactions of small RNAs with mRNA.

“When I started working in *E. coli*, it was because that’s where the genetics was; it was the model organism in which molecular biology developed and you could ask fairly sophisticated questions about mechanisms,” said Gottesman. “Not by coincidence, the principles we discover often end up being applicable to other systems.”

### Dancing with the Chaperone

Daniel Schu, Ph.D., arrived as a postdoctoral fellow in 2009. He first met Gottesman when she came to give a lecture at Virginia Tech, where he was completing his Ph.D. Schu was working on a plant

pathogen and had identified a novel small RNA involved in its virulence.

“I had two different motives to talk to her: 1) to pick her brain about what role this small RNA could be playing in our pathogen and 2) to find out what her lab was like and if there were any openings in the near future,” said Schu. Six months later, he came to the NIH to join Gottesman’s laboratory.

With a background in protein biochemistry, Schu became interested in the regulation of small RNAs by the protein Hfq. Gottesman suggested that he look at how this protein was facilitating pairing with mRNAs, which was the key step in regulation by small RNAs.

“Over the past decade, we and others have found an ever growing number of small RNAs and mRNAs that interact with Hfq,” said Schu. “Because they are in constant competition, it didn’t make sense that there would be only one pathway for binding and regulation.”

“Over the past decade, we and others have found an ever growing number of small RNAs and mRNAs that interact with Hfq.”

“For me, one of the great things about being at the NIH is the strong community of researchers working on bacteria.”

Schu and Gottesman, in collaboration with Zhang and Storz, have instead identified different classes of small RNAs with different modes of interaction with the chaperone. Hfq forms a ring structure with three different faces with which RNAs can interact.

“There’s a kind of gymnastics going on with the RNAs for interaction with Hfq,” said Schu. “We’ve shown that one face always has small RNAs binding to it, but while one class of small RNAs will interact with the second face, another class interacts with the third. Small RNAs of the first class are usually transiently activated by stress; the other class seems more stable and appears to be involved in functions like metabolism.”

“Depending on which class they belong to, we’re finding that the small RNAs bind to this chaperone very differently and their fate is different. We think that has physiological consequences,” said Gottesman. “We’re hoping that understanding this will give us more insight, generally, into how cells sort small RNAs and choose which to use and maybe give us insight into how this family of chaperones is used in other organisms, certainly in other bacteria.”

## New Challenges

“For a while, we were the only reference for anyone doing small RNA work in bacteria. Labs were packing up their students and postdocs, and coming here to sit down with Susan and Gigi to learn how they could bring this work to their favorite organism. It was like riding the crest of a wave; it was

very exhilarating,” said Majdalani.

Now, Gottesman characterizes her laboratory as being in transition. “For the last few years, we’ve done a lot of tracking down of small RNAs and their targets. Interesting stories have emerged, but I don’t think we’ll be doing that for long.”

Among the new explorations under way in the laboratory are attempts to work with different organisms. Clearly the molecular mechanisms of bacterial regulation they have discovered and characterized could be applied to many pathogens.

For example, in response to an outbreak of *Klebsiella* in the NIH Clinical Center three years ago, Gottesman got involved in a collaboration to look for solutions. Given the parallels between *Klebsiella* and *E. coli*, the concept is to use the tools of *E. coli* genetics to engineer a more easily manageable organism that would mimic the *Klebsiella* surface proteins to use in high-throughput screening.

In another collaboration with colleagues at the National Institute of Allergy and Infectious Diseases, Gottesman’s laboratory is looking at the immune response to bacteria in mice. “We know that small RNAs regulate a lot of bacterial cell surface proteins, but we don’t know why. Maybe it’s to help protect the bacteria from host immunity,” said Gottesman.

To test this, the antibody response of mice to bacteria with deletions of small RNAs is being compared to the response to wild-type bacteria. One problem is again engineering the mouse commensal strains to delete small RNAs. “These strains are giving

us the same problems as *Klebsiella*; the normal *E. coli* engineering techniques are not working,” said Majdalani.

“In *E. coli*, we can do almost anything,” said Gottesman. “The difficulties start to arise when we get out of our comfort zone.”

“It’s never boring,” said Majdalani, “In part, because of Susan’s attitude towards research. She’s always dynamic and never stops recruiting students.”

“For me, one of the great things about being at the NIH is the strong community of researchers working on bacteria. I see that community every week at a seminar series called lambda lunch, which began before I came to the NIH as a postdoc,” said Gottesman.

Anyone watching Gottesman at lambda lunch or at seminars put on by the NIH-wide RNA Biology Interest Group or the CCR RNA Initiative might notice her scribbling in the back of a little green notebook, which she carries with her to capture the “random thoughts” she has during such scientific exchanges.

“I believe it is Susan’s established record of scientific achievement that attracts many talented people to her laboratory,” said Schu. “But I think it is her mentoring that has really fueled her continued success. She has two or three students, from high school, college or beyond, coming through the lab every year, either for the summer or as postbacs for a year or two. She allows them to take on their own projects, build confidence in their techniques, and really get a taste for how science is done.”

To learn more about Dr. Gottesman’s research, please visit her CCR website at <https://ccr.cancer.gov/susan-gottesman>.