

Eukaryotic cell membranes are organized into functional lipid and protein domains, the most widely studied being membrane rafts. Opposite to bulk fluid-like membrane they are more solid-like domains. Basic form of these domains in eukaryotic cell plasma membrane are resting state rafts (20 nm in diameter and half-life ~1 sec), therefore invisible in classical optical and confocal microscopes. These domains are enriched in sphingolipids and cholesterol and contain several types of membrane proteins such as stomatin and flotillins, which are their “permanent” residents, along with several others, often only transiently associated with rafts such as receptors, including growth factor receptors. Membrane rafts organize receptors and their downstream molecules to regulate a number of intracellular signalling pathways thus participating in regulation of many basic cellular processes, such as proliferation, growth, migration as well as some endocytotic pathways. They also are known as site of interaction of various pathogens, e.g. viruses with a cell.

Although the structure and function of membrane rafts had been studied for almost 20 years the problem of biological mechanism of their organization and regulation in cell membrane was not a subject of intensive studies. Our previous projects led to the discovery of a new molecular mechanism of resting state membrane raft organization in erythrocytes and erythrocyte precursor-derived cells. We showed that for this process membrane palmitoylated protein-1 (MPP1) is responsible. MPP1 is a major palmitoylation substrate in the erythrocyte and gene coding for this protein is expressed to high level in the other blood cells and their precursors but not in many other cells of human origin.

Unexplained molecular mechanism(s) underlies organization of resting state membrane rafts in cells other than blood cells which do not express *MPP1* gene to high levels. Knowing that membrane rafts play important roles in regulation of many biological processes, major question we are asking is: what is the molecular mechanism of resting state membrane raft organization and regulation in cells other than blood cells and their precursors? In our hypothesis we assume that the molecular mechanism of resting state raft organization is similar to the one described by us for blood cells and their precursors, but the protein responsible for organization of these domains is different. We assume that this protein interacts with flotillin-1 or -2 and their heterodimer) similarly as MPP1. Therefore we plan to analyze flotillin “interactome”, i.e. to identify cellular protein(s) binding to flotillins, using the “pull-down approach” where the bait is recombinant flotillin-1 or -2 or their heterodimer bound to the solid phase chromatographic resin. Other standard approach is yeast two hybrid system based on genetic mechanism to discover interactions between chosen protein and other proteins in a living cell. Flotillin-1 or -2-binding proteins will be identified by using advanced chemical, cellular and molecular biology techniques.

Selected in result of mentioned procedures proteins will be studied towards their participation in the raft organizing mechanism mainly via inactivation of genes encoding studied proteins or silencing their expression and analyzing the effect of elimination or marked decrease in the protein in the cell on plasma membrane physical properties by using advanced methods of imaging of membrane physical properties. Mainly FLIM (fluorescence life-time imaging microscopy) and svFCS (spot variation fluorescence correlation spectroscopy) will be used. We also shall perform analyses of the effects of eliminating or decreasing studied protein level on cell membrane function, functions of the membrane receptors, cell survival and mobility. Interactions of recombinant candidate protein(s) with flotillins in model systems and reconstituted membranes will also be studied in order to gain knowledge on molecular mechanism of resting state raft organization.

Our preliminary studies seem to confirm our hypothesis, namely using a pull-down approach of flotillin-2 partner with an aid of mass spectrometry-based proteomic technique we have identified protein EFR3A, which fulfils criteria of resting state membrane raft organizer in HeLa cells. Its interaction with flotillin-2 remained unknown. Important conclusion from these studies is that chosen by us experimental approach and verification system of function in resting state raft organization proved successful.

Obtained in a result of the proposed studies data should facilitate understanding a general mechanism and molecular basis of resting state membrane rafts organization and regulation in cells and as we anticipate their role in cellular signalling pathways regulating proliferation and motility, particularly important in neoplasia and metastasis. Therefore might provide an opportunity to discover new target for developing drugs.

Proposed project is characterized by multidisciplinary approach of cellular and molecular biology, including classic biochemistry and first of all biophysics, mainly using advanced methods of living cells imaging. Taking into account our previous experience in the pioneering work on erythroid cell membrane raft organization we expect to gain results permitting formulation of original, general model of an important biological event.