Description for general public

Eukaryotic mRNA has a unique structure on its 5' end (m^7GpppN), known as a cap. It is formed of 7-methylguanosine (m^7G) bound to the next nucleotide (N) via an atypical (for nucleic acids) 5'-5' triphosphate bond (ppp). The cap plays a crucial role at various stages of mRNA functioning in the cell: from the protective role against degrading enzymes, through splicing and intracellular transport, to protein synthesis. Reports from the last few years point to a huge variety of cap structures and protein factors that interact with cap in cellular processes.

Cap removal results in processive degradation of the transcript by specific exonucleases. One of the best-known enzymes that remove the cap from 5' end of mRNA (decapping enzymes) is a Dcp2 protein, with Nudix motif responsible for binding and effective hydrolysis of substrates. Recently, a series of other proteins from the Nudix family has been described. Their activity is similar to that of Dcp2, but their role in mRNA degradation is poorly understood. The aim of this project is to increase knowledge about the molecular basis of mRNA degradation – the process that is crucial for the stability of this molecule in the cell. The obtained information will be of practical importance related to the use of mRNA in biotechnology and medicine. Over the last few years, mRNA has been intensively studied for its therapeutic use as a drug. Upon introduction to a cell, such therapeutic mRNAs become a template for the production of specific proteins to e.g. supplement missing proteins or replace their defective forms. This strategy is designed to relieve or lead to withdrawal of disease symptoms. One of the most important applications, currently under advanced phase of clinical trials, is the so-called „mRNA vaccine" coding for antigens aimed at stimulating the immune system to fight tumor cells. To ensure therapeutic efficiency of mRNA, the molecules must be resistant against degradation in the cell, which would expand the time of mRNA therapeutic action.

Using a broad spectrum of biophysical and biochemical methods, we plan to study representative Nudix proteins for their structural features important for specific interaction with natural and chemically modified cap structures from our unique collection of modified cap analogs. The activity of Nudix proteins will be tested against a series of naturally occurring diverse cap structures, which would allow us to determine their substrate preferences. To further characterize the specificity of Nudix enzymes, we will use fluorescence and microcalorimetry titration which allow to precisely determine the effectiveness of the protein interaction with different cap analogs. Another significant research direction will be determination of Nudix proteins structure based on crystal rentgenography. Crystallographic studies will be supplemented with investigation on the protein structure and dynamics in solutions, using a series of unique techniques, such as small angle X-ray scattering (SAXS), ultracentrifugation or circular dichroism (CD). An interesting scientific challenge will be attempt to analyze a pool of dinucleotide cap analogs isolated from cellular extracts were expression of chosen Nudix proteins was increased or silenced. Comparing such a cap pool with control samples provide the basis to search for structures, the content of which is most intensively influenced by the lack or overexpression of a given enzyme. Within this project, we will also perform analysis of mononucleotide cap analogs as potential inhibitors of one of Nudix proteins engaged in degradation of 6-thiopurine nucleotides used as anticancer medications and immunosuppresants.

Our studies will provide important information on the mechanism of interaction between Nudix proteins and cap analogs, which will allow to increase our knowledge about the function of these enzymes. The obtained results will also have applicable meaning. They will be used to design the most effective mRNA (more specifically, cap) modifications to make it resistant against degradation by a broad spectrum of enzymes, which will allow for wider application in the studies on biological processes and, in a further perspective, in medicine.