Structure of human N4BP1 ribonuclease together with RNA decapping proteins

Templates for production of proteins in cells are gene transcripts, which are RNA molecules that carry information about the amino acid sequence of proteins. One of the processes negatively affecting the level of transcripts in cells is their degradation by enzymes that hydrolyze RNA molecules, which belong to RNases. Mechanisms of RNA degradation are an important element of maintaining the equilibrium in cell, but also play a significant role in counteracting unfavorable factors e.g. during the regulation of inflammation. Some of RNA degradation processes are related to the presence of granular structures in cells, such as stress granules and RNA processing bodies. In the case of stress granules, they are also a storage place for transcripts that can be subsequently released when the stress factors disappear, thus transcripts can be recirculated and contribute to the synthesis of new proteins. One of the main properties of RNA processing granules is the presence of the RNA decapping complex at the 5' end of transcripts. The multi-protein decapping complex is associated with the EDC4 protein, which is a scaffold for other components of the complex, including the DCP2 protein, which performs hydrolysis of RNA cap. The existence of RNA cap in eukaryotic organisms protects transcripts from premature degradation. The consequence of RNA cap hydrolysis is a cleavage of transcript, which is typically performed by RNase - exoribonuclease 1 (XRN1). Due to the activity of XRN1, transcript is successively shortened one by one nucleotide, until completely degraded.

Previous studies have shown that RNase N4BP1 is involved in regulation of the inflammatory response by degrading transcripts of pro-inflammatory cytokines. Our preliminary results show that N4BP1 is a nuclease associated with the decapping complex, and represents an alternative way of transcripts degradation that is independent to XRN1 activity. Using the immunoprecipitation method, the N4BP1 complexes were isolated from the cell, followed by the identification of the components of the complexes by mass spectrometry. Interestingly, the results of preliminary analyzes revealed the participation of N4BP1 in the complex with EDC4 and other decapping proteins. Our research aims to show the structural basis of the interaction of N4BP1 with EDC4 and RNA using the methods of structural biology. We suppose that by interacting with EDC4, the N4BP1 nuclease targets specific RNA loops in transcripts at their 5' ends. We plan to solve the 3D structure of N4BP1 by X-ray crystallography and cryo-electron microscopy. During the project, we will use cell cultures to produce recombinant proteins with specific properties and sequences. Recombinant proteins will be isolated from cells and purified by chromatographic techniques. Subsequently, the structural characterization of N4BP1 will be performed through crystallization, along with the determination of the structure of the tested protein on the basis of X-ray diffraction patterns of the protein crystal. In addition to the analysis of N4BP1 itself, we will perform research on its complexes with EDC4 and other decapping proteins. We expect to obtain information about the architecture of the N4BP1 active site and to learn the details of how this protein selectively binds specific RNA sequences or structures present in certain transcripts. In addition, using biochemical techniques, we will check the substrate selectivity of N4BP1 nuclease and the influence of the components of the decapping complex on the activity of N4BP1. As substrates for studies of N4BP1-driven transcripts degradation, we will use RNA with a specific sequence and structure that models untranslated regions in transcripts. The results of planned experiments will reveal the mechanism of action of N4BP1 in RNA-processing granules. We aim to determine the structure of N4BP1 and the details of catalysis of transcripts by N4BP1. These results can be used in the future for intelligent design of the inhibitors of N4BP1 that modulate inflammatory processes. In addition, N4BP1 degrades RNA of some retroviruses including HIV 1. Therefore, determining the molecular mechanism of action of N4BP1 will be possibly important in developing new methods to enhance the primary inflammatory response to prevent some viral infections.