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ABSTRACT

Regulation of gene expression by small non-coding RNA (sRNA) molecules in bacteria is often dependent on RNA chaperones. This means that to properly and effectively interact with mRNAs, sRNAs cooperate with appropriate proteins. The best studied bacterial RNA-binding chaperone is Hfq. It interacts with hundreds of transcripts, whether sRNA, mRNA or tRNA, which makes it a central element of many molecular networks and regulatory or adaptive mechanisms. Recent discoveries have allowed for the recognition of anew, much more diverse family of bacterial RNA chaperones -proteins with the FinO domain. FinO domain proteins differ not only in their structure, but also in numbers of bound RNAs, some of them bind many, others only a few. Despite the significant progress that has been made in understanding the biology of FinO domain proteins, the details of their interaction with RNA remain largely unknown.

From transcriptome profiling techniques (CLIP-seq, RIL-seq and RIP-seq) data previously published by other teams, it is known that the only structural motif in RNA recognized by the ProQ proteins, belonging to the FinO domain proteins, is the Rho-independent transcription terminator. Interestingly, this motif is also the only known one in the RNA structure bound by the Hfq protein. However, the pools of RNAs bound by ProQ and Hfq remain mostly separate. This raises the question of a motif in the terminator structure that could determine which protein, ProQ or Hfq, a given RNA will interact with. In the ligand pools of the Hfq and ProQ proteins bound through the Rho-independent transcription terminator region, no motifs in its sequence or structure that are distinct have been found to date. Therefore, in the first part of the work, I decided to investigate the nucleotide composition of the 10-nucleotide sequence on the 5' side of the RNA terminator hairpins bound by ProQ and Hfq in three bacterial species: *E. coli*, *S. enterica* and *N. meningitidis*. Data on RNAs bound by Hfq and ProQcame from experiments performed by the teams of Prof. Gisela Storz and Prof. Jörg Vogel. I analyzed data from: RIL-seq and CLIP-seq for ProQ from *E. coli*, RIL-seq for Hfq from *E. coli*, CLIP-seq for ProQ and Hfq from *S. enterica*, CLIP-seq for ProQ from *N. meningitidis* and with RIP-seq for Hfq from *N. meningitidis*. I showed that RNAs containing the Rho-independent transcription terminator bound by the ProQ and Hfq proteins in *E. coli* and *S. enterica* show a statistically significant difference in the nucleotide composition of the sequence on the 5' side of the terminator hairpin. ProQ protein ligands were enriched in adenosine while those of Hfq ligands in uridine. The presence of adenosine in this region turned out to be a motive that weakens binding by Hfq, thus it is an element of competition for ligands between ProQ and Hfq. On the other hand, in *N. meningitidis*, the RNA pools bound by the ProQ and Hfq proteins show the same enrichment in adenosine in the sequence on the 5' side of the terminator. Interestingly, this enrichment is a feature of the entire *N. meningitidis* transcriptome. The resulting question of why the ligand pools of *N. meningitidis* ProQ and Hfq proteins are mostly distinct remains open.

In the second part of the work, I decided to investigate the yet unknown details of the interaction between the ProQ protein from *N. meningitidis* and RNA. In *in vitro* experiments, I determined the equilibrium dissociation constant during the binding of the ProQ protein from *N. meningitidis* to a set of RNA molecules, either known as its ligands and those that were not known to interact with ProQ. I showed that *in vitro* the ProQ protein from *N. meningitidis* specifically recognizes and binds the structure of the Rho-independent transcription terminator in RNA, and that its availability at the 3' end is critical for the binding strength. Depriving the RNA molecule of the terminator structure led to the loss of interaction with the protein. In addition, the extension of the 3'-terminal sequence beyond the oligo(U) tail resulted in a decrease in affinity for the *N. meningitidis* ProQ protein. The sequence on the 5' side of the terminator hairpin also participates in the interaction, although the extent of its effect on

binding may vary between ligands. In the case of AniS sRNA, shortening it to 6 nucleotides completely abrogated the binding, while the sequence of the same length allowed for still strong interaction of *rpmG*-3'UTR with ProQ. Experiments with the double-stranded region of the terminator hairpin truncated by the upper pairs of nucleotides did not show a decrease in the binding strength of the RNA to the *N. meningitidis* ProQ protein, which indicates that the interaction is mediated by its lower part. In addition, I proved the key role of the 3'-terminal sequence of the oligo(U) tail in this interaction, because its shortening below six uridine residues resulted in a decrease in the binding strength.

Overall, in the first part of the work, I showed that in *E. coli* and *S. enterica* RNAs bound by the ProQ protein are enriched in adenosine in the sequence on the 5' side of the terminator hairpin, while RNAs interacting with Hfq are enriched in uridine in the same region, which may be an element of the mechanism of competition for ligands bound in the Rho-independent transcription terminator region by both proteins. However, in *N. meningitidis* no such difference was observed. In the second part of the work, I proved that the lower part of the terminator hairpin together with the adjacent sequence on the 5' side and the 3'-terminal sequence of the oligo(U) tail participate in the interaction with the ProQ protein from *N. meningitidis.*