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**The recognition of RNA molecules by the FinO domain of the ProQ protein in *Escherichia coli***

Abstract:

Bacterial small noncoding RNAs (sRNAs) contribute to adaptation to changing environmental conditions. By interaction with specialized proteins, noncoding RNAs impact translation and target mRNA stability. One of the best known RNA binding proteins is Hfq, however recent reports point out the role of FinO-domain proteins in this kind of regulation. ProQ protein present in *Escherichia coli*, is a member of this family. ProQ consists of two globular domains, RNA-binding N-terminal FinO domain (NTD) and C-terminal Tudor domain (CTD) connected via unstructured linker of about 50 aa. ProQ binds numerous RNAs in *E. coli*, however little is known yet about its ligand recognition fashion.

In order to explain how the ProQ protein recognizes RNA molecules, in the first part of my PhD thesis I investigated which features of the sequence and structure of RNA molecules are necessary for their binding by the FinO domain of the ProQ protein, and in the second part of the thesis I examined how mutations in the domain FinO affect the binding of various RNA molecules. In the first part of my research, I established the characteristics of RNA molecules that determine their binding to NTD. From RNAs bound by ProQ *in vivo*, I selected six molecules: MicA, SibA, *cspE*-3', *malM*-3', *hupA*-5' and *lpp*-5', and I measured their binding to purified full-length ProQ protein and its FinO domain (NTD). The analysis of RNA molecules and their mutants binding indicated that the structural element recognized by NTD is the Rho-independent transcription terminator. For the binding relevant are a polyuridine tail of at least four nucleotides at the 3' end and a double-stranded hairpin region of at least two base pairs. The introduction of a mismatch above the fourth base pair of the double-stranded region of the terminator hairpin does not affect the interaction with the NTD. Furthermore, I found that the adenosine-rich sequence motif located on the 5'-side of the terminator hairpin of RNA molecules bound by ProQ plays a role in competition between ProQ and another protein binding the 3' end of RNA, which is the Hfq protein. The substitution of adenosine for uridine caused the RNA molecules to bind to NTD less or the same as to RNA of natural sequence. However, the binding of such mutant RNA molecules to the Hfq protein was clearly improved. The results suggest that the adenosine-rich motif prevents the binding of RNA molecules to the Hfq protein and facilitates their binding to ProQ.

In the second part of my research I indicated amino acids within the N-terminal domain of ProQ important for interaction with RNA molecules. Based on the analysis of the evolutionary conservation of amino acid sequence and the available literature I selected ten amino acid residues and checked their effect on the binding of seven RNAs (*cspE*-3', *cspE*81-3', *malM*-3', *gapA*-3', RybB, SibA and SibB) *in vitro*. Substitutions at positions R58, Y70 and R80 resulted in inhibition of protein binding to RNA. Significant for the interaction are also positions K54 and R62, which substitutions significantly weakened RNA binding, while mutations at positions R32, D41 and T65 had the least effect on binding. Interestingly, mutations of the K35 and R69 residues had a diverse effect on binding of different RNAs, the least on the binding of the *cspE*-3', and the greatest on the binding to SibB sRNA. This may suggest differences in the interaction between RNAs and the FinO domain of the ProQ protein. To indicate which regions of RNA molecules are responsible for the differential effect of the K35A and R69A substitutions in NTD on RNA binding, I examined the binding of RNA molecules containing mutations that: deprived them of single-stranded regions adjacent to the terminator hairpin, changed the sequence of the terminator hairpin, or changed the length of the linker between the terminator hairpin and the nearest double-stranded element on the 5' side of the hairpin. The effects of introducing these mutations in RNA did not indicate direct contact of the mutated RNA sequences with amino acid residues at positions 35 and 69, therefore it is possible that the K35A and R69A substitutions have an indirect effect on RNA binding, e.g. by changing the conformation of the protein.

In conclusion, the results of the experiments showed that the N-terminal domain of the ProQ protein recognizes RNA molecules by binding to them within the junction between the lower part of the transcription terminator hairpin and the surrounding single-stranded sequences. In addition, studies of the effects of mutations introduced into the FinO domain suggest that amino acid residues located in the central part of the concave side of the FinO domain are crucial for RNA binding, while amino acid residues located at the edges of this domain or on the convex side are less important for RNA binding or, as in the case of K35 and R69 residues, have a diverse effect on the binding of different RNAs. The presented results allow to explain the mode of interaction of the ProQ protein with RNA molecules, and thus to better understand the role of this protein in the process of gene expression dependent on regulatory RNAs in bacteria.