Spatio-temporele aspecten van G-eiwitsignalering
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Summary

The experimental work conducted for this thesis is aimed towards a better understanding of the fundamental aspects of G protein signaling at the plasma membrane and beyond. The use of advanced microscopy techniques in living cells allows the collection of quantitative information on reaction kinetics, protein-protein interactions and subcellular localization of proteins in their native environment, with single cell resolution. We provide a description of the development and characterization of an optimized FRET biosensor to investigate G-protein coupled receptor (GPCR) signaling towards the Gαi subfamily of heterotrimeric G proteins. Moreover, we describe the characterization of canonical heterotrimeric G protein signaling at the four known histamine receptor isoforms. We have investigated the recruitment and activation mechanisms of the three different ARHGEF25 isoforms; p63RhoGEF^{580}, p63RhoGEF^{619} and GEFT. It is shown that GPCR mediated activation of p63RhoGEF^{580} leads to GTP loading of RhoA at the periphery, and that plasma membrane localized RhoGEF activity leads to actin polymerization, whereas RhoGEF activity in the cytoplasm does not. This provides evidence for a model where the plasma membrane is the subcellular location of RhoGEF mediated actin polymerization by RhoA. Furthermore we report on efforts to elucidate the molecular mechanism behind the oncogenic potential of the small RhoGEF TGAT. Together, the results reported in this thesis add valuable tools and fundamental knowledge to the study of interactions between different classes of G proteins at the plasma membrane and the understanding of spatiotemporal regulation of G protein signaling in general.