

The competition between RNA binding proteins ProQ, Hfq and FinO in *Escherichia coli*

Small regulatory RNAs play important roles in gene expression regulation in bacteria. These RNAs contribute to bacterial adaptation to changes in environmental conditions, participate in maintenance of intracellular homeostasis, and contribute to the control of bacterial virulence. In RNA-dependent gene expression regulation in *Escherichia coli* participate proteins ProQ and Hfq, which bind numerous RNA molecules, and plasmid-encoded FinO protein, which recognizes few RNA molecules. The Hfq protein forms a ring consisting of six identical subunits, which contains on its surface three RNA binding sites. On the other hand, ProQ and FinO belong to the family of FinO-domain proteins, which are widely distributed in β - and γ -proteobacteria. The ProQ protein consists of the FinO domain connected by a linker to the C-terminal Tudor domain, while the plasmid-encoded FinO protein contains the FinO domain connected to the N-terminal region enriched in positively charged amino acids.

Recent global profiling studies showed that ProQ and Hfq proteins bind distinct as well as overlapping RNA pools in *E. coli* and *Salmonella enterica*. Because Rho-independent transcription terminator structures are major binding motifs recognized by both proteins, it poses an interesting question of how these proteins recognize distinct pools of RNAs. An unexpected recent discovery in our lab has been the observation that A-rich sequence motifs, present in top RNAs bound by ProQ in *E. coli*, prevent the binding of these RNAs by the Hfq protein. This suggests that the ability of ProQ to recognize specific RNAs depends not only on the binding of ProQ to appropriate RNA structure motifs, but also on the competition for binding to these RNAs against the Hfq protein.

To explain the role of the competition between ProQ, Hfq and FinO proteins for binding RNA molecules, in the proposed project we plan to explore how the overexpression of each protein in *E. coli* cells affects what RNA molecules are bound by other proteins. In these studies we plan to use methods based on global sequencing of RNA molecules in bacterial cells. Additionally, we plan to explain what features of RNA molecules determine, whether they will be bound exclusively by one of the proteins, or rather will be susceptible to the binding competition between them in *E. coli*. To achieve that, we will compare the binding of ProQ, Hfq and FinO proteins to those RNA molecules, which preferably bind to one of these proteins in *E. coli*, and to those that are most susceptible to the competition between them. We will also compare the binding of these proteins to such RNA molecules, in which we will introduce mutations in the regions that are expected to be important for interactions with the competing proteins.

We expect that the studies planned in this project will allow explaining the role of the competition between RNA binding proteins ProQ, Hfq and FinO in the tuning of gene expression regulation in bacteria. Additionally, they will help to better understand the biological functions of proteins belonging to the widely distributed and evolutionarily conserved family of FinO-domain proteins.