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TESTING OF PHOTOCYCLIC ROD OPSIN VARIANTS USING REPORTER CELLULAR TEST-SYSTEM

Losev S.D. (**SPbSU**), **Meshalkina D.A.** (IEPB RAS)
Scientific adviser – Dr. Firsov M.L.
(IEPB RAS)

Introduction. Optogenetic retinal prosthetics is one of the promising approaches to restore vision loss due to retinal degeneration. In such cases, candidates for prosthetics are bipolar cells which can be introduced with light-sensitive proteins in order to give the cells photoreceptor function. We consider the 3 rhodopsin variants described by Kazumi Sakai et al, 2022 to be promising variants of such molecules. The mutants they obtained are capable of returning retinal from the trans-conformation back to the cis-conformation without the need to replace the ligand molecule after excitation by light (i. e. the mutants are photocyclic). It is likely that they will not require retinal replacement, which may provide an advantage for prosthetic applications, since bipolar cells are unable to synthesize their own retinal, and it is unknown whether they can interact with pigment epithelium cells, normally producing it..

Main part. The aim of the study is to obtain 3 variants of human rod opsin containing mutations that would give them photocyclic properties along with additional thermostablity and accelerated reaction with retinal. The mutants were synthesized using the PCR-assembly method. The variants were tested on a reporter cell line HEK293, transfected with plasmids containing chimeric Gso-protein (interacting with the rod opsin as Go-protein and elevating cAMP level upon the interaction as Gs-protein) and red fluorescent protein mRuby2 under cAMP-responsive promoter.

The activity of rod opsins was estimated based on the level of red fluorescence since mRuby2 was expressed in response to the rise of cAMP. Gso-protein would cause the rise of cAMP levels in response to light-dependent rod opsin activation.

Cells that were illuminated for 24 hours have shown higher red fluorescence levels that the cells that were kept in the dark throughout the experiment. The synthesized rod opsins have demonstrated a different ability to increase cAMP levels in the cells. The most active turned out to be photocyclic rod opsin with mutations that made it thermostable and accelerated its reaction with retinal. Wild-type rod opsin, photocyclic and thermostable photocyclic rod opsins did not show any significant difference in activity levels between each other.

Also we made a hypothesis that rod opsin could interact with Gi-proteins of HEK293, decreasing the level of cAMP. However, transfecting the cells with plasmids without Gso only resulted in no significant difference in red fluorescence levels between Gso-containing cells and the cells without this chimeric G-protein, that refuted the hypothesis.

Conclusion. The obtained photocyclic, thermostable photocyclic and thermostable with accelerated photocycle rod opsins do show the ability to increasethe level of cAMP in cells in the presence of Gso-protein. Validation of the test system with and without illumination showed that our variants have photoreceptor properties and the introduced mutations affect their function. However, the affinity of the obtained rhodopsins to other G-proteins, their ability to change the membrane potential of cells, and their testing on the model of blind mice with degenerated retinas remain to be studied.

Sources:

1. Ballister E. R., et al. A live cell assay of GPCR coupling allows identification of optogenetic tools for controlling Go and Gi signaling // BMC Biology. 2018. № 1 (16). P. 16.
2. Sakai K., et al. Creation of photocyclic vertebrate rhodopsin by single amino acid substitution // eLife. 2022. (11). P. 16.