Journal of Life Sciences & Biomedicine, vol. 5(78), No 1, p. 80-86 (2023)

Subtle mechanisms of the visual cascade: searching for critical amino acid residues in signal transduction of the visual receptor rhodopsin

Azad Abdurahimov[1], Ilham Shahmuradov[1,2], Karim Gasimov[1*]

[1]Institute of Biophysics, Ministry of Science and Education of the Republic of Azerbaijan, 117 Zahid Khalilov Str., AZ1073, Baku, Azerbaijan
[2]Institute of Molecular Biology & Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan, 11 Izzat Nabiyev Str., AZ1141, Baku, Azerbaijan

***For correspondence:** karim.gasimov@science.az*
10.5281/zenodo.8004455
Received: February 23, 2023; Received in revised form: March 5, 2023; Accepted: April 8, 2023

Rhodopsin is the first G protein-coupled receptor (GPCR) whose three-dimensional structure has been resolved using X-ray crystallography. The crystal structure of rhodopsin revealed the molecular mechanism of photoreception and signal transduction in the visual system. Although several other GPCR crystal structures have been reported over the past two decades, the structure of rhodopsin remains an important model for understanding the structural and functional properties of other GPCRs. This review summarizes the structural properties and photoactivation of rhodopsin and details the contact points between rhodopsin and visual G-protein transducin during the initiation of visual signaling.

Keywords: *Visual transduction, GPCRs, transducyn, cristall structure*

INTRODUCTION

The human visual system is astonishing for the amount and quality of information it provides about the world surrounding it. A cursory glance is enough to describe the location, size, shape, color and composition of objects, and if the objects are moving, their direction and speed.

The organ of vision - the eye, consists of a fluid-filled sphere surrounded by three layers of tissue. Only the retina, the innermost layer of the eye, has neurons that are sensitive to light and capable of transmitting visual signals to central targets. The tissue layer immediately adjacent to it consists of three different but continuous structures, which together form the uveal tract.

The most anterior part of the uveal tract is the iris. It has two sets of muscles whose opposing movements allow it to adjust pupil size under the control of the nervous system. The sclera is the outer layer of tissue in the eye and is made up of tough white fibrous tissue. At the front of the eye, this opaque outer layer becomes the cornea, a special transparent tissue. Outside the cornea, light rays pass through two different fluid media before entering the retina. In the anterior chamber, behind the cornea and in front of the lens is the aqueous humor, a clear, watery fluid that supplies both of these structures with nutrients (Sultanov and Alieva, 1983). Aqueous humor is formed by ciliary processes in the posterior chamber.

The retina or neural part of the eye is in fact, a part of the central nervous system. The retina is formed of the inner wall of the optic cup, and the pigment epithelium of the retina is formed of the outer wall (Purves et al., 2004).

Photoreceptor cells in the human retina are divided into two types - rod cells and cone cells which are the primary receptors of visual stimulation. While cones perform colour vision, rod cells can be stimulated by a wide range of wavelengths of light starting with a dim light such

10.5281/zenodo.8004455
Available online 30 June 2023

as moonlight. Photoreceptor cells signal (synapse) with overlapping layers of interneurons innervated by different combinations of photoreceptor cells (Nakanishi, 2000). All these signals are processed and transmitted through the visual thalamus to the part of the brain called the visual cortex, where they are interpreted. Signal reception and its five-step processing are carried out by a number of protein and non-protein components included in a signal transmission chain, and this process is called "Phototransduction activation" (Fig. 1). In step 1 of this process, the light is absorbed in the eye and activates rhodopsin through a conformational change from cis-retinal to trans-retinal in the rod disc membrane (Fig. 2), and it dissociates from the opsin molecule, which opens transducin binding sites on the opsin protein. In step 2, the opsin rebinds to transducin molecules and GDP release catalyzes transducin activation by binding cytoplasmic GTP to it. In step 3, the GTP-bound subunit then removes the PDE alpha subunit and activates it. In Step 4 the activated PDE hydrolyzes cGMP to GMP. In step 5, the decrease in cGMP levels causes the closing of gated ion channels, which prevents the influx of Na^+ and Ca^{2+}, thereby depolarizing the cell.

Rod cells sense light through a light-sensitive GPCR called rhodopsin. Rhodopsin is composed of an opsin protein with a visual GPCR structure and a light-absorbing pigment called retinal covalently attached to it (Hofmann et al., 2009; Nakanishi, 2000). Found only in rod cells, rhodopsin is located in the ~1500 flattened membrane discs that form the outer segment of these rod-shaped cells (Fig. 2). A single human rod cell contains about 4×10^7 molecules of rhodopsin. A trimeric G protein (Gt) called transducin that binds to rhodopsin has a G_α subunit called $G_{\alpha t}$, like rhodopsin, $G_{\alpha t}$ is found only in rod cells.

Rhodopsin (R) differs from other GPCRs in that ligand binding does not activate the receptor. Conversely, the absorption of a photon of light by the retina coupled to the receptor is the activating signal (Calvert et al, 2006; Hofmann et al., 2009). Upon absorption of a photon, the retinal moiety of rhodopsin is immediately converted from the cis isomeric form (known as 11-cis-retinal) to the all-trans isomeric form, causing a conformational change in the opsin protein (Fig. 2). This is equivalent to the activation of a conformational change by ligand binding in other G protein-coupled receptors, which allows rhodopsin to bind to the $G_{\alpha t}$ subunit of its G protein-coupled transducer, causing GDP to be replaced by GTP in its G_α subunit. The resulting activated rhodopsin, denoted as R*, is unstable because its covalent bonds with retinal are spontaneously broken. Since retinal-free opsin cannot find the transducer, the initiation of visual signal transduction is broken at this point. Free all-trans-retinal is converted back to 11-cis-retinal in the dark through a series of steps involving enzymes in the rod cells and adjacent retinal pigment epithelium cells (Smith, 2010). The resulting 11-cis-retinal moves to the rod cells, where it combines with opsin again to form rhodopsin, and completes the visual cycle.

Fig. 1. Initiation of phototransduction of the visual cascade in vertebrates.

Fig. 2. Retinal isomerization and rhodopsin activation cycle.

Structure of rhodopsin

Rhodopsin is the first GPCR to have its crystal structure obtained (Palchewski et al., 2000). To obtain the crystal structure, bovine

rhodopsin was purified by separating it from the outer segmental membranes of rod cells and crystallized from nonyl-thiol-glucoside detergent solution to which amphiphilic heptane 1,2,3-triol was added (Okada et al., 1998; 2000).

Rhodopsin, an integral membrane protein, is composed of three topological domains: an extracellular surface domain, a membrane-embedded transmembrane domain, and an intracellular surface domain (Figure 3). Since rhodopsin is located in the disc membranes of the rod outer segment (Fig. 2), the extracellular domain is often called the intradiscal domain. The amino terminal of the protein is in the extracellular part and the carboxyl terminal is in the intracellular part. The membrane-bound domain consists of seven transmembrane segments (H1-H7) dominated by an α-helix. The helical segments form a compact bundle and contain a retinal (RET) binding site (Menon et al., 2001).

In addition to the NT segment, the extracellular surface domain has three extracellular interhelical loops: E1 loop of 101–106 amino acids and connects H1 and H2, E2 loop of 174–199 amino acids and connects H4 and H5, E3 loop consists of amino acids residues 278-285 and connects H6 and H7.

Using site-directed mutagenesis, it has been shown that the extracellular loops and NT of bovine rhodopsin are required for the proper folding of the receptor which plays important roles in cell processing, chromophore binding (Doi et al., 1990) and in cell trafficking (Borjighin and Nathans, 994).

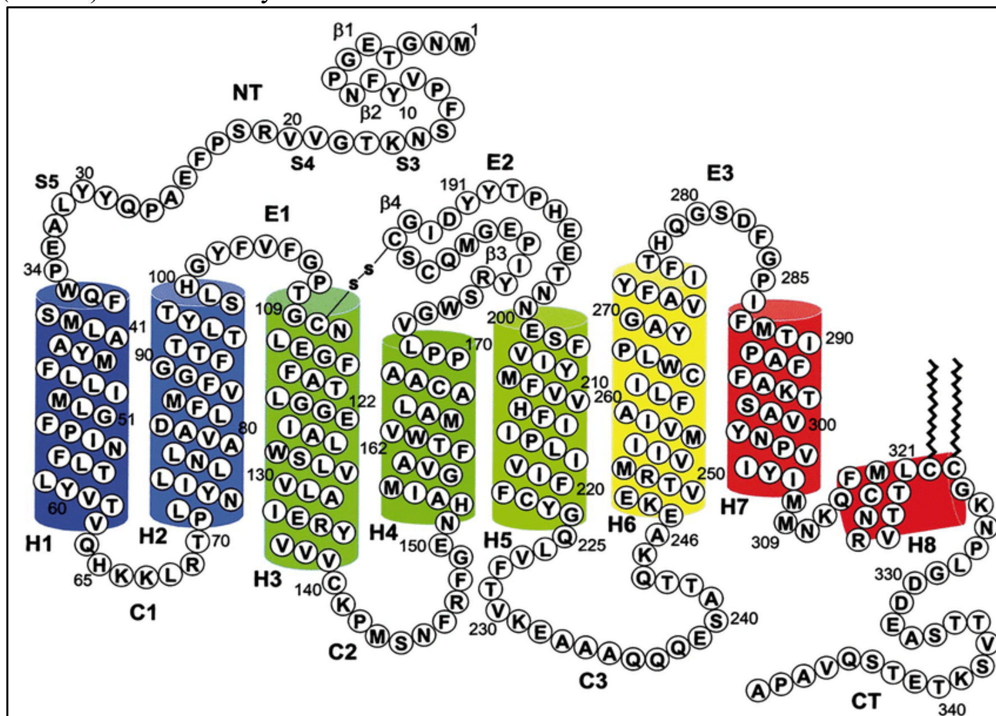

Fig. 3. Secondary structure of rhodopsin. Regional schematic diagram of transmembrane helices, intradiscal regions and cytoplasmic regions. The position of each residue is depicted in a single-letter code.

Rhodopsin is known to be glycosylated at Asn-2 and Asn-15 residues of NT. Non-glycosylated rhodopsin has been shown to be defective in the light-dependent activation of Gt (Kaushal et al., 1994).

Two conserved cysteine residues in the extracellular domain, Cys-110 and Cys-187, have been shown to be important for the folding of the opsin protein (Karnik et al., 1988). Elegant studies by site-directed mutagenesis have shown that these two residues are involved in the formation of disulfide bonds (Karnik and Khorana, 1990). It appears that this disulfide bond stabilizes the ground-state structure of the chromophore-binding pocket.

Extracellular surface domain of rhodopsin

The extracellular surface domain is composed of an amino-terminal tail (NT) and three interhelical loops (E1, E2, and E3). The extracellular domain contains significant secondary structural elements and several intra- and inter-domain interactions. The NT extends from the amino terminus to Pro-34 and consists of a distorted strand labeled as β1, β2, S3, S4, and S5 (Figure 3).

The membrane-embedded domain of rhodopsin

The crystal structure of rhodopsin shows that 194 of the 348 amino acid residues of bovine rhodopsin make up its seven membrane-embedded (H1-H7) segments (Menon et al., 2001). These are H1 (residues 35-64), H2 (71-100, H3 (107-139), H4 (151-173), H5 (200-225), H6 (247-277), and H7 (286-306). The crystal structure shows that this domain has multiple bends and disordered structures in individual transmembrane segments, but the overall secondary structure is α-helical (Menon et al., 2001).

The chromophore moiety of rhodopsin binds to retinal (RET) in its chromophore-binding pocket. RET is a derivative of vitamin A1 and consists of 20 carbon atoms. The RET chromophore bound to the rhodopsin crystal structure is in the 6-s-cis, 11-cis 12-s-trans conformation (Menon et al., 2001). The RET chromophore binding site is located in the membrane-embedded part of the receptor. All seven transmembrane segments and part of the extracellular domain are involved in the interaction of the opsin protein with the chromophore. The chromophore is located closer to the extracellular side of the transmembrane domain than to the intracellular side. Chromophore polyene of C6 and C11 runs almost parallel to H3. This transmembrane segment provides most of the amino acid side chains involved in the chromophore-binding pocket: Glu-113, Gly-114, Ala-117, Thr-118, Gly-120, and Gly-121. The extracellular-facing polyene chain of the receptor is covered by β4-sheet amino acid residues (Ser-186 to Gly-121) of the E2 loop.

Cytoplasmic domain of rhodopsin

The cytoplasmic domain of rhodopsin consists of three cytoplasmic loops and a carboxyl terminal tail:C1 (amino acids 65-70), C2 (140-150), C3 (226-246), and CT (307-348). CT is divided into two structural domains. C4 extends from Ile-307 to Gly-324 at the end of H7, just beyond vicinal Cys residues (Cys-322 and Cys-323), which are post-translationally palmitoylated (Menon et al., 2001). The remaining part of CT extends from Lys-325 to Ala-348 at the carboxyl terminus of rhodopsin. A characteristic feature of the C4 loop is that it forms an α-helix, denoted as H8 (Fig. 3). H8 lies almost perpendicularly on H7.

The C1 loop exhibits a rigid conformation involving the basic residues His-65, Lys-66, Lys-67, and Arg-69. Lys-66 and Arg-69 side chains are facing the membrane bilayer. His-69 is located close to the C4 loop within H8. And Lys-67 interacts with CT located approximately parallel to C1. C2 resembles an L-shaped structure when viewed from a viewpoint parallel to the putative membrane plane. A short β-barrel structure extends from Met-143 to Phe-146.C2 and C3 lie at approximately the same level on the cytoplasmic border of the receptor.

The main characteristic of C4 is that it has an α-helical structure, designated H8 as shown above. H8 binds to H7 by the tripeptide Met-309/Asn-31Lys-311 and extends perpendicularly to it.

Rhodopsin residues responsible for interaction with G-protein transducin

Transduction and amplification of the signal from rhodopsin (R*) to G_t occurs as a result of specific interactions between the cytoplasmic surface domain residues of R* and at least two regions of G_t – the C-terminus of the α-subunit and the C-terminus of the γ-subunit (Garcia, et al., 1995; Helmreich and Hoffman, 1996, Kisselev, 1999).

The most studied receptor-G protein interactions have been between bovine rhodopsin and bovine G_t which can be easily isolated and purified or expressed recombinantly. Extensive and accurate biochemical and biophysical analyzes of R*-G_t interactions were performed by mutagenesis of cytoplasmic domains of bovine rhodopsin.

Initial mapping of the G_t-recognizing

rhodopsin residues was performed in the late 1980s using site-directed antipeptide antibodies (Weiss et al., 1987). These antibodies were generated specifically for the putative cytoplasmic domain of rhodopsin. Studies have shown that the potential G_t-recognizing residues of bovine rhodopsin are located in cytoplasmic loops 3, 4 and the C-terminus. Ala-scanning mutational studies were able to detect several transducin-recognizing rhodopsin residues only in the C3 loop (Shi et al., 1995).

A More extensive mapping of Gt-recognizing rhodopsin residues was performed using a pre-chemically activated cross-linking reagent N-succinimidyl 3-(2-pyridyldithio) propionate examining the contact sites that interact between light-activated rhodopsin and transducing (Itoh et al., 2001). In these studies, cross-linking with Tα was demonstrated for rhodopsin mutants K141C, S240C and K248C. The major site of cross-linking in transducin is within the Leu-19-Arg-28 peptide sequence in the N-terminal region of Tα. Both the N and C termini of Tα have been shown to be closer to the third cytoplasmic loop of rhodopsin in the Rho-T complex, and cytoplasmic loop 2 residue Lys-141 and cytoplasmic loop 3 residues, Ser-240 and Lys-248are critical residues for transducin recognition.

Complete mapping of transducin-recognizing rhodopsin residues was carried out in N. Artemyev's laboratory (Natochin et al., 2003). In this study, a gain-of-function mutational approach was used to identify rhodopsin residues critical for G_t activation. The target regions of rhodopsin for mutagenesis included the C4 loop, the non-helical parts of the C2 and C3-loops. Three mutant opsins with residues 140–148 in loop C2, 229-244 in loop C3, and 310–320 in loop C4 (helix H8) were substituted with poly-Ala sequences of equivalent length and used as templates for mutagenesis. In each of the templates, Ala residues were replaced by original rhodopsin residues. Template mutants with poly-Ala substitutions in the C2 and C3 loops produced 500-nm-long absorbing pigments but failed to activate transducin. In each of the templates, back-replacement of the Ala residues with the original rhodopsin residues (recovery of function) Cys140/Lys141 and Arg147/Phe148 residues in the C2 loop significantly (~50%) restored rhodopsin/transducin binding. In the C3 loop, residues Thr229/Val230 and Ser240/Thr242/Thr243/Gln244 completely restored Gt activation. These studies revealed the role of Asn310/Lys311 residues in 11-cis-retinal binding and Phe313 and Met317 residues in Gt activation in the C4 template mutant.

Recent advances in structural biology have made it possible to obtain high-resolution structures of protein complexes of GPCR-G proteins, including cryoelectron microscopy structures of 4.5 Å engineered Rho-bound G-protein (Kang et al., 2018) and dominant-negative G-protein mutants (Liang et al., 2018; Draper-Joyce et al., 2018; Garci'a-Nafri'a et al., 2018; Kang et al., 2018). Use of binding partners such as nanobodies (Rasmussen et al., 2011; Zhang et al., 2017; Liang et al., 2017, 2018) or antibody fragments (Koehl et al., 2018; Kang et al., 2018; Krishna Kumar et al., 2019) has revolutionized the analysis of the G-protein-rhodopsin complex. A cryo-EM structural image of a fully functional and signal-transmitting Rho-Gt complex is presented in the presence and absence of engineered nanobodies that do not interfere with G-protein activation (Gao et al., 2019). These studies showed that light-activated Rho forms extensive contacts with Gt, resulting in an interface area of 1042 Å2. Formation of this interface is provided by the α5 helix, α4-β6 loops, β2-β3 loops, and the αN-β1 eGαt loop in the G protein, as well as the H3, H5, H6 transmembrane helices, intracellular loop 2 (ICL2) and H8 of rhodopsin.

Thus, studies using many different approaches and methods have revealed the important role of certain residues in the three cytoplasmic loops of rhodopsin (C2, C3 and C4), especially those adjacent to the H3, H5, H6 transmembrane helices and the intracellular H8, in the formation of the complex of rhodopsin with transducin and initiation of phototransduction via transducin activation.

REFERENCES

Borjigin J., Nathans J. (1994) Inserftional mutagenesis as a probe of rhodopsin's

topography, stability, and activity. *J. Biol. Chem.,* **269:** 14715-14722.

Calvert P., Strissel K.J., Schiesser W.E., Pugh Jr.E.N., Arshavsky V.Y. (2006) Light-driven translocation of signaling proteins in vertebrate photoreceptors. *Trends Cell Biol.,* **16:**560–568.

Doi T., Molday R.S., Khorana H.G. (1990) Role of intradiscal domain in rhodopsin assembly and function. *Proc. Natl. Acad. Sci. USA*, **87:** 4991-4995.

Draper-Joyce C.J., Khoshouei M., Thal D.M., Liang Y.L., Nguyen A.T.N., Furness S.G.B., Venugopal H., Baltos J.A., Plitzko J.M., Danev R. et al. (2018) Structure of the adenosine-bound human adenosine A1 receptor-Gi complex. *Nature,* **558:** 559–563.

Gao Y., Hu H, Ramachandran S., Erickson J.W., Cerione R.A., Skiniotis G. (2019) Structures of the Rhodopsin-Transducin complex: Insights into G-protein activation. *Molecular Cell,* **75:** 781–790; doi: 10.1016/j.molcel. 2019.06.007.

Garcia P.D., Onrust R., Bell S.M., Sakmar T.P., Bourne H.R. (1995) Transducin-alpha C-terminal mutations prevent activationof rhodopsin: a new assay using recombinant proteins expressed in cultured cells. *EMBO J.*, **14:** 4460-4469.

Helmreich E.J., Hoffman K.P. (1996) Structure and function of proteins in G-protein-coupled signal transfer. *Biochem. Biophys. Acta*, **1286:** 285-322.

Hofmann K.P., Scheerer P., Hildebrand P.W., Choe H-W., Park J.H., Heck M., Ernest O.P. (2009) A G protein-coupled receptor at work: the rhodopsin model. *Trends Biochem. Sci.,* **34:** 540–552.

Itoh Y., Cai K., Khorana H.G. (2001) Mapping of contact sites in complex formation between light-activated rhodopsin and transducin by covalent cross linking: Use of a chemically preactivated reagent. *Proc. Natl. Acad. Sci. USA,* **98 (9):** 4883-4887.

Kang Y., Kuybeda O., de Waal P.W., Mukherjee S., Van Eps N., Dutka P., Zhou X.E., Bartesaghi A., Erramilli S., Morizumi T. et al. (2018) Cryo-EM structure of human rhodopsin bound to an inhibitory G protein. *Nature*, **558:**553–558.

Karnik S.S., Khorana H.G. (1990) Assembly of functional rhodopsin requires a disulfide bond between cysteine residues 110 and 187. *J. Biol. Chem.,***265:** 17520-17524.

Karnik S.S., Sakmar T.P., Chen H.B., Khorana H.G. (1988) Cysteine residues 110 and 187 are essential for the formation of correct structure in bovine rhodopsin. *Proc. Natl. Acad. Sci. USA,* **85:** 8459-8463.

Kaushal S., Ridge K.D., Khorana H.G. (1994) Structure and function in rhodopsin: the role of asparagine-linked glicosilation. *Proc. Natl. Acad. Sci. USA,* **91:** 4024-4028.

Kisselev O.G., Kao J., Ponder J.W., Fann Y.C., Gautan N., Marshall G.R. (1999) Light-activated rhodopsin indices structural binding motif in G protein alpha subunit. *Proc. Natl. Acad. Sci. USA,* **96:** 4898-4903.

Koehl A., Hu H., Maeda S., Zhang Y., Qu Q., Paggi J.M., Latorraca N.R., Hilger D., Dawson R., Matile H. et al. (2018) Structure of the μ-opioid receptor-Gi protein complex. *Nature*, **558:** 547–552.

Krishna Kumar K., Shalev-Benami M., Robertson M.J., Hu H., Banister S.D., Hollingsworth S.A., Latorraca N.R., Kato H.E., Hilger D., Maed S. et al. (2019) Structure of a signaling cannabinoid receptor 1-G protein complex. *Cell*, **176:** 448–458.

Liang Y-L., Khoshouei M., Glukhova A., Furness S.G.B., Zha P., Clydesdale L., Koole C., Truong T.T., Thal D.M., Lei S. et al. (2018) Phase-plate cryo-EM structure of a biased agonist-bound human GLP-1 receptor-Gs complex. *Nature*, **555:** 121–125.

Menon S.T., Hann M., Sakmar T.P. (2001) Rhodopsin: Structural basis of molecular physiology. *Physiological Reviews*, **81(4):** 1659-1688.

Nakanishi K. (2000) Recent bioorganic studies on rhodopsin and visual transduction. *Chem. Pharm. Bull.,* **48(10):**1399-1409.

Natochin M., Gasimov K.G., Moussaif M., Artemyev N.O. (2003) Rhodopsin determinants for transducin activation: A gaın-of-functıon approach. *J. Biol. Chem.,* **278(39):**37574–37581

Okada T., Le Trong I., Fox B.A., Behnke C.A., Stenkamp R.E., Palchewski K. (2000) X-ray diffraction analysis of three-dimensional cristals of bovine rhodopsin obtained from mixed micelles. *J. Structural Biol.*, **130:** 73-80.

Okada T., Takeda K., Kouyama T. (1998) Highly selective separation of rhodopsin from bovine and rod outer segment membranes using combination of divalent cation and alkyl(thio)glicoside. *Photochem. and Photobiol.,* **67:** 495-499.

Palchewski K., Kumasaka T., Hori T., Behnke C.A., Motoshima H., Fox B.A., Le Trong I., Teller D.C., Okada T., Stenkamp R.E., Yamamota M., Miyano M. (2000) Cristal structure of rhodopsin: a G-protein-coupledreceptor. *Science*, **289:** 739-745.

Purves D., Augustıne G.J., Fıtzpatrıck D., Hall W.C., Lamantıa A-S., McNamara J.O., Wıllıams S.M. (2004) Neuroscience. Third edition. USA, Massachusetts, Sinauer Associates Inc. Publishers Sunderland: 773 p.

Rasmussen S.G.F., DeVree B.T., Zou Y., Kruse A.C., Chung K.Y., Kobilka T.S., Thian F.S., Chae P.S., Pardon E., Calinski D. et al. (2011) Crystal structure of the b2 adrenergic receptor-Gs protein complex. *Nature*, **477:**549–555.

Sultanov M.Yu., Alieva Z.A. (1983) Lacrimal drainage physiology. Azerneshr: 31 p. (in Russian).

Shi W., Osawa S., Dickerso C.D., Weiss E.R. (1995) Rhodopsin mutants discriminate sites important for the activation ofrhodopsin kinase and Gt. *J. Biol. Chem.,* **270(5):** 2112–2119.

Smith S.O. (2010) Structure and activation of the visual pigment rhodopsin. *Annu. Rev. Biophys.,* **39:** 309-328.

Weiss E.R., Kelleher D.J., Johnson G.L. (1987) Mapping sites of ınteraction between rhodopsin and transducinusing rhodopsin antipeptide antibodies. *J. Biol. Chem.,* **266(13):**6150-6154.

Zhang Y., Sun B., Feng D., Hu H., Chu M., Qu Q., Tarrasch J.T., L.S., Sun Kobilka T., Kobilka B.K., Skiniotis G. (2017) Cryo-EM structure of the activated GLP-1 receptor in complex with a G protein. *Natur*e, **546:** 248–253.

Citation: Abdurahimov Azad, Shahmuradov Ilham, Gasimov Karim, Subtle mechanisms of the visual cascade: searching for critical amino acid residues in signal transduction of the visual receptor rhodopsin. *Journ. Life Sci. & Biomed.,* **78(1):** 80-86

A.Abdurahimov ORCHID: https://orcid.org /0009-0004-8035-1018
I.Shahmuradov ORCHID: https://orcid.org /0000-0001-5533-5906
K.Gasimov ORCHID: https://orcid.org 0009-0009-2306-0114