Abstract

Calcium (Ca^{2+}) is an essential second messenger that regulates and modulates a variety of cellular functions. In particular, local changes of the calcium concentration play a key role in physiologically relevant processes. The elucidation of temporal and spatial dynamics underlying Ca^{2+} signaling will improve knowledge to understand intracellular signaling mechanisms and the impact of Ca^{2+} on such mechanisms. Therefore, imaging of Ca^{2+} signals is an important field of current investigation. Until recently, synthetic Ca^{2+}-sensitive dyes were the only available agents to visualize Ca^{2+} dynamics in living cells. Nowadays genetically encoded Ca^{2+} indicators, e.g. GCaMP3.0, are often used as an alternative to monitor calcium signaling.

The expression of GCaMP3.0 in eukaryotic cells mainly leads to a homogenous, cytosolic distribution of the protein. To enable the measurement of localized Ca^{2+} signals in different subcellular compartments, the GCaMP3.0 encoding gene was genetically modified as a part of this thesis. Adding short targeting motifs to GCaMP3.0 led to specific subcellular distribution of the protein either in the plasma membrane, the mitochondrial matrix or the mitochondrial outer membrane as well as the golgi apparatus. Additionally, another sensor variant was targeted to the nucleus. Any impairment of the fluorescence properties of the modified sensor proteins was ruled out by *in vitro* biophysical characterization. Furthermore, HEK293 cells were used as a model system to investigate the spatial localization and functionality of modified as well as non-modified GCaMP3.0. Apart from that, the targeted GCaMP3.0 variants were designed to study temporal and spatial Ca^{2+} dynamics in neurons. Therefore the genes encoding the modified GCaMP3.0 variants were used to generate recombinant Adeno-associated viruses. These viruses were used as gene ferries to deliver GCaMP3.0 variants to primary cultures derived from rat cortex. In both cell types, HEK293 cells and rat cortical neurons, the targeted sensor variants allowed time resolved measurements of localized Ca^{2+} signals.