The complex nature of multidomain proteins: the unmasked activity of human Dicer ribonuclease

Multidomain proteins can potentially serve multiple functions in the cell. Presumably, multidomain proteins have evolved by the fusion of two or more ancestral single domain proteins or have emerged as a result of recombination events such as domain duplication or swapping. New combinations of individual domains are of great importance in protein evolution as they may ensure direct linkages between proteins that act in a coordinated manner; for example, successive enzymes in the same pathway. What is important, such consolidation of protein domains allows time and the cell's energy saving. An excellent example of multidomain proteins is the ribonuclease Dicer. Dicer ribonucleases are widely known for their important role in the biogenesis of small regulatory RNAs: microRNAs (miRNAs) and small interfering RNAs (siRNAs). Aberrant Dicer forms initiate pathological processes, including carcinogenesis, as well as neurodegenerative, immune system and rheumatic disorders. Dicers belong to the family of ribonuclease III (RNase III) endoribonucleases, the enzymes that specifically cleave double-stranded RNAs (dsRNAs). A typical Dicer-type ribonuclease consists of a helicase domain, a domain of unknown function (DUF283), Platform, a PAZ domain, two RNase III domains and a dsRNA-binding domain; however, the domain composition of Dicers varies among species. Dicer and its homologues developed only in eukaryotes; nevertheless, the two enzymatic domains of Dicer, helicase and RNase III, display high sequence similarity to their prokaryotic orthologs. Evolutionary studies indicate that a combination of the helicase and RNase III domains in a single protein is a eukaryotic signature and is supposed to be one of the critical events that triggered the consolidation of the eukaryotic RNA interference (RNAi); miRNA and siRNA generated by Dicer proteins are essential to components to the RNAi pathway. The enzymatic activity of the RNase III domains of Dicer proteins is well known; they perform substrate cleavage. Nevertheless, the enzymatic activity of the helicase domain of the vertebrate Dicers is still poorly understood. In this project, we will focus on the helicase domain of human Dicer (hDicer). The hDicer helicase has well-conserved ATPase motifs; however, it has been suggested that ancestral Dicers in the hDicer lineage had decreased ATP binding affinity and thus lost ATPase activity, in contrast to plant or arthropod Dicers. Thus, it remains a mystery why hDicer has preserved well-conserved ATPase motifs? Interestingly, our new data indicate that the helicase domain of hDicer is capable of ATP hydrolysis. To the best of our knowledge, this is the first time this activity has been reported for vertebrate Dicers. In light of these findings, new questions and scientific challenges arise, which we would like to address during implementation of this project. One of the questions is: what purposes, i.e. molecular and cellular pathways, can the ATPase function of hDicer serve? To address this question, by using the mass spectrometry techniques, we will determine the cellular protein interactome of the helicase domain of hDicer and, consequently, possible molecular and cellular pathways that may depend on the ATPase activity of hDicer.

In vitro, the ATPase activity of hDicer can only be observed under low-turnover conditions. We hypothesize that after a single round of ATP hydrolysis, the products of hydrolysis stay bound to the hDicer helicase domain and block the next round of reaction. *In vivo*, a product release may be stimulated by a yet unknown factor or factors that are missing in our *in vitro* reactions. The ATPase activity of some chaperone proteins may be influenced by interdomain contacts. Thus, it is possible to hypothesize that interdomain interactions, between hDicer and its partner proteins, may as well affect the ATPase activity of hDicer. Interestingly, it has so far been shown that RNA can stimulate the ATPase activity of some invertebrate Dicers by increasing their ATP affinity. Importantly, hDicer has been found to bind various cellular RNAs, including mRNAs and long non-coding RNAs, “passively”, i.e. without further cleavage. Passive binding of hDicer to transcripts is presumably mediated by its helicase domain. Considering the presented facts and hypotheses, to get a better insight into the ATPase activity of the hDicer helicase domain, we would like to determine which factors: partner proteins, interacting RNAs, or both, can assist hDicer in ATP hydrolysis. To address these issues, by using advanced microscope imaging techniques, we will validate interactions between hDicer and both selected partner proteins and selected RNA targets. By using the *in vitro* biochemical assays, we will determine whether the particular protein or RNA can support or block the ATP hydrolysis by hDicer. Further, to get a deeper understanding of the character of interactions between individual hDicer’s domains and the selected factors influencing ATP hydrolysis, we will apply the cryo-EM technique.

The results of this project will provide new, valuable insights into the molecular and cellular pathways regulated by hDicer. Given the complex nature of multidomain proteins, it comes as no surprise that Dicer is implicated in many complicated diseases, including cancer, neurodegenerative, immune system and rheumatic disorders. Therefore, the new knowledge on the cellular pathways linked to Dicer proteins may expand our understanding of the etiology of many diseases, including those mentioned above.