The importance of the N-terminal fragment of the Gαi3 subunit in the process of membrane localization of trimeric G protein

Trimeric G proteins, composed of Gα subunit and Gβγ dimer, are an essential partner for G-proteins coupled receptors (GPCR). Those metabotropic receptors are highly relevant for pharmacology due to their prevalence and involvement in many processes such as mood and behavior, homeostasis (i.e., water balance), immune response and inflammation, autonomic nervous system, senses (sight, taste, smell), and cancer. Therefore G protein interaction with GPCRs in the cell membrane also modulates the overall outcome of the processes regulated by those receptors. The complexity of the membrane environment affects the cell response due to various interactions between all components. This impact is more profound in the case of transmembrane proteins like GPCRs. Nevertheless, it also affects peripheral proteins like G proteins. The presence of G proteins on the surface of the inner leaflet of the cell membrane is ensured by lipid modifications of Gα and Gγ subunits. Additionally, the positively charged region in the amino acid sequence (polybasic motive) localized near lipidation enhances attachment. Specific localization in the cell membrane is, therefore, the result of a few signals. Presented studies analyze the importance and impact of all these signals for one representative of the trimeric G proteins - Gαi3. Confocal imaging of Gαi3 fused with citrine revealed the major impact of the two lipidations in Gαi3 (palmitoylation and myristylation) in the process of membrane association for various Gβγ dimers. Moreover, FLIM-FRET measurements revealed changes in localization within the cell membrane between the D2 receptor and Gi3 triggered by the partial loss of the positive charge in Gαi3. Measurements of the intracellular cAMP levels determined the impact on the functionality of those three membrane association signals in Gαi3. Additionally, the relevance of the polybasic region in lipid attachment of Gαi3 was determined in vitro for liposomes with different phospholipids, sphingomyelin, and cholesterol, and in silico with bioinformatic docking analysis of Gαi3 with four phospholipids. In a similar manner, this work also shows the impact of lipidations and the polybasic region for Gαs. Therefore presented results compare two different classes of the G proteins in membrane localization and reveal the significant importance of lipid anchors for Gαi3, whereas for Gαs – Gβγ dimer and polybasic motif.