Final report of the K 105006 project
supported by NKFIH

The main goal of our research to investigate the physiological importance of inositol lipids in general, but during this five-year support we concentrated mainly on the plasma membrane phosphatidylinositol 4-phosphate (PI4P) and phosphatidylinositol 4,5-bisphosphate (PIP2). Within this project, three main fields can be distinguished.

1. Further development of inositol lipid sensors

An important aim of the project was to target the Venus fluorescent protein into various membrane compartments including the subcompartments of plasma membrane (ordered and non-ordered), which was achieved by using the targeting sequence of various proteins such as Lck (L10), Fyn, K-Ras, H-Ras (ordered), c-Src (S15), K-Ras (non-ordered). On the other hand we became more and more familiar with the application of the T2A peptide, which allows the equimolar expression of two proteins. By combining these two molecular approaches, we created a set of BRET-based lipid probes that allow the reliable measurement of plasma membrane inositol lipids (PI4P, PIP2, PIP3) in attached live cells. Since the originally FRET-based cAMP, IP3 and calcium ion sensors were also modified by replacing the Cerulean fluorescent protein by a Luciferase enzyme, now we have a complete set of sensors that makes possible to have a complete screen of the intracellular pathway of various G-protein coupled receptor activation on a single 96 well plate.

Pulications:

Gulyás et al. Measurement of inositol 1,4,5-trisphosphate in living cells using an improved set of resonance energy transfer-based biosensors, PLOS ONE 10:(5) Paper e0125601. (2015)

Péter Várnai, Tamás Balla, Monitoring Membrane Lipids with Protein Domains Expressed in Living Cells, In: Jin Zhang, Sohum Mehta, Carsten Schultz (szerk.), Optical Probes in Biology. Boca Raton FL: CRC Press - Taylor and Francis Group, 2015. pp. 89-137.

Várnai et al, Quantifying lipid changes in various membrane compartments using lipid binding protein domains, CELL CALCIUM 64: pp. 72-82. (2017)

Matuska Rita, Gulyás Gergő, Hunyady László, Várnai Péter: A plazmamembrán PtdIns(3,4,5)P3 szintjének mérésére kifejlesztett BRET bioszenzorok vizsgálata emlős sejtekben, FAME 2016, Pécs, 2016. június 1-4., 2016

Although up to date we were not able to detect any microdomain-specific change of the various plasma membrane inositol lipids, which was an important aim of the project, the extremely high sensitivity any working range of the BRET-based sensor made possible even the increase of the already high level of plasma membrane PI4P and PS. These measurements led to the discovery of a protein kinase C dependent PI4 kinase activation that can compensate the usage of the inositol lipids upon the EGF or Gq-coupled GPCR activation. In another project the sensors allowed the direct measurement of lipids as the consequent of the function of the newly described lipid exchange proteins (Orp 5/8).

Publications:

Tóth at al, BRET-monitoring of the dynamic changes of inositol lipid pools in living cells reveals a PKC-dependent PtdIns4P increase upon EGF and M3 receptor activation, BBA MOLECULAR AND CELL BIOLOGY OF LIPIDS 1861:(3) pp. 177-187. (2016)

Sohn et al, Lenz-Majewski mutations in PTDSS1 affect phosphatidylinositol 4-phosphate metabolism at ER-PM and ER-golgi junctions, PNAS, 113:(16) pp. 4314-4319. (2016)

2. Acute manipulation of the plasma membrane level of inositol lipids

Using the previously introduced target sequences, we evoked microdomain specific plasma membrane recruitment of the inositol lipid modifying 5-phosphatase enzyme. Unfortunately, similar to the microdomain specific detection, our efforts to see any microdomain related effect of the acute lipid depletion have been failed.

These results were published in the BBA paper as part of the validation of the BRET-based lipid sensors.

An important aim of the project was the generation of a genetically modified mouse that contains the components of the most promising rapamycin-induced PIP2 depletion system. After careful consideration and investigation of the PIP2 depleting ability we selected the Fyn-FRB(T2098L)-mRFP-T2A-FKBP-5-phosphatase construct to create a transgenic mouse using the Sleeping Beauty system. Surprisingly, despite the CMV promoter only keratinocytes and most probably the acinar cells of the pancreas contained detectable fluorescence. Unfortunately, the functional test (termination of calcium signal) revealed that even in these high expressing lines the expression level is not high enough to rich effective PIP2 depletion. Based on the data of genotyping we created a mathematical model, which predicts of the increase of protein expression, so we decided to select the animals with the highest copy numbers in each generation and use them as parents of the further generations.

Publication:

Gergő Gulyás, Bence Szalai, Miklós Geiszt, László Hunyady, Tamás Balla, Péter Várnai: Generating a Transgenic Mouse Line Containing the Plasma Membrane Phosphatidylinositol 4,5-Bisphosphate Depletion System, EB2017, Chicago, 2017. április 22-26., 2017

3. Effects of the acute depletion of plasma membrane PI4P and PIP2

Investigating the effects of plasma membrane inositol lipids we found that plasma membrane localization of several peripheral membrane proteins can be modified by lipid depletion. A screen was performed, in which we fused the membrane targeted motifs of many proteins including small G proteins and protein kinases to the fluorescent protein, Venus. We found that in the case of either the CAAX domain of K-Ras or the N-terminal target sequence of Src kinase the Venus protein translocated from the plasma membrane to the ER and Golgi upon acute plasma membrane PI4P and PIP2 depletion. Since the localization of full length K-Ras was found to be also inositol lipid dependent, this mechanism may serve as a link between GPCR and K-Ras signalization.

Publication:

Gulyas et al, Plasma membrane phosphatidylinositol 4-phosphate and 4,5-bisphosphate determine the distribution and function of K-Ras4B but not H-Ras proteins., JBC 292(46) 18862-18877 (2017)

Previously we published that rapamycin-induced PIP2 depletion inhibited the internalization of various G-protein coupled receptors, but it remained an unsolved question whether this effect can be reached by other mechanism of PIP2 depletion. Therefore, we created a protein construct in which the FRB domain was fused to the C-terminal of AT1 receptor. This construct allows two distinct mechanisms for plasma membrane PIP2 depletion. It serves as docking site for the 5-phosphates and therefore PIP2 can be reduced upon rapamycin treatment, or it can be activated by angiotensin II, the agonist of the receptor. To avoid the interaction of the receptor with the endocytic machinery its non-internalizing mutant was used. Although the level of PIP2 depletions measured by PLCδ1-PH were similar, the agonist-induced PIP2 depletion alone did not inhibit the endocytosis of the β2 adrenergic receptor, while in the presence of the A1 compound (recently developed inhibitor of PI4Kα) we detected a significant inhibition suggesting the importance of local inositol lipid resynthesis. Interestingly, in

another functional assay (inositol lipid regulation of the capacitative calcium influx) we got exactly the same conclusion.

Publication:

Dániel J. Tóth, József T. Tóth, Bernadett Tallósy, László Hunyady, Péter Várnai: The Effects of Hormone-induced PtdIns(4,5)P2 Depletion on Endocytosis Suggest the Importance of Local Regulation of Inositol Lipid Signalling, #P737 ECE 2014, 3-7 May, Wroclaw, Poland, 2014

Szabó Kamilla, Tóth József, Hunyady László, Várnai Péter: A plazmamembrán foszfoinozitidek szerepe a kapacitatív kalcium beáramlás szabályozásában, MÉT Vándorgyűlés, P3.8 Szeged, 2015. május 27-30., 201

As planned, we also created additional endocytic route markers, and followed the internalization of AT1R upon biased activation. In collaboration, using the same BRET approach to follow the molecular events of receptor activation we investigated the coactivation between AT1 and β2 receptors.

Publications:

Szakadati et al, Investigation of the fate of type I angiotensin receptor after biased activation, MOLECULAR PHARMACOLOGY 87:(6) pp. 972-981. (2015)

Tóth et al, Angiotensin type 1A receptor regulates β-arrestin binding of the β2-adrenergic receptor via heterodimerization, MOLECULAR AND CELLULAR ENDOCRINOLOGY 442: pp. 113-124. (2017)

Other scientific achievements

Using our expertise regarding protein targeting into the surface of various organelles including mitochondria and the application of rapamycin-induced rapid dimerization, several high impact papers with collaborators have been published. The papers 1.) contain new data regarding the ultrastructure of mitochondria, 2.) show that adjacent mitochondria exhibit coordination of inner mitochondrial membrane cristae at inter-mitochondrial junctions serving the structural basis to enhance the propagation of intracellular bioenergetic and apoptotic waves through mitochondrial networks within cells, 3.) describe the existence of redox nanodomains at the ER-Mito contact sites, 4.) allow molecular insights into the VDAC2-dependent apoptosis and details of plasma membrane receptor trafficking.

Publications:

Weaver et al, Distribution and Apoptotic Function of Outer Membrane Proteins Depend on Mitochondrial Fusion, MOLECULAR CELL 54:(5) pp. 870-878. (2014)

Picard et al, Trans-mitochondrial coordination of cristae at regulated membrane junctions, NATURE COMMUNICATIONS 6: Paper 6259. 8 p. (2015)

Naghdi el al, Motifs of VDAC2 required for mitochondrial Bak import and tBid-induced apoptosis, PNAS, 112:(41) pp. E5590-E5599. (2015)

Booth et al, Redox Nanodomains Are Induced by and Control Calcium Signaling at the ER-Mitochondrial Interface., MOLECULAR CELL 63:(2) pp. 240-248. (2016)