Rola oddziaływań białka Hfq z sekwencją kodującą mRNA w regulacji translacji

(The coding sequence of mRNA as a target for translation regulation by the Hfq protein)

The synthesis of proteins by ribosomes programmed with mRNA template is finely tuned [1, 2] and subject to regulation at several steps of this process [3]. One of the mechanisms of translation regulation in bacteria involves *trans*-encoded small noncoding RNAs (sRNAs), which are essential for the bacterial cell's adaptation to changing environmental conditions and affect the virulence of bacterial pathogens [4]. These riboregulators are functional analogues of eukaryotic miRNAs, because they control translation by binding to partly complementary sequences in selected mRNAs. Most of sRNAs need the Hfq protein to exert their effect on translation. Hfq is homologous to eukaryotic Sm-like proteins. It has a shape of a six-membered ring with separate RNA binding sites on the opposite surfaces and the rim of this ring. Our recent data show that sRNAs differ in the competition for binding to Hfq [5], and that their competition efficiency is dependent on structural features of sRNAs [6].

The canonical mode of action of sRNAs involves the regulation of translation initiation by binding to untranslated mRNA regions overlapping the ribosome binding sites. However, the recent Hfq profiling data of mRNAs from *E.coli* K12 and pathogenic EHEC strains showed that almost 40% of recovered reads mapped to the coding sequence of mRNAs [7], which suggested that the elongation phase of translation could also be an important target for regulation by sRNAs. Some, but not all, of the identified Hfq binding sites were associated with known or predicted binding sites of sRNAs. It suggests that the role of Hfq in some of these sites could be related to sRNA-dependent regulation. However, binding of Hfq to other sites could have sRNA-independent roles, such as direct involvement in translation regulation or influence on mRNA folding.

The aim of this project is to understand the function of the binding of Hfq to the coding sequence of mRNAs. To achieve that the influence of Hfq on translation progress of selected mRNAs will be monitored in *E.coli* S30 translation extracts and in bacterial cells using reporter constructs. These results will be compared with such properties as Hfq binding affinity to mRNA, Hfq-dependent acceleration of sRNA-mRNA annealing rates, and its influence on mRNA structure to reveal how these properties affect the regulation of mRNA translation. The methodology used will involve the measurements of the binding affinities using filter retention assays, the monitoring of sRNA annealing to mRNA using electrophoretic mobility shift assays (EMSA), and the RNA secondary structure determination using structure-specific RNases, or, in the case of longer RNA molecules, using SHAPE method. The mechanism of Hfq-dependent regulation of translation will be further analyzed using Hfq variants with mutations in RNA binding sites. The results of these experiments will

help to elucidate the role of Hfq binding to the coding sequence of mRNA, and may also provide new insights into mechanisms regulating translation during the elongation phase.