Endoribonuclease Dicer is mostly known for its important role in the biogenesis of small regulatory RNAs. During this process, Dicer cleaves single-stranded precursors adopting stem-loop structures (pre-miRNAs) and double-stranded RNAs (dsRNAs) into short RNA duplexes containing functional microRNAs (miRNAs) or small interfering RNAs (siRNAs), respectively. miRNA or siRNA, together with Argonaute proteins, form the RNA-induced silencing complex (RISC) which mediates post-transcriptional gene silencing. Growing evidence shows that apart from this canonical role, Dicer serves a number of other functions either in the cytoplasm or in the nucleus. For example, one interesting report has demonstrated that in nematode *Caenorhabditis elegans*, a caspase-cleaved form of Dicer moves from the cytoplasm to the nucleus, where it acts as a deoxyribonuclease that introduces breaks in chromosomal DNA during apoptosis. Moreover, the emerging evidence points to possible cleavage-independent roles of Dicer: in *C. elegans* and human cells, Dicer has been found to bind various RNAs passively, i.e., without further cleavage. Importantly, our recent findings show that human Dicer (hDicer) may as well bind RNA and DNA G-quadruplexes *in vitro*, and that this interaction influences hDicer ability to process its canonical substrates. Using the immunoprecipitation-based techniques, we have already identified in human cells many transcripts harboring the guanine-rich sequences adopting the G-quadruplex structures in the region bound by hDicer.

Accumulating evidence indicates that G-quadruplexes serve important regulatory roles in fundamental biological processes such as DNA replication, transcription, and translation, while aberrant G-quadruplex formation is linked to genome instability and cancer. Understanding the biological functions played by G-quadruplexes requires detailed knowledge of their protein interactome. Our newest finding that hDicer can bind RNA and DNA G-quadruplexes opens new avenues of research on the cellular functions of Dicer. Consequently, in light of the importance of the G-quadruplexes in the regulation of various essential cellular processes and pathways, new questions and scientific challenges arise, which we would like to address during implementation of this project. One of the questions is: **what are the potential functional implications of *in vivo* interactions between hDicer and RNA molecules adopting the G-quadruplex structures?**

Additionally, taking into consideration reports describing Dicer association with chromatin DNA, and the fact that hDicer binds to human telomeric DNA repeats *in vitro*, **we hypothesize that hDicer can bind to the DNA G-quadruplex structures *in vivo* as well.** Already, it has been demonstrated that hDicer is a target for caspases during apoptosis. However, contrary to *C. elegans* Dicer, movement of the apoptotic caspase-cleaved hDicer from the cytoplasm to the nucleus has never been reported. Therefore, we ask the questions: **Whether hDicer, similar to *C. elegans* Dicer, can move from the cytoplasm to the nucleus upon induction of apoptosis? Whether nuclear hDicer can bind to the telomeric G-quadruplex structures, and other G-quadruplex structures formed within chromatin? What are the potential functional implications of *in vivo* interactions between hDicer and DNA G-quadruplexes?**

To **prove that hDicer binds to the G-quadruplex structures in the cell**, we will apply Forster Resonance Energy Transfer (FRET)-based assays. FRET is based on the fact that if emission spectrum of a donor matches the absorption spectrum of an acceptor fluorophore, then the energy transfer can occur. By excitation of the donor, we can induce the emission of the acceptor, provided that it is nearby (<10 nm). Moreover, imaging co-localization experiments will be performed with the MINFLUX nanoscope, a newest technology which enables not only an identification of the interaction and the distance between the two targets, but also allows for unambiguous verification whether the two targets are next to each other, and hence whether they indeed interact. Given the nuclear-cytoplasmic localization of Dicer proteins, and the fact that hDicer binds to human telomeric DNA repeats *in vitro*, **we would like to reveal the DNA pool bound by hDicer in the cell**. To this end we will apply the chromatin immunoprecipitation-sequencing (ChIP-seq) techniques. Based on the all collected data, **the potential functional implications of Dicer's interactions with the RNA and DNA molecules adopting G-quadruplex structures will be inferred**.

The knowledge gained during the project implementation will **broaden our understanding of the molecular mechanisms behind Dicer activity outside the small regulatory RNA biogenesis pathways**. As G-quadruplex structures frequently occur in genomes and transcriptomes, and have important regulatory roles, comprehensive **identification of G-quadruplex-interacting proteins is essential to dissect functions of these non-canonical structures**. In addition, the data on the interaction between hDicer and the RNA/DNA G-quadruplexes formed in the cell may provide important insights into the pool of the G-quadruplexes which are regulated by hDicer. This output may be important for a wide group of researchers and clinicians focusing on the problem of the misregulation of cellular processes resulting from the aberrant G-quadruplex formation; e.g. genome instability leading to cancer.