The mechanisms of recognition and matchmaking of small noncoding RNAs and regulated mRNAs by the ProQ protein in *Escherichia coli*.

Small regulatory RNAs (sRNAs) contribute to bacterial cell's adaptation to changes in the external environment, participate in the maintenance of internal homeostasis, and affect bacterial virulence during the infection of the host organisms. They affect gene expression by binding to complementary sequences in mRNAs, which leads to changes in mRNA stability of translation. The regulation by sRNAs is often dependent on the homohexameric Hfq protein which facilitates the pairing of sRNAs to regulated mRNAs. However, recent studies based on the global analysis of RNA binding with proteins in bacterial cells showed that numerous sRNAs are bound by another protein ProQ.

The ProQ protein belongs to the ProQ/FinO domain family of proteins. The structure of these proteins is typically modular and besides the ProQ/FinO domain also include N-terminal or C-terminal extensions, which may provide them with additional functions. The ProQ/FinO domain proteins are present in numerous β- and γ-proteobacteria, including species important for human health. The subject of studies in this project is the ProQ protein from *Escherichia coli*, which consists of two domains connected by a linker: N-terminal ProQ/FinO domain, and C-terminal domain, which is homologous to the Tudor domain. This protein binds numerous sRNAs and mRNAs in *E. coli* but the mechanism of RNA recognition by ProQ and the reason why ProQ and Hfq proteins recognize different sRNAs remain unclear. Another important questions is whether beyond binding RNA molecules ProQ also assists them in pairing with regulated mRNA molecules.

The aim of this project is to explain what elements of structure of RNA molecules and the ProQ protein contribute to their mutual recognition, and also how the ProQ protein participates in the interactions between sRNAs and regulated mRNAs. To explain that we plan to address three specific aims. The first one is to dissect the structure of RNA molecules that were identified as ProQ ligands *in vivo* to explain which sequence and structure elements determine that these RNAs are bound by the ProQ protein, and not by the Hfq protein. The second one is to identify those amino acid residues on the surface of ProQ, which are most important for binding RNA molecules, and also to explain if ProQ uses the same amino acid residues to bind different RNA molecules. The third one is to analyze the kinetic steps of formation of the complex between an RNA and a regulated mRNA in the presence and absence of ProQ to reveal what is the mechanism of the contribution of ProQ to the pairing of sRNAs to their mRNA targets.

In the proposed studies we plan to measure the thermodynamic stability and the kinetics of RNAs binding to ProQ protein or to complementary RNAs using the electrophoretic mobility shift assay, the results of which will be analyzed globally to determine the rates of the individual reaction steps. In the studies of the first aim we will use this method to reveal the effect of mutations in RNA molecules on binding to ProQ, and in the studies of the second aim the effect of mutations in ProQ on binding to RNAs. Additionally, the effect of mutations in ProQ on the binding of RNA molecules to this protein in bacterial cells will be studied using co-immunoprecipitation of ProQ mutants with bound RNAs. In the studies of the third aim we plan to analyze the detailed kinetic mechanism of the pairing of sRNAs to mRNAs in presence of ProQ to test, which of the possible models describing the role of ProQ in the interactions between sRNAs and mRNAs best explains the experimental data. Moreover, we will test the effect of mutations in ProQ on the interactions between sRNAs and mRNAs in *E. coli* using a RIL-seq method, which enables the identification of RNA molecules simultaneously binding the ProQ protein.