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ABSTRACT

Bacterial organisms have envolved a variety of mechanisms that enable rapid adaptation to changing environmental conditions. Small non-coding RNAs (sRNAs) play a crucial role in post-transcriptional gene regulation in bacteria, participating in the regulation of most cellular pathways, metabolic processes and stress responses. The mechanism of sRNA action relies on interactions with their target mRNAs, which often require the presence of RNA-binding proteins. These proteins contribute, among other functions, to the stabilization of RNA structure, protection against degradation and facilitation of RNA-RNA interactions.

The most characterized bacterial RNA-binding protein is Hfq. In recent years, a new family of RNA-binding proteins containing the FinO domain has also been identified. Proteins that belong to the FinO family are highly conserved and widely distributed among various β- and γ-proteobacteria, such as *Escherichia coli*, *Salmonella enterica* and *Neisseria meningitidis*, which are significant for human health. *Escherichia coli* cells encode two FinO-domain proteins: the plasmid-encoded FinO protein and the chromosomally encoded ProQ protein.

Global analyses of RNA binding in *Escherichia coli* and *Salmonella enterica* have shown that ProQ and Hfq interact with distinct but partially overlapping pools of RNA molecules. It has been demonstrated that ProQ, Hfq and FinO bind RNA within internal regions of transcription terminator hairpins. Furthermore, the presence of adenosine-rich motifs in ProQ-regulated RNAs has been shown to limit their binding by Hfq. However, the factors that determine the specificity of these proteins toward their target RNAs, as well as the impact of competition and mutual interactions between these proteins on RNA selection, remain largely unknown.

The aim of this doctoral thesis was to investigate the mechanisms underlying selective RNA recognition by selected RNA-binding proteins in *Escherichia coli* and to identify factors determining competition and mutual interactions between these proteins. This work focused on three complementary research directions: identification of sequence and structural features of RNA molecules responsible for their recognition by FinO-domain proteins, analysis of the role of selected amino acid residues within the FinO domain of ProQ in RNA binding in *Escherichia coli* cells, and investigation of the significance of competition and mutual interactions between ProQ and Hfq in shaping RNA binding *in vivo*.

In the first part of this work, I demonstrated that the nucleotide sequence at the base of the transcription terminator hairpin is critical for the specific recognition of RNA molecules by ProQ and FinO. Even minor nucleotide differences in this region can alter RNA-binding preferences of the studied protein. In the second part of the thesis, I examined the role of selected amino acid residues within the FinO domain of ProQ in RNA binding under in vivo conditions. RIP-seq analysis revealed that mutations within the FinO domain differentially affect the binding capacity of ProQ toward distinct RNA molecules. A mutation of the arginine residue at position 80 proved to be particularly significant, as it resulted in a substantial

reduction in the binding of many RNAs, especially antisense RNAs and RNAs that belong to toxin-antitoxin systems, such as members of the Sib RNA family.

Further structural analyses and mapping of binding sites on SibA and SibC RNAs showed that ProQ and its FinO domain bind within the terminator hairpin region as well as adjacent secondary structure elements, inducing structural changes in the RNA. Moreover, using SibA and SibC sRNAs as examples, I demonstrated that the FinO domain recognizes RNAs containing a transcription terminator, whereas full-length ProQ is also capable of binding RNAs lacking this structure. This suggests that regions outside the FinO domain contribute to RNA-protein complex formation. In the final part of this work, I investigated competition and interactions between ProQ and Hfq in *Escherichia coli* cells. RIL-seq analysis showed that these proteins form a dynamic regulatory system in which they influence each other. Changes in the cellular level of one protein resulted in alterations in RNA-RNA pairs bound by the other protein, with the magnitude and direction of the effect depending on the specific RNA-RNA pair.

In summary, the results of this doctoral thesis demonstrate that the selectivity and range of interactions between ProQ, FinO and Hfq and their associated RNA molecules are determined in a multilayered manner. Single nucleotide differences at the base of RNA transcription terminator structures alter binding preferences, while mutations within the FinO domain of ProQ modulate interactions with specific RNA molecules in the cell. In addition, competition and mutual interactions between ProQ and Hfq shape the pool of bound RNAs in *Escherichia coli*, representing an important regulatory mechanism that enables cellular adaptation to changing environmental conditions.