

Opsin: To See or Not to See

Cedarburg SMART Team: Abigail Arnholt, Abigale Butt, Olivia DeBuhr, Meggie Griffin, Ethan Janecek, Isabelle Kalmer, Lauren Ketelhohn, Jake Levy, Nicole Minerva, Joshua Wankowski

Teacher: Karen Tiffany
Mentor: Alison Huckenpahler
Medical College of Wisconsin

Abstract

Colorblindness, a genetic disorder affecting millions of people worldwide, is caused by mutations in a retinal protein, opsin. Opsin is a G-protein coupled receptor which binds to light sensitive retinal. When light enters the cell, the retinal isomerizes from the *cis* form to the *trans* form, and opsin is activated; nerves then signal the brain. The opsin complex, sensitive to specific wavelengths of light, is identified by the wavelength that activates it; long (L), middle (M), or short (S). Mutations in opsin may lead to visual problems, including the inability to distinguish between red and green. The Cedarburg SMART (Students Modeling A Research Topic) Team has used 3D printing technology to construct a model of opsin which contains two monomers consisting of several helices that are stabilized by interactions between Lys231-Glu247 and Tyr223-Arg135. Two openings in opsin can be found in the retinal-binding pocket; one allows the *cis* form of retinal to enter and bind opsin, while another between allows the *trans* form to exit opsin. Common forms of colorblindness are characterized by mutations in amino acid residues. Three specific mutations, identified by using the single letter amino acid codes LIAVA, LIAVS, and LVAVA, involve the amino acids at positions 153, 171, 174, 178 and 180. These residues are particularly important for differentiating between M cone opsin and L cone opsin. Because the effect of different opsin mutations on the cell is unknown, a major research focus is to determine whether these mutations can be overcome to restore spectral sensitivity in colorblind people.

Mutations in the protein opsin are a leading cause of colorblindness across the world.

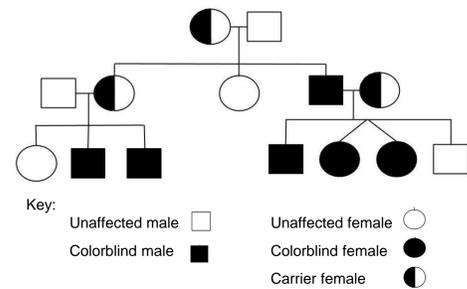


Figure A: Pedigree showing the inheritance pattern of red-green colorblindness.

According to the Color Blind Awareness Company, 4.5% of the world population is colorblind. Red-green color blindness, the most common type of colorblindness, is an X-linked recessive trait, so males are more likely to be colorblind than females. People with this kind of colorblindness, for example, would have a hard time distinguishing from red and green on a traffic light, which can be a fatal problem.

Opsin is a protein responsible for spectral sensitivity.

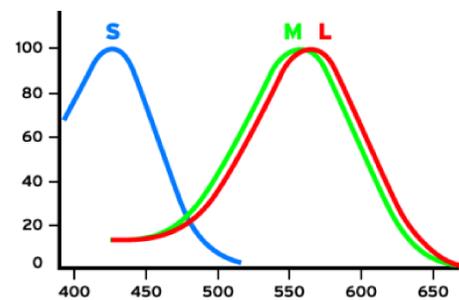
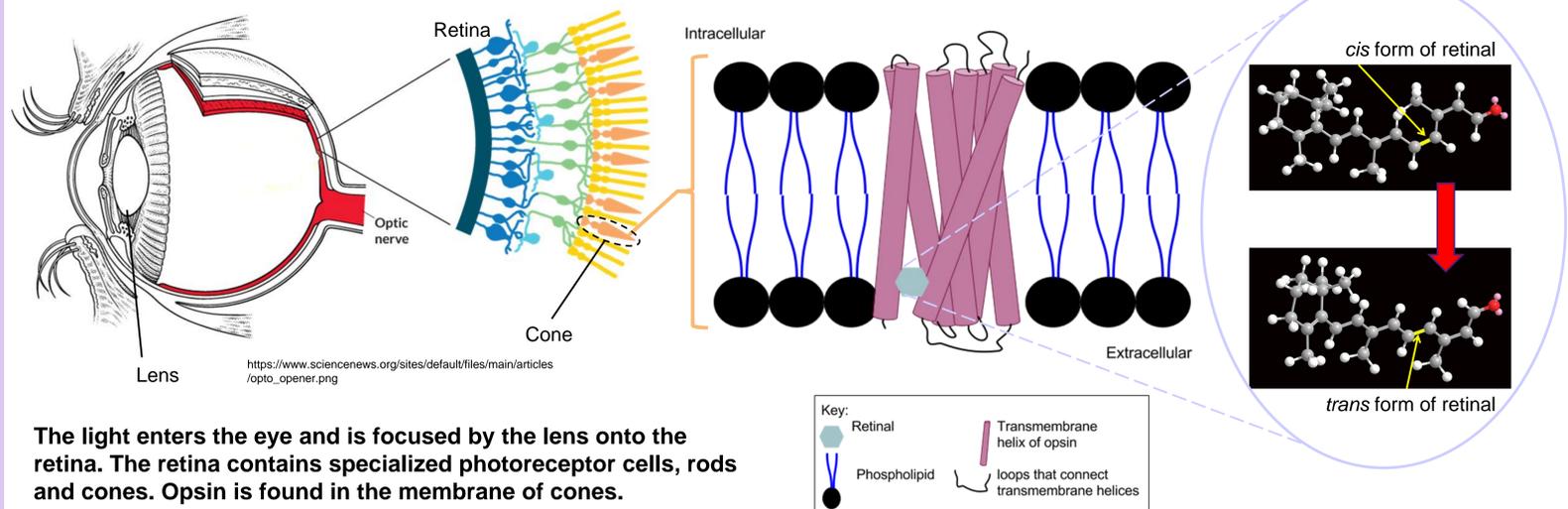


Figure B: The percent (%) of specific wavelengths (nm) that the short (S), middle (M), and long (L) opsin absorb.

Opsin is a protein present in the cones of the eye. It translates waves of light into electrical signals that are interpreted by the brain. The cones are located inside the retina of the eye and detect differences in wavelengths of light. There are three types of cones, each with an opsin protein adapted to absorb specific wavelengths of light: short (S), middle (M), and long (L).

Opsin, found in retinal photoreceptors, mediates the conversion of light photons into electrochemical signals. This involves the isomerization of *cis*-retinal to *trans*-retinal.

Retinal is a light sensitive molecule that binds opsin. When retinal is in the *cis* form, opsin is not active. When light hits the retina, retinal switches to the *trans* form and opsin is activated. This results in the cones signaling the brain and the brain perceiving color. Once the retinal bound to opsin is in the *trans* form, opsin cannot be activated again; the *trans*-retinal then must leave opsin to allow more *cis*-retinal to bind opsin.



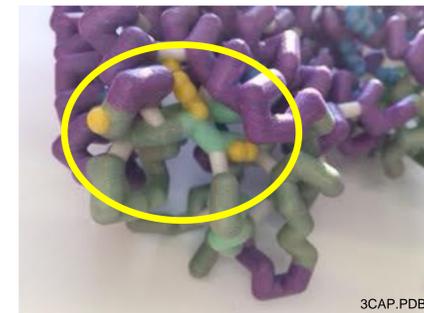
The light enters the eye and is focused by the lens onto the retina. The retina contains specialized photoreceptor cells, rods and cones. Opsin is found in the membrane of cones.

The structure of opsin is important for cone function.

Opsin is a G-protein coupled receptor (GPCR) and an opsin monomer has the typical GPCR structure comprised of seven transmembrane helices which are shown in purple. The beta sheets can be seen in light blue-green. Opsin functions as a dimer.

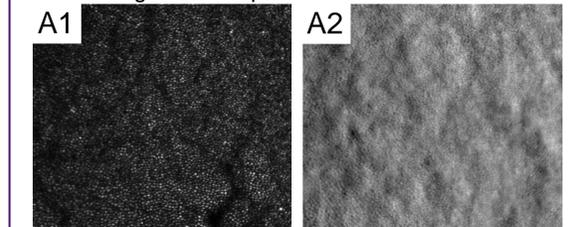
Although retinal was not present in the crystal structure, amino acids important for binding retinal (Glu113, Glu181, Tyr191, Met207, Phe261, Trp265, Lys296, and Phe293) are included in our model and shown in blue.

Thousands of different types of mutations result in the red-green form of colorblindness. The most common mutations that result in red-green color blindness involve the five amino acids at positions 153, 171, 174, 178, and 180: LIAVA (Leu153, Ile171, Ala174, Val178, Ala180), LIAVS (Leu153, Ile171, Ala174, Val178, Ser180), and LVAVA (Leu153, Val173, Ala174, Val178, Ala180). These mutation sites in the opsin model are colored yellow.

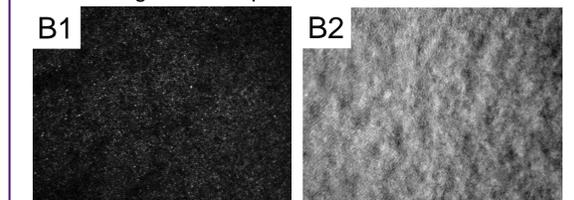


Advanced Optics (AO) images show that cones are present but not functioning in individuals who are colorblind.

Retinal images from a person with normal color vision.



Retinal images from a person with colorblindness.



The split detector images (A2 and B2) provide evidence that patients with colorblindness have a comparable number of cones as individuals with normal color vision. Confocal imaging (A1 and B1) of the same retinal area shows many dark areas in the colorblind patient (B1), indicating non-functional cones. Lighted areas in the confocal image are functioning cones.

Conclusion

- Opsin mutations decrease spectral sensitivity.
- L/M opsin mutations cause red-green colorblindness.
- Using advanced ocular imaging programs, differences in eye structure for colorblind individuals can be visualized.
- Individuals who are colorblind have cones, but they are non-functioning. Finding a way to fix opsin mutations could lead to fully functioning cones.

References and Acknowledgements

Jung Hee Park, Patrick Scheerer, Klaus Peter Hofmann, Hui-Woog Choe, and Oviver Peter Ernst. 2008. Crystal Structure of the Ligand-free G-protein-coupled Receptor Opsin. Nature, v454, 183-188.

Joseph Carroll, Alfredo Dubra, Jessica C. Gardner, Lilianna Mizrahi-Meissonnier, Robert F. Cooper, Adam M. Dubis, Rick Nordegren, Mohamed Genead, Thomas B. Connor, Jr, Kimberly E. Stepien, et al. The Effect of Cone Opsin Mutations on Retinal Structure and the Integrity of the Photoreceptor Mosaic. ARVO, v53, 8006-8015.

The Cedarburg Smart Team thanks Kathleen Pollock (M.S.) for her assistance in digital graphics design for the retinal depiction, and members are indebted to Brian Higgins, lab coordinator of the Advanced Ocular Imaging Program, and Alex Salmon, graduate student, for their help in obtaining AO images.