Optogenetic tools for manipulation of cyclic nucleotides, functionally coupled to CNG-channels

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Abstract

Background and Purpose The cyclic nucleotides cAMP and cGMP are ubiquitous second messengers participating in the regulation of several biological processes. Interference of cNMP signalling is linked to multiple diseases and thus is an important component of pharmaceutical research. The existing optogenetic toolbox in *C. elegans* is restricted to soluble adenylyl cyclases, the membrane-bound *Blastocladiella* CyclOp and hyperpolarizing rhodopsins, yet missing are membrane-bound photoactivatable adenylyl cyclases and hyperpolarizers on the basis of K^+-currents. **Experimental Approach** For the characterization of the photoactivatable nucleotidyl cyclases, we expressed the proteins alone or in combination with cyclic-nucleotide gated channels in *C. elegans* muscle cells and cholinergic motor neurons. To investigate the extent of optogenetic cNMP production and the ability of the systems to de- or hyperpolarize the cells, we performed behavioural analyses (locomotion, muscle contraction) and measured the cNMP content in vitro. **Key Results** We implemented *Catenaria* CyclOp as a new tool for cGMP production, allowing fine-control of cGMP levels. As photoactivatable membrane-bound adenylyl cyclases, we established YFP::BeCyclOp(A-2x) and YFP::CaCyclOp(A-2x), enabling more specific optogenetic cAMP signalling compared to soluble ACs. For the hyperpolarization of excitable cells by K^+-currents, we introduced the cAMP-gated K^+-channel SthK from *Spirochaeta thermophila* with either bPAC or BeCyclOp(A-2x), and the *Blastocladiella emersonii* cGMP-gated K^+-channel BeCNG1 with BeCyclOp. **Conclusion and Implications** We established a comprehensive suite of optogenetic tools for cNMP manipulation for the nematode, which will be useful for applications in many cell types, including sensory neurons which use mainly cGMP as second messenger, and for potent hyperpolarization using K^+-ions.

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