UKE Paper of the Month Juli 2018

Rhodopsin-cyclases for photocontrol of cGMP/cAMP and 2.3 Å structure of the adenylyl cyclase domain

Ulrike Scheib, Matthias Broser, Oana M. Constantin, Shang Yang, Shiqiang Gao, Shatanik Mukherjee, Katja Stehfest, Georg Nagel, Christine E. Gee*, Peter Hegemann*

**equal contribution*

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ABSTRACT: The cyclic nucleotides cAMP and cGMP are important second messengers that orchestrate fundamental cellular responses. Here, we present the characterization of the rhodopsin-guanylyl cyclase from Catenaria anguillulae (CaRhGC), which produces cGMP in response to green light with a light to dark activity ratio > 1000. After light excitation the putative signaling state forms with T= 39 ms and decays with T= 570 ms. Mutations (up to 6) within the nucleotide binding site generate rhodopsin-adenylyl cyclases (CaRhACs) of which the double mutated YFP-CaRhAC (E497K/C566D) is the most suitable for rapid cAMP production in neurons. Furthermore, the crystal structure of the ligand bound AC domain (2.25 Å) reveals detailed information about the nucleotide binding mode within this recently discovered new class of enzyme rhodopsin. Both YFP-CaRhGC and YFP-CaRhAC are favorable optogenetic tools for non-invasive, cell-selective and spatio-temporally precise modulation of cAMP/cGMP with light.

STATEMENT: Signalling via the intracellular messengers cAMP and cGMP is crucial for all cells and aberrant signalling is implicated in a host of human disorders including but not limited to cancers, cardiac insufficiency, deafness and blindness. Tools to directly manipulate cAMP and cGMP in individual cells or subcellular regions have been lacking, impeding our understanding of the cell autonomous functions of these molecules. This study presents two new optogenetic tools that can be expressed in cells of interest to specifically raise cAMP or cGMP in response to light. These differ from existing tools in that they are membrane-anchored, single component systems that do not require endogenous enzymes for their function so we believe they will be widely usable in future studies and may have uses for vision or hearing restoration. The neuronal characterization was all performed at the UKE as part of an ongoing collaboration with the group of Peter Hegemann in Berlin. The group of Georg Nagel Würzburg also collaborated in this study.

BACKGROUND: Oana Constantin joined the group of Christine Gee in the Institute of Synaptic Physiology, ZMNH at the UKE to characterize these photoactivatable cyclases for her Master's thesis (University of Bremen). After completing her Master's she opted to move to Hamburg and enrol as a PhD student at the UKE where she is now studying the role of adenylyl and guanylyl cyclases in neuronal synaptic plasticity. This work was supported by the DFG (SPP1665, FOR2419) and the Landesforschungsförderung Hamburg and these tools will be very important for the new SFB1328 'Adenine nucleotides in immunity and inflammation'.