Project title: FAST protein interactions in cell death regulation

Principal Investigator: Daria Dawidziak, *MSc. Eng.*

The aim of the project is to characterize the interactions of the FAST protein (also called Fas-activated serine-threonine phosphoprotein) with other macromolecules in the process of cell death regulation. This molecule is considered a survival protein because under conditions of environmental stress (e.g. subjecting cells to UV radiation), it migrates to stress granules, interacts with the TIA-1 protein and enhances the production of inhibitors of apoptosis. These molecules stop apoptosis - a programmed mechanism for removing used and damaged cells that can be compared to controlled cell suicide for the benefit of the whole organism. FAST and TIA-1 proteins also meet in the cell nucleus, where they affect the alternative splicing of Fas receptor. This receptor can occur in our bodies in two forms - pro-apoptotic (enhancing cell death) or anti-apoptotic (silencing it). Both FAST and TIA-1 promote the emergence of the pro-apoptotic version of Fas, which shows that FAST can regulate cell death in two opposite ways. However, this protein interacts with another important molecule - the eukaryotic translation initiation factor 4E (eIF4E) - and it's possible that it does it in the same way as maskin and cup proteins, i.e. by binding to eIF4E and TIA-1 and promoting formation of a loop on mRNA. Such a loop stabilizes the silenced mRNA until conditions improve and a protein can safely form from the mRNA.

Importantly, these interactions and processes have not been mapped together to create a coherent model of FAST function in the post-transcriptional regulation of gene expression and have not yet been studied in the light of the RNA binding properties of FAST alone. In this project, we would like to explore the possibility of formation of the abovementioned loop, and also check whether FAST helps in the interaction of TIA-1 with Fas death mRNA during alternative splicing.

In our laboratory, we were the first to produce recombinant FAST protein by expression in bacteria and purification by chromatographic methods. This material was used to conduct *in vitro* experiments that confirmed the ability of FAST to bind RNA. In this project we will also check FAST ability to bind TIA-1 and eIF4E, and we will select a short RNA molecule preferably bound by FAST. In addition, we will check whether FAST has TIA-1 kinase activity, and therefore whether it can attach a phosphate residue to the TIA-1 molecule, which can affect its activity. These studies will be conducted using molecular, biochemical and structural biology methods. The recombinant FAST will be combined with its cellular partners, as well as with RNA and the architecture of such complexes will be analyzed using X-ray crystallography and electron cryomicroscopy.

It is suggested that FAST protein, due to its ability to regulate Fas-induced cell death, is involved in several forms of inflammatory diseases mediated by the immune system. Interestingly, this molecule is overexpressed in patients with various diseases of this origin, including rheumatoid arthritis, systemic lupus erythematosus, autoimmune diabetes and multiple sclerosis. Overexpression or misregulation of FAST can contribute to these autoimmune syndromes by regulating Fas-induced apoptosis, which is a prelude to autoimmune disease in several experimental systems. Our research will broaden the understanding of the role of FAST in apoptosis and alternative splicing that appears to affect each other at many levels of RNA regulation by this molecule. Understanding the structure of FAST may enable the design of drugs directed against autoimmune diseases such as asthma and rheumatoid arthritis.