The cellular RNA interactome of the human Dicer helicase domain

The discovery of gene expression silencing induced by short RNA molecules: microRNA (miRNA) and small interfering RNA (siRNA), termed "RNA interference", was a landmark discovery at the end of the last century. The researchers, who defined and described the RNA interference phenomenon, A. Z. Fire and C. C. Mello, were awarded the Nobel Prize in Medicine and Physiology in 2006. In the biogenesis of short regulatory RNAs, an important role is played by Dicer ribonucleases. Dicer ribonucleases are multidomain proteins, usually consisting of: N-terminal helicase domain, domain of unknown function 283 (DUF283), Platform and PAZ domains, two RNase III domains (RIIIa and RIIIb) and a C-terminal double stranded RNA binding domain (dsRBD). This project focuses on the helicase domain of human Dicer (hDicer). The helicase domain is responsible for recognizing the precursors of miRNA and siRNA molecules: pre-miRNA and pre-siRNA, respectively. Additionally, this domain serves as a platform for the binding of various hDicer protein partners. It is also known, that hDicer helicase contains the DExD/H-box motif, which is responsible for ATP binding and hydrolysis. However, the activity of hDicer has never been found to be an ATP-dependent. Interestingly, recently published data indicate, that hDicer helicase may play an important role in antiviral defense.

The research conducted in recent years has significantly enriched our knowledge of the functions performed by Dicer ribonucleases. For example, Dicer has been shown to be involved in the metabolism of different RNAs, including tRNAs and snoRNAs, removal of toxic double-stranded RNAs, and maintenance of genome stability. Importantly, *in vivo* Dicer has been shown to bind to specific sites within transcripts in a "passive" manner, that is without performing dicing. Such Dicer binding sites present within transcripts are termed "passive sites". It is believed, that the passive sites function as a buffering system to control the cleavage activity of Dicer by sequestering it from pre-miRNAs. Moreover, it is suggested that the passive binding of cellular transcripts by Dicer may be an important element of cellular pathways involved in the regulation of RNA metabolism. Presumably, passive binding of hDicer to cellular transcripts is mediated by its helicase domain.

The role of the hDicer helicase domain in the antiviral defense in human cells remains the subject of intense debate. The cellular interaction network between proteins and hDicer helicase is also intensively studied; however, the cellular RNA interactome of this domain is still unexplored. **Accordingly, the main aim of this project is to look at the cellular interaction network between the hDicer helicase domain and RNAs.**

In our research we will use human embryonic kidney cell line (HEK 293T) and the derivative cell lines producing: (i) hDicer; (ii) hDicer lacking the helicase domain; (iii) hDicer with a mutation in the helicase domain, in the motif responsible for the ATP hydrolysis. Based on the results collected from the immunoprecipitation of RNA-protein complexes targeting the C-terminal domains of hDicer and followed by NGS sequencing of RNA pools bound *in vivo* by hDicer, we will infer about **the possible role of the helicase domain in the interactions between hDicer and cellular transcripts, as well as the role of ATP for these interactions.**

Furthermore, we would like to perform a detailed biochemical characterization of the activity displayed by the helicase domain of hDicer. For this purpose, we will use a diverse group of RNA molecules, which differ in length and secondary structure. To study RNA-protein interactions, we will apply: (i) electrophoretic mobility shift assay (EMSA) and (ii) biolayer interferometry (BLI). Additionally, we will investigate whether the hDicer helicase domain exhibits an annealing activity and the ability to unwind RNA duplexes. Each of these activities will be tested in the context of the ATP dependence.

We believe that the recognition and better understanding of the molecular mechanisms behind Dicer activity outside small regulatory RNA biogenesis pathways would give a valuable impact towards understanding of the numerous phenomena, including cell functioning. The data on the interaction between hDicer helicase and cellular RNAs may be important for a wide group of researchers and clinicians focusing on the problem of the misregulation of cellular processes which leads to disease development, including carcinogenesis. Taking into consideration the documented importance of the hDicer helicase domain in antiviral defense, findings from this project will also contribute to a better understanding of virus-caused diseases and the role of hDicer in host-virus interactions.