Abstract:

Giardia intestinalis is an anaerobic protozoan pathogen, agent of the disease known as giardiasis. The regulation of gene expression during giardia cell- and life-cycle has been poorly studied so far, with the exception of variable surface proteins, which constitute the immunoprotective coat of the cell. In this diploma thesis, we focus on the possible role of the 3´ untranslated region (3´UTR) of mRNA that mediate stability and localization of mRNA transcripts. We use RNA binding proteins of PUF family, which control the function of the target transcripts by their repression, activation or sequestration, to monitor and verify the role of 3´UTRs. These only eukaryotic proteins are highly evolutionarily conserved. Each of them contain highly conserved C-terminal domain, which specificly binds to 3´UTR of mRNAs.

We have identified five different PUF proteins in the genome of *G. intestinalis* (GiPUF), cinfirmed their expression in *G. intestinalis* trophozoites and located all five proteins in the cytoplasm. GiPUF2, GiPUF3 and GiPUF5 show an additional affinity to the surface of the endoplasmic reticulum. We have identified the C-terminal binding domain in protein sequences of all GiPUF. The most conserved GiPUF4 contain eight binding sites, nearly identical to the binding site of human Pum1 protein, yeast PUF3p, FBF1 of *C. elegans*, PUM in *D. melanogaster* and PUF1 in *P. falciparum*, which suggest a close evolutionary relationship. For this reason we focus other studies only on GiPUF4. Cytoplasm localization of this protein was confirmed by cell fractionation of giadia cells containing HA-tagged GiPUF4 protein. Using bioinformatics 19 theoretical cognate mRNAs of GiPUF4 protein were. 3D structural model of the protein with the binding of RNA confirmed the presence of the binding motif and overall structural similarity of the protein, which like most PUF proteins form the tertiary structure of crescent shape, in which inner fold binds 3'UTR of mRNA. Specific mRNA partners of GiPUF4 protein will be identified by the native isolation of GiPUF4 protein the bound mRNA.

The effect of 3'UTR VSP genes at the level of their expression is the only previously known mechanism of gene regulation in *G. intestinalis*. This effect was confirmed by experiments with the Sec20 protein. Moreover, the results indicate that, the localization of proteins may depend not only the information contained in the actual molecules (target sequence, etc.), but also on the rate of the protein expression.