Role of Rhodopsin in Retinitis Pigmentosa

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Abstract
Rhodopsin is a 348 amino acid protein composed of seven transmembrane alpha helices located within the rod photoreceptor cells of the retina and is chiefly responsible for stimulating the nervous system of humans and other vertebrate animals to see light. Rhodopsin is a member of the much larger class of G protein-coupled receptors (GPCRs), which are comprised of cell surface signaling receptors that convert extracellular signals into intracellular signaling pathways through the activation of G proteins. There are about 800 different human GPCRs and members of this superfamily are responsible for our sense of smell, taste, and vision. The normal “wild type” form of rhodopsin has a sequence with no mutations. This form folds normally and uses its retinal pigment, a derivative of vitamin A, in conjunction with its G protein partner, transducin, to detect light, particularly in night vision. Retinal serves as the “cofactor” of rhodopsin that directly absorbs a photon, causing its isomerization and a change in the conformational structure of rhodopsin, leading to an intracellular signaling cascade that results in a signal being sent through the optic nerve to the brain that light has been detected. Any one of over 150 rhodopsin mutations already documented in humans are known to result in loss of Rhodopsin’s light-sensing function, leading to Retinitis Pigmentosa (RP). RP is a slow progressive rare genetic disease that damages the retina and can cause blindness, particularly among young adults. The most common cause RP is mutation-induced misfolding of rhodopsin.

Function
The rod outer segment (ROS) contains a tall stack of thin membrane discs comprised of 700-1000 discs and each disc contains hundreds of rhodopsin molecules. The many forms of proposed treatment of RP are largely focused on improving the folding of rhodopsin and inducing photoreceptor function — the process in the cell that promotes correct folding and quantities of proteins. Pharmacological chaperones, such as retinos or non-isomerizable rhodopsin analogs directly target protein structure and have produced lab results that show enhanced folding and protection of the retina from the effects of mutated rhodopsin. However, replicating this in living organisms has not been possible yet.

Mutations
Various mutations of the RHO gene can result in the development of retinitis pigmentosa, a progressive degeneration of rod cells and loss of night vision early on and the destruction of cones and loss of daytime vision later [4]. Five RHO missense mutations that encode amino acid changes in rhodopsin, p.G90D, p.H94Q, p.E113K, p.A295V, and p.A295V that lead to autosomal dominant congenital stationary night blindness (adCSNB) and often result in a lack of detectable rod function and scotopic vision. While most mutations are constant in their impact on rhodopsin in rod cells with time, the same mutations can lead to retinitis pigmentosa later in life as the cone rhodopsin is still negatively affected. Two other missense RHO mutations that cause adRP involve amino acid changes p.E150K and M235L, as well as two nonsense mutations that prematurely terminate the protein chain, p.W161ter and p.E244ter, are associated with autosomal recessive retinitis pigmentosa phenotype, which constitutes the vast majority of RHO mutations. In a genetic analysis of five unrelated people who were diagnosed with sector retinitis pigmentosa, three RHO missense mutations (p.T17M, p.L131Q, and p.G160R) were identified [1]. RHO mutations can be classified by both their clinical presentation and their impact on GPCR function. Rhodopsin is composed of three subunits: alpha, beta, and gamma subunit. Bonding to rhodopsin occurs at the G alpha subunit, and the phototransduction cascade relies on this subunit to transmit the signals needed to create an action potential within the retina.

Disease and Treatment
There are about 150 different disease mutations in the RHO gene, the gene that is mutated in rod cells, resulting in a progressive degeneration of rod cells and loss of night vision. As the patient grows older, blind spots develop in the peripheral vision. There is no cure for RP; however, scientists are developing several treatments to lessen the complications of the disease. Gene therapy has been largely successful in the lab and is currently in clinical trials. The method of gene therapy differs based on the specific mutation in the RHO gene. For autosomal dominant RP, gene therapy works either to prevent the production of the mutant protein or counter the protein’s expression. Scientists are also currently working on stem cell treatment for human RP. Embryonic stem cells have successfully repaired rats with retinitis pigmentosa. The stem cells were not able to completely replace the damaged retina, but they were found to differentiate into photoreceptors specific to the location of the retina. One approach being explored is to use pharmacological chaperones to restore proteostasis by improving the folding of mutant rhodopsin. Other substances, such as vitamin A or osmotopes, can also promote proper protein folding. In January 2020, the Air School of Ophthalmology at Central South University will be starting a clinical trial using a rhodopsin injection of autoreg, a serum that contains active factors to stimulate the creation of aqueous humor (the school has found that patients with RP have decreased amounts of aqueous humor), to treat RP patients.

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Figure 1. Normal retina versus a collapsed retina affected by retinitis pigmentosa.

Figure 2. The rod outer segment contains a tall stack of thin membrane discs. The membranes are comprised of cell surface rhodopsin and G protein complexes (GPCRs). The activated G protein triggers a signal through the cell, into Guanosine Monophosphate (GMP). PDE (Guanosine Monophosphate Phosphodiesterase)’s function is to convert cGMP, which regulates sodium ion channels by opening them through the cell membrane. PDE is a decrease in the cell’s concentration of cGMP and subsequent increase in the concentration of GMP, causing many sodium ion channels to close up as a result of their “GMP-phobic” state. Without the sodium ion channels open to allow for a free ion flow, the rod is now shut off. This sodium ion channel blockage causes a decrease in the number of neurotransmitter molecules produced. A majority of the photoreceptor rhodopsin in the retina is first evident in childhood with loss of night vision. As the patient grows older, blind spots develop in the peripheral vision. These blind spots eventually merge, causing tunnel vision. Progressing throughout adulthood, RP begins to affect central vision, impeding activities such as reading or driving. Eventually, it leads to complete blindness. The rate of progression of RP varies between the members of a single family. Overall, the disease progresses slowly - many patients are able to live in an active adulthood. Most do not experience complete blindness until they are around sixty-five years old.

Figure 3. A model of the propagation of calcium ions in retinal cells. Calcium ions, which are released from intracellular stores, are also released from the retinal cells and the photoreceptor complex to activate the ryanodine receptor. This, in turn, leads to an increase in intracellular calcium levels. The increase in intracellular calcium levels in turn activates the ryanodine receptor, which in turn releases calcium ions from the intracellular stores. This process is repeated several times, leading to a sustained increase in intracellular calcium levels.

Figure 4. The phototransduction pathway of rhodopsin. G-coupled protein receptors, or GPCRs, are a family of transmembrane proteins that act as the cell membrane receptors in endocrine signaling transduction pathways. The activation of seven alpha helices embedded in the hydrophobic core of the membrane that are connected by amino acid loops on the cytoside and the extracellular side of the membrane. One of the loops on the cytoside side of the GPCR is extended to provide a binding interface for the G protein, which coordinates with the GPCR to be activated by the external ligand. The activated G protein is induced to release its co-constituent bound guanine diphosphate (GDP) and then bind guanine triphosphate (GTP), causing the G protein to break apart into two subunits, one of which triggers the intracellular signal transduction pathway. This pathway serves to amplify the chemical signal of the ligand to induce the cellular responses more rapidly and on a larger scale than could be accomplished without the receptor complex. GPCRs are an extremely diverse family of receptor proteins and are involved in regulating many sensory and nearly all non-sensory physiological processes. To cite two examples, one GPCR, chemokine receptor CXCR4, coordinates with its external ligand chemokine CXCL12 to regulate immune surveillance, specifically relating to glioma, a glioblastoma found in the brain. The primary binding interface for the GPCR is proposed to be between loops V and VI. Another GPCR, GPR87, is involved in the intracellular release of G proteins, which are involved in the suppression of some enzymatic pathways through allosteric binding. For many years, it was considered an orphan receptor, but the ligand was discovered in 2007 to be theophylline, a drug that is very similar in structure to that of the GPCR phototransduction pathway of rhodopsin.