Role of Tollip protein in embryonic development and protein homeostasis in the model of zebrafish (*Danio rerio*)

Cells of an organism produce specific molecules – proteins, which are essential for the all biological processes. There are different types of proteins with different functions (e.g. structural, immune, and signaling proteins). A gene (a small piece of DNA) consisting of nucleotides (chemical compounds) carries the information concerning the composition and the structure that an organism requires to synthesize a protein. Faulty, damaged and redundant proteins are degraded in an organism. Therefore, proper protein homeostasis is maintained. Proteins might be degraded by other proteins (enzymes) found in small intracellular vesicles (lysosomes). Under stress conditions (for example in case of lack of nutrients), this process, called autophagy, causes proteins are to be cleaved into small peptides, that enables cells to recover nutrients. The second machinery responsible for protein degradation is the ubiquitin-protesome system. In both systems molecules directed to destruction are tagged with a small protein called ubiquitin which acts as a signal and initiates protein degradation to polypeptides and amino acids. These two degradation pathways actually interact and regulate each other. Upon proteasome or autophagy failure, tagged proteins are not removed, so they accumulate and tend to aggregate. Cellular accumulation of faulty proteins is a characteristic not only for neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease, but also for other pathologies including Gaucher's disease. Abnormal aggregates of proteins that develop inside nerve cells may cause the impairment of life processes.

Many of protein functions and structures are still unexplored. One of the proteins that are still investigated is Tollip. It was shown to play a role in activating immune responses but most studies were conducted using cultured cells, e.g. epithelial cells, derived from a multicellular organism. Importantly, Tollip contains a specific site (the so called CUE domain) that binds to ubiquitin. Therefore, Tollip may influence the degradation of other proteins. Our recent studies have revealed a new role of Tollip as a negative regulator of Wnt signaling. The Wnt pathway is responsible for cell fate decisions. Thus, it plays a role in development and functioning of an organism. Our results obtained using a model organism (zebrafish) have revealed that Tollip protein deletion at early stages of life may have serious consequences on development resulting in numerous defects of an embryo.

The presented project aims to investigate the role of Tollip in vertebrate development, with a particular focus on its role in protein homeostasis (proteostasis). A common approach to discover the function of a protein is its depletion followed by investigation of processes impaired by its deficiency. The proposal is based on generation of Tollip-deficient zebrafish lines in which the gene of Tollip will be altered, resulting in a non-functional protein. Modifications of the gene will be transmitted to the next generation. In the presented project innovative tools, CRISPR/Cas9 method of genetic engineering and SPIM microscopy for 3D imaging of developing embryos, will be used. Next, detailed characterization of the mutant lines will be performed including studies on autophagy and the ubiquitin-proteasome system. In order to investigate a potentially beneficial role of Tollip in protein clearance, a fish model of Gaucher's disease will be used. In this model inclusions of ubiquitinated proteins in the brain and symptoms related to swimming defects are observed. The results of this project will show if Tollip protein is needed for early development and for degradation of proteins by autophagy and the ubiquitin-proteasome system. Moreover, the studies will verify a hypothesis related to a potentially beneficial function of Tollip protein in clearance of protein aggregates typical for Gaucher's disease in a model vertebrate organism.