# RNA reflections: converging on Hfq

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As the journal *RNA* celebrates its 20th anniversary, the role of non-coding RNAs as regulators is firmly established in a broad range of organisms. Among these are the many bacterial RNAs that pair with their targets and regulate mRNA stability and translation. In *Escherichia coli* and other gram-negative organisms, these small RNAs (sRNAs) depend on Hfq, a protein that chaperones pairing. Twenty years ago, Hfq was resuscitated after 15 years of obscurity and the extent and roles of bacterial regulatory RNAs were about to become appreciated to go well beyond the few examples in the literature.

## Serendipitous discovery of small RNAs

Both of us entered the RNA biology field somewhat by chance by unexplained or unexpected results in experiments meant to lead elsewhere. We were fortunate to have the freedom at the National Institutes of Health to pursue these observations that led us both to non-coding regulatory RNAs.

Gigi Storz discovered the sRNA OxyS in 1985 as a second year graduate student when she did a flawed experiment. She was intending to examine the levels of the mRNA encoded by the OxyR transcription factor to determine if the levels increased upon treatment with hydrogen peroxide. Instead of running an agarose gel and hybridizing with a strand specific probe, which would have been more appropriate, Gigi ran an acrylamide gel and hybridized with a long double stranded DNA fragment. The *oxyR* band was barely detected and did not really change, but a 109 nucleotide band was strongly induced by hydrogen peroxide. The band corresponded to the divergently encoded OxyS RNA. Through gradual characterization of OxyS, including protein gels that showed changes upon OxyS overexpression and a MuD-lac screen carried out by Shoshy Altuvia when she was a postdoc in the Storz lab, it became clear that OxyS was acting by base pairing with mRNAs. This work was finally published in 1997.

For Susan Gottesman, the experiments that led to the discovery of the sRNA DsrA began in the early 1990s as work directed at understanding the regulation of an unstable transcriptional regulator, RcsA, part of the cascade that regulates capsular polysaccharide synthesis in *E. coli*. A plasmid carrying a fragment of the bacterial chromosome including the *rcsA* gene was found to cause cells to overproduce capsule. That was expected. What was not expected was that a mutant derivative of the plasmid, with a transposon insertion in the *rcsA* gene, also overproduced capsule. The project was set aside for some years after the expected interpretations of this observation were ruled out. A postdoc in the lab, Darren Sledjeski, finally pinned down the activation of capsule to a transcribed but not translated region on the plasmid. This new gene was named *dsrA* for downstream of *rcsA*. In 1995, Darren reported DsrA was a small non-coding RNA that positively regulated capsule by negatively regulating a global transcriptional silencer H-NS. Subsequently, as reported in 1996, DsrA was found to activate translation of the RpoS, a stationary phase sigma factor with broad roles in protecting cells from a variety of stresses, and in 1998, Nadim Majdalani, as a postdoc in the Gottesman lab, demonstrated that this was via direct pairing of DsrA with the *rpoS* leader.

The publications on OxyS and DsrA and papers that followed led our group and others to devise ways to define other small RNAs in *E. coli*; by 2001 the list of these sRNAs had grown to dozens. As more and more of these sRNAs were characterized, it became clear that most act by base pairing and required Hfq, a protein that was previously known from studies of bacteriophage.

## Hfq rediscovered

Hfq, the RNA chaperone that facilitates bacterial sRNA base pairing with their targets, was first discovered and characterized in the mid-1970s as one of the host factors that allowed the in vitro replication of the RNA bacteriophage Qβ. Much of early molecular biology, before the event of cloning, focused on viral systems; the viruses had smaller genomes that could be manipulated in a test tube. Thus experiments were carried out to understand how Qβ replicated and to determine what was necessary for its replication. Franze de Fernandez and colleagues showed that Host Factor I (HF-1,

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later renamed Hfq, host factor for Qβ), a small, heat stable protein, was required for optimal Qβ replication both in vivo and in vitro. These studies initially were followed up by a number of biochemical studies by multiple labs that showed Hfq preference for binding specific RNA sequences and association with ribosomes. However, little was published on this protein after 1980, and what it did for the bacterial cell was unknown.

This lack of interest in Hfq began to change when the lab of Malcolm Winkler reported pleiotropic phenotypes associated with an *E. coli hfq* mutant in 1994. The labs of Thomas Elliott and Regine Hengge reported, in 1996 and 1997, that Hfq was necessary for translation of RpoS. OxyS had been found to regulate *rpoS*; could these effects be related? To examine the effects of OxyS on *rpoS*, Aixia Zhang, then a postdoc, tested OxyS activation of an *rpoS-lacZ* fusion in mutants lacking various of the proteins known to affect RpoS expression, including Hfq. OxyS activation of the fusion turned out to be completely dependent on Hfq, and Aixia went on to show that OxyS co-immunoprecipitates with Hfq and shifts Hfq in gel mobility assays, and, in a subsequent study, facilitates base pairing between OxyS and another OxyS target *fhlA*. These sets of experiments laid the foundation for recognizing Hfq as an essential chaperone for multiple small RNAs, including DsrA and OxyS.

## What has been learned?

Since the re-discovery of Hfq, many laboratories have characterized its role for sRNA-mediated regulation both in vivo and in vitro. The structure of Hfq has been solved with RNA fragments and, most recently, full-length sRNAs, revealing that the ring-like Hfq hexamer has at least three distinct RNA binding sites. Insights into how the protein facilitates base pairing and how RNAs compete for each other on Hfq are also being obtained. It has become clear that Hfq-binding sRNAs are broadly prevalent and are integral parts of many regulatory networks. Their discovery and characterization has explained a number of regulatory mysteries such as how transcription activators could act negatively and transcription repressors could act positively by controlling the expression of an sRNA.

## What next?

Despite all of the recent work on Hfq, a number of interesting and important questions remain: How do base pairing sRNAs find their targets within seconds or minutes among thousands of mRNAs? This is not a problem that is unique to bacterial cells. What other factors influence the ability of Hfq to bind RNAs and facilitate base pairing and regulation? Aside from having a very conserved core fold, Hfq in different organisms can have different embellishments (length of the C-terminus, charge distribution) and some organisms have multiple Hfq genes. How do these differences impact Hfq function? Finally, a number of bacteria lack Hfq entirely. Are the alternative chaperones in these organisms and are there other chaperones in bacteria that have Hfq?

## Acknowledgments

We would like to express our appreciation for the collaborative atmosphere that has been part of the work on Hfq and regret that we can only allude to a small part of the work of our colleagues in our brief reflection. It has been exciting for us to see many of our former lab members make critical contributions to this field in their own groups. Finally, we would like to thank Aixia Zhang and Nadim Majdalani for sustaining the Hfq projects in our own labs. Research discussed here was supported in our labs by the Intramural Research Programs of the NIH, National Cancer Institute, Center for Cancer Research and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.