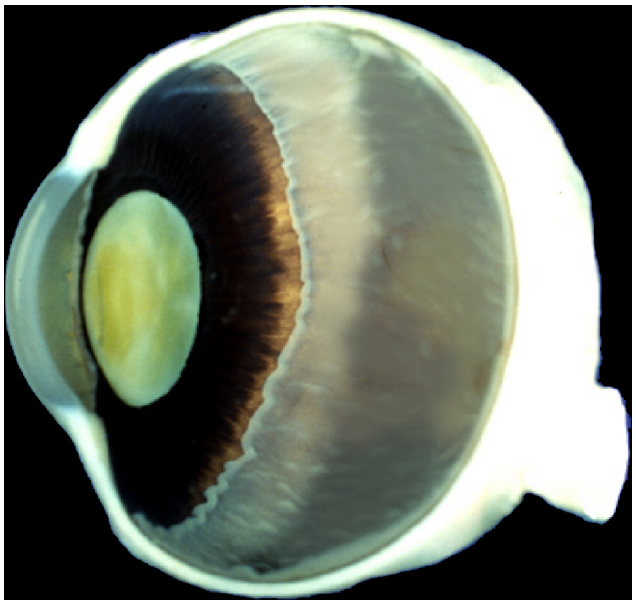
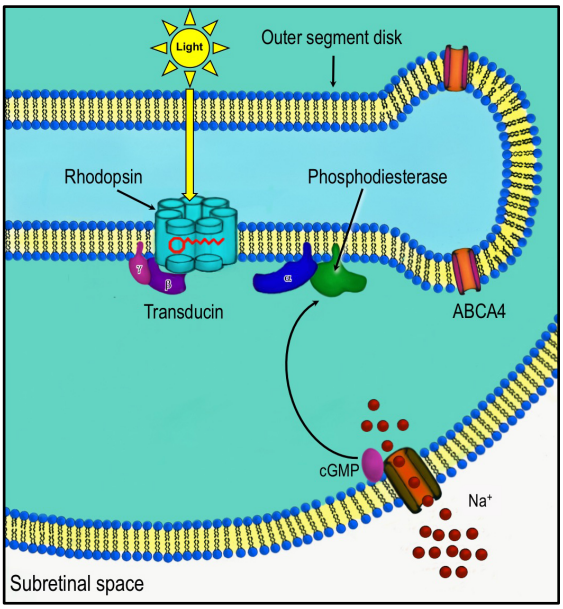
Anatomy and Physiology of the Retina

Relevant to Inherited Retinal Disease





a tutorial from

StoneRounds.org

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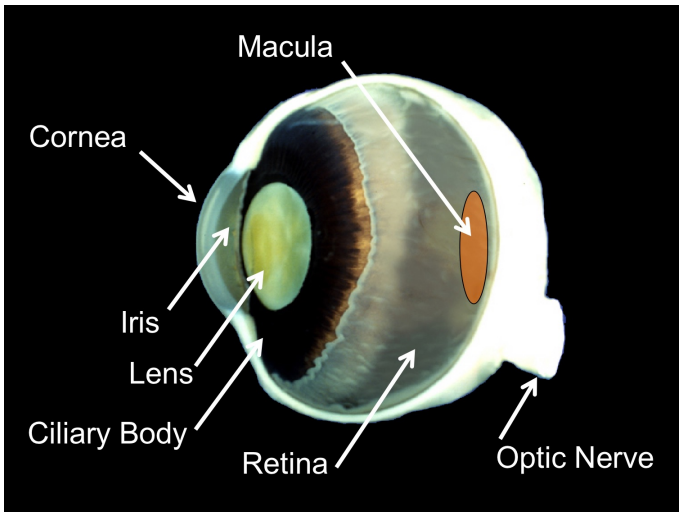
Editor

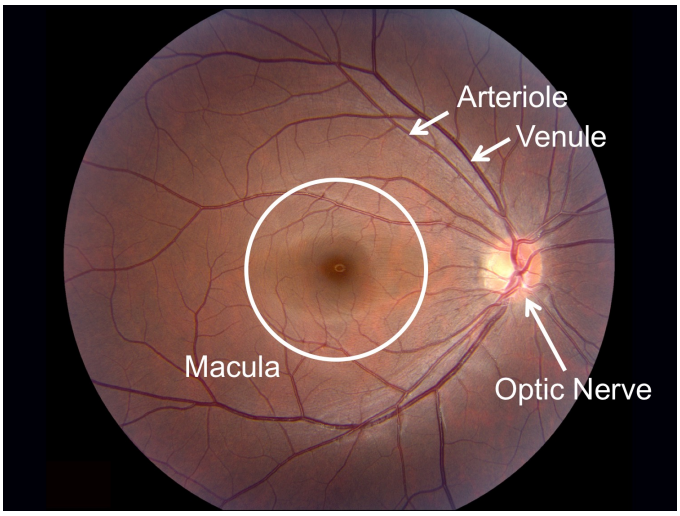
This is the right eye of a human donor viewed from above. You can tell that it's the right eye because the optic nerve exits the eye on the nasal side of center.



The transparent front wall of the eye is known as the cornea and the curved surface of this structure provides about 75% of the converging lens power needed to focus images on the back of the eye.

The remaining 25% of the focusing power comes from the flexible crystalline lens, which actually becomes thicker when a person looks a near objects due to contraction of a circumferential muscle known as the ciliary body. The thin colored structure between the cornea and the lens is the iris which functions to control the amount of light entering the eye.

The posterior two thirds of the eye is lined by a very thin layer of nerve tissue – the retina. In life, the retina is completely transparent and the brownish or orange color that you see when looking into the eye with an ophthalmoscope is due almost entirely to the blood filled capillaries and pigmented structures beneath it.



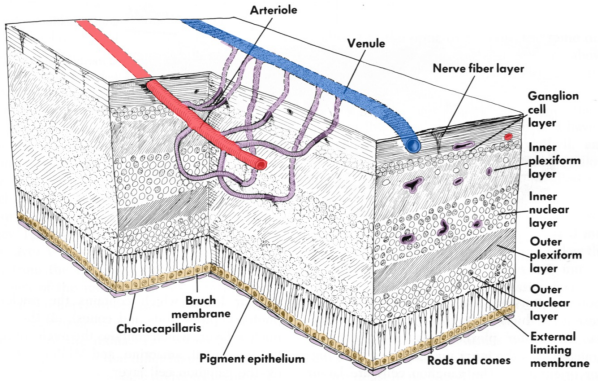
The central portion of the retina - shown here in orange - is known as the macula.

This is a color photograph of the retina of the right eye of a normal individual.

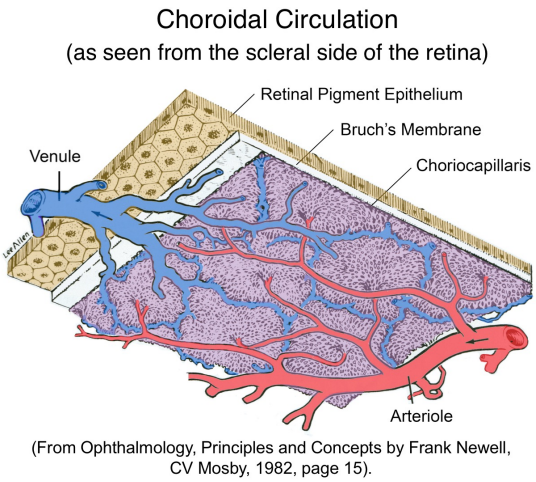
The optic nerve is 1.5 mm in diameter and the blood supply of the inner aspect of the retina emanates from the center of the nerve and sweeps around the macula.

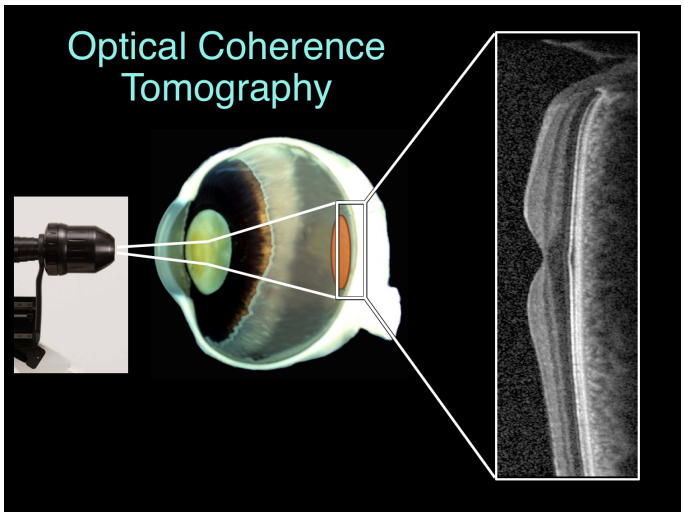
The venules can be distinguished from the arterioles because of their thicker caliber and darker purple color. These large caliber vessels supply and drain capillary plexuses that lie primarily in the ganglion cell and inner nuclear layers.

Retinal Circulation
(as seen from vitreous cavity)



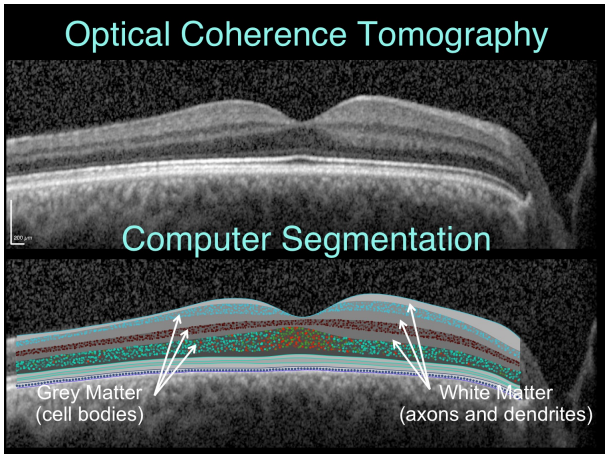
(From *Ophthalmology, Principles and Concepts* by Frank Newell, CV Mosby, 1982, page 28).



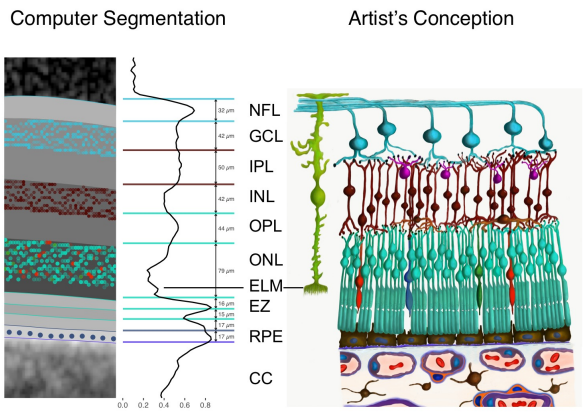


The retina has a second distinct blood supply that lies between the retinal pigment epithelium and the sclera. The fine capillaries adjacent to the RPE are known as the choriocapillaris and these vessels provide the tremendous amount of oxygen needed by the photoreceptor cells.

Optical coherence tomography is a clinical imaging technique that can provide images of the retina in living individuals with near-histologic levels of resolution.



Computerized image analysis of these images can "segment" the tomogram into layers that correspond to the different anatomical layers of the retina. As in the gross anatomy of the brain the darker grey layers correspond to collections of cell bodies while the lighter grey areas correspond to the densely packed axons and dendrites that connect these neurons to each other.

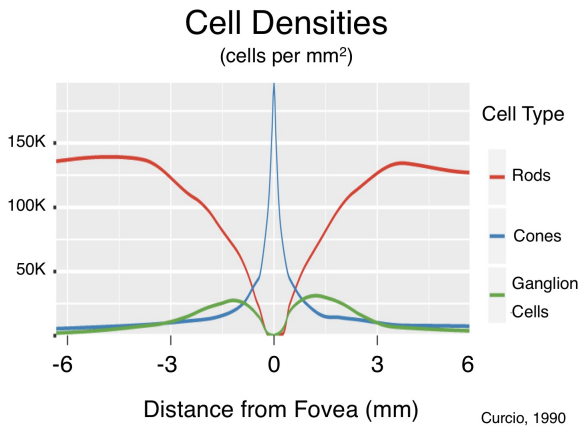


Zooming in on a small section of the retina we can illustrate our current understanding of the anatomic correlations with the varying intensities of the OCT image. With proper controls, one can detect anatomical changes with a resolution of a few microns using this instrument.

Here is an artist's conception of the most important retinal layers that makes the relationships of these cells a little easier to visualize.

It is important to note that there are 20 times more rod photoreceptors than cones in the human retina, and that the short wavelength sensitive cones are the least numerous of all.

The lowest ratio of photoreceptors to ganglion cells occurs in the fovea – approximately 5 to one -- which enables the very high acuity vision associated with this region. In the periphery, a single ganglion cell can receive input from more than 1000 photoreceptors.



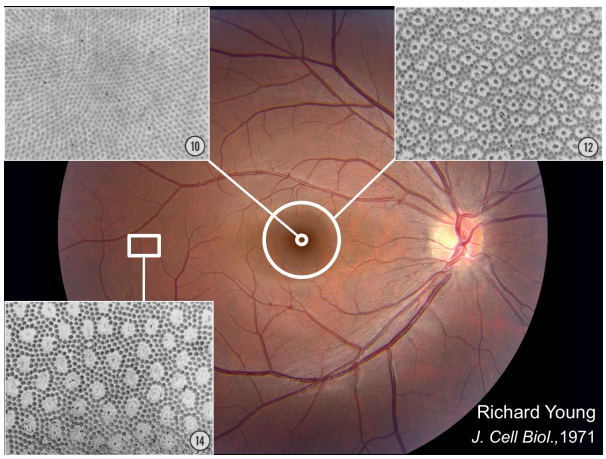
Here is another view of the variation in density of various retinal neurons with increasing eccentricity from the fovea.

The very high density of cone photoreceptors in the fovea helps explain why cone-selective diseases are often associated with decreased acuity.

The rods' peak density occurs more than 3 millimeters from the foveal center and is likely the explanation for the ring scotomas that are characteristic of the rod-selective disease retinitis pigmentosa.

The ganglion cells are the most numerous in the central 5 millimeters of the retina. One of the reasons that our initial photoreceptor precursor transplants will be placed in this region is because of the greater chance these transplants will have to establish synaptic connection with the relatively numerous inner retinal neurons of this region.

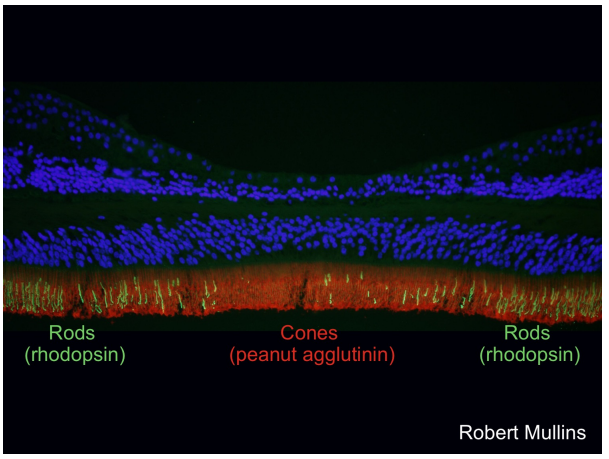
Richard Young's en face photomicrographs of the primate retina give us another view of how the retinal mosaic changes with increasing eccentricity. The foveal center consists entirely of unusual cones with very slender outer segments.



One millimeter from the foveal center, a ring of rods can be seen surrounding each cone and outside the macula, the ratio of rods to cones is even higher.

If we take a immunohistochemical section through the fovea of a human donor eye we can see the absence of rhodopsin-containing rods near the foveal center as well as the way in which the

ganglion cells that are synaptically related to the foveal cones are displaced laterally. You can tell that this section does not pass through the absolute center of the fovea because if it did, the inner nuclear layer would also be displaced laterally.



For descriptive purposes, the macula has been divided into six concentric zones and the dimension of the outer boundary of each of these zones is shown here. Clinically, the foveola and foveal avascular zone are essentially synonymous. The fovea is the same diameter as the optic nerve head – 1.5 millimeters. The prefix "para" means "next to" and the term "parafovea" is most appropriately used for findings that lie in a zone greater than one but a bit less than two disk diameters centered on the foveal light reflex. The prefix "peri" means "around" and the term "perifovea" is used for a ring one disk diameter in thickness at the outermost edge of the macula.

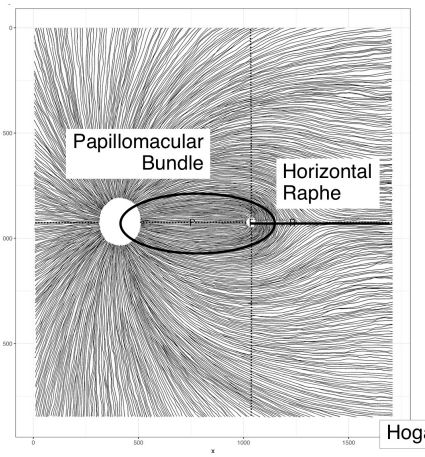
The reason that we are interested in the uneven distributions of the various cell types in the retina is that it is likely that the regional differences in retinal anatomy play an important role in the disease mechanisms of many of the conditions we're interested in and an understanding of these mechanisms will be important in the successful treatment of these conditions.

Macular Regions and Sizes

Region	Size (mm)	Size (deg)
Perifovea	5.5	18
Parafovea	2.5	8
Fovea	1.5	5
FAZ	0.5	1.6
Foveola	0.35	1.2
Umbo	0.15	0.5

One other type of regional specialization we should touch on briefly involves the innermost layers of the retina – the ganglion cells and their axons.

Hogan's classic drawing shows how the axons of ganglion cells nasal to the fovea approach the optic nerve head fairly directly while those temporal to the fovea sweep above and below the macula to minimize the amount of tissue lying between the incoming photons and the macular photoreceptors.



Hogan, 1971

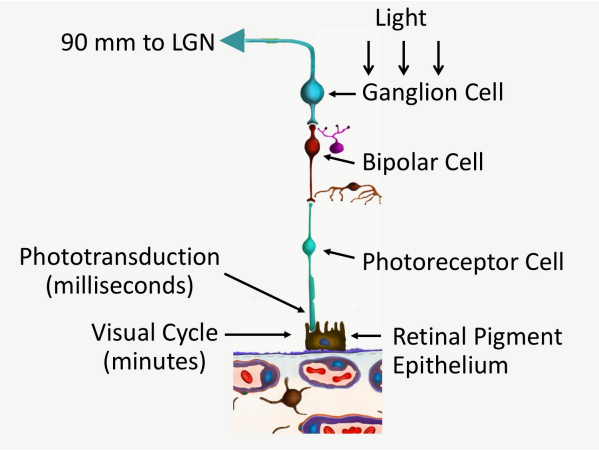
One of the practical effects of this anatomic specialization is that ganglion cells that are quite close to each other in the temporal retina can send their axons to the optic nerve by quite different routes. This is the anatomical explanation for the "nasal step" observed in the visual fields of many glaucoma patients.

The line along which this superior/inferior route decision is made is known as the horizontal raphe.

Because of the low ratio of photoreceptors to ganglion cells in the macula, there are a large number of axons that originate there and go directly to the optic nerve. These axons are required for high acuity vision and are known as the papillomacular bundle.

Now, let's spend a few minutes reviewing some of the physiology of the more important cell types in the retina. The basic wiring of the retina consists of just three neurons the photoreceptor cell the bipolar cell and the ganglion cell. The axons of the photoreceptors and bipolar cells travel only a few dozen microns before synapsing on the next cell but the axons of the ganglion cells travel over 90 millimeters – three and a half inches -- to get to the lateral geniculate nucleus of the thalamus.

All three of these neurons use glutamate as their neurotransmitter.



The photoreceptors and bipolar cells have graded inputs and outputs but the ganglion cells convert their graded inputs into trains of action potentials that travel down myelinated neurons. To maintain the transparency of the nerve fiber layer, the myelin doesn't begin until the axons exit the eye.

Some people wonder why evolution placed the light detecting photoreceptors closest to the sclera and all of the other neurons between them and the incoming light .

The reason is because of the essential functions performed by the retinal pigment epithelium. The RPE is the retina's primary source of the light sensitive chromophore 11-cis retinal.

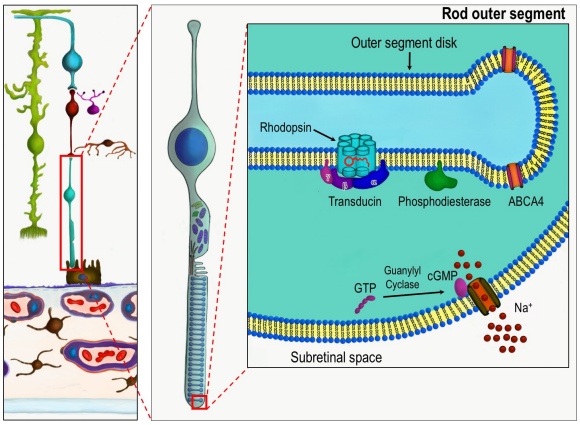
As we'll see in more detail in just a minute, the process that recycles 11-cis-retinal from the all-trans-retinal released from the photoreceptors is known as the visual cycle and it takes place over a period of tens of minutes. This is the process that you experience when your eyes "get used to the dark" in a movie theatre or walking on a trail at night on a camping trip.

The process that converts a stream of photons to an increase in photoreceptor membrane potential is known as phototransduction.

This process is four orders of magnitude faster than the visual cycle – less than 1/30th of a second in cone photoreceptors.

Let's look at the mechanism of phototransduction in a bit more detail.

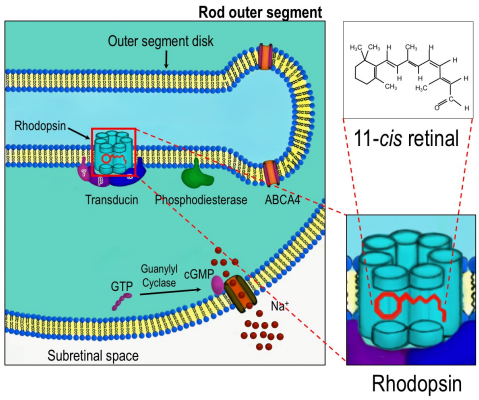
To do this we'll "mag up" on the outer segment of a rod photoreceptor, showing a flattened lipid-bilayer disk containing a transmembrane protein known as rhodopsin.



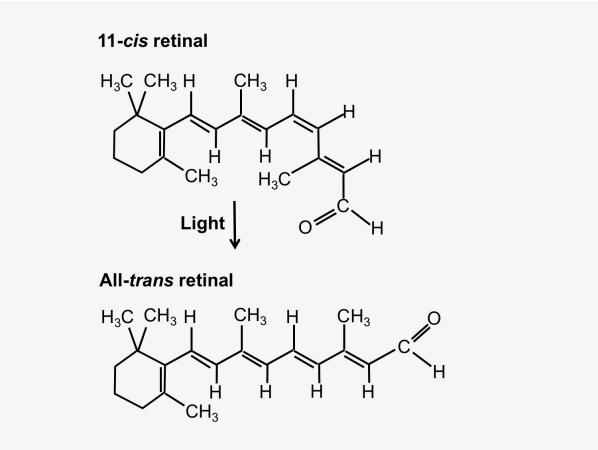
Rhodopsin is a "G-protein-coupled receptor" that mediates the first step of phototransduction.

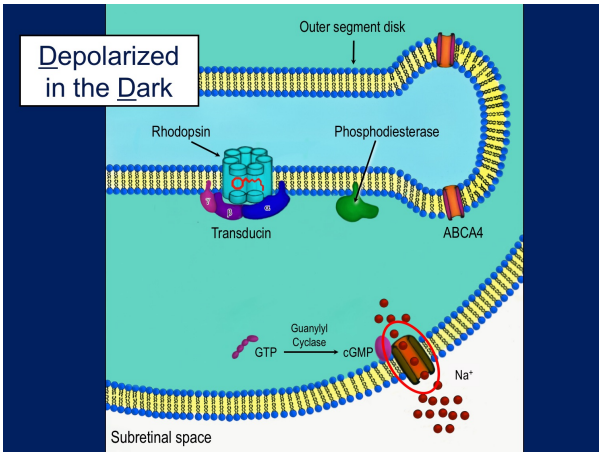
The light sensitive form of rhodopsin contains a molecule of vitamin A known as 11-cis-retinal covalently linked to it at lysine 296.

When 11-cis retinal absorbs a photon, it isomerizes to all trans retinal. That isomerization causes a conformational change in rhodopsin which sets off the remainder of the phototransduction cascade.

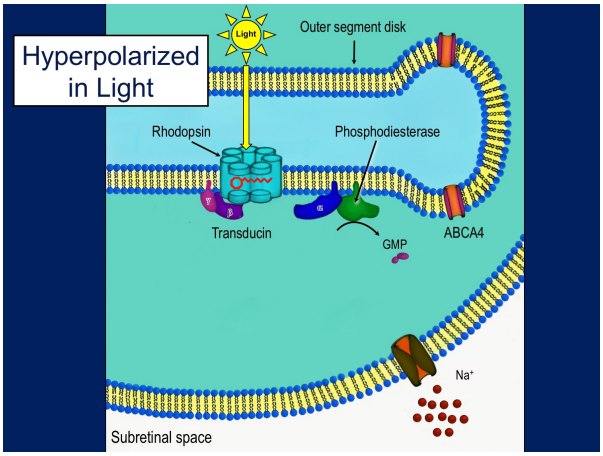


Let's return to the diagram of the outer segment disk and cell membrane to see the main steps in the cascade.





In the dark, a cyclic nucleotide gated cation channel is open and sodium ions flow in freely, thereby lowering the membrane potential.



When light strikes a vitamin A molecule bound to rhodopsin, it isomerizes the vitamin A to all-trans, which causes a conformational change in the protein.

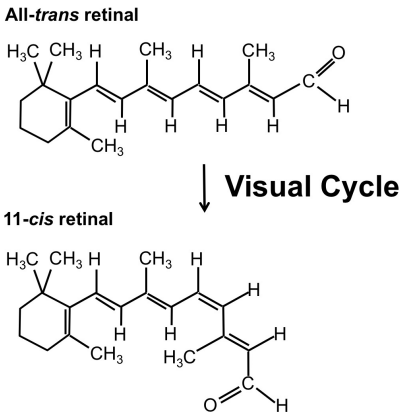
This conformational change dissociates rhodopsin's trimeric G protein, known as

transducin. The alpha subunit of transducin activates a phosphodiesterase which cleaves the cyclic GMP that has been keeping the cation channel open. When the channel closes, the resting membrane potential rises – diminishing the release of neurotransmitter from the synaptic terminals of the photoreceptor axon.

So to review the main proteins in the phototransduction cascade: light falling on the 11-cis retinal bound to rhodopsin causes it to change conformation, this releases alpha transducin which activates phosphodiesterase, lowering cyclic GMP, closing the cation channel, elevating the membrane potential, and reducing neurotransmitter release.

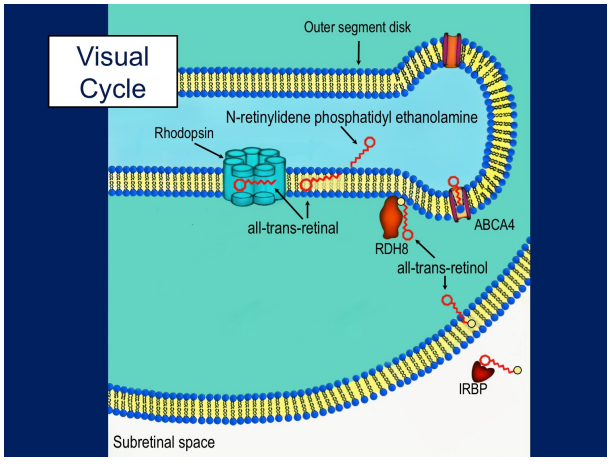
The evolutionary purpose of this elaborate cascade is low-noise amplification – a single photon falling on a rod photoreceptor can increase its membrane potential by a millivolt – this represents at least a one million fold amplification of the input signal.

The by-product of phototransduction is all-trans-retinal. For this molecule to be able to detect another photon, it needs to be re-isomerized to 11-cis-retinal.



This process is known as the visual cycle and as you will see, some of the steps take place outside the photoreceptors. Also, the process is a bit different for rods and cones.

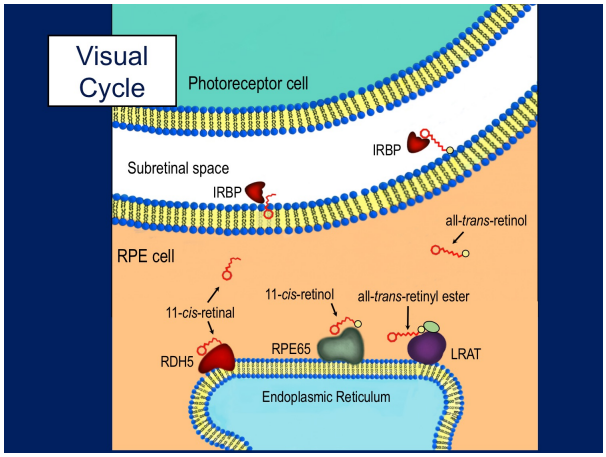
The process begins when all-trans-retinal is released from rhodopsin into the lipid bilayer of the outer segment disks. There, the aldehyde group of the retinal is exposed to the amino groups of the polar heads of some of the membrane lipids and a reversible covalent bond forms creating a molecule known as N-retinylidene phosphatidyl ethanolamine or N-ret-PE.



The protein encoded by the gene ABCA4 is an ATP-binding cassette transporter that flips N-Ret-PE to the outer leaflet of the disk membrane.

There, the all trans retinal is met by a dehydrogenase -- RDH8 -- that removes it from the phospholipid and converts it to a less reactive alcohol.

It is this all -trans-retinol form of vitamin A that leaves the photoreceptor for transport to the retinal pigment epithelium or Mueller cells bound to a protein known as the interphotoreceptor retinoid binding protein or IRBP.



After crossing the plasma membrane of the retinal pigment epithelium the all trans retinol moves to the endoplasmic reticulum where it is esterified to all trans-retinyl ester by the lecithin retinol acyl transferase, commonly referred to as LRAT.

These all-trans-retinyl esters are in turn acted upon by RPE65, which de-esterifies the retinol and re-isomerizes it to the light sensitive 11-cis isomer of vitamin A.

The 11-cis retinol is then oxidized to 11-cis retinal by membrane bound retinol dehydrogenases such as RDH5. The 11-cis-retinal then moves to the cell surface, crosses the plasma membrane and is returned to the photoreceptor cell by IRBP.

The visual cycle is complete when the 11-cis retinal returns to the binding pocket of rhodopsin, forms a covalent bond with lysine 296, and restores rhodopsin to the light sensitive state.

To summarize the key steps of the visual cycle:

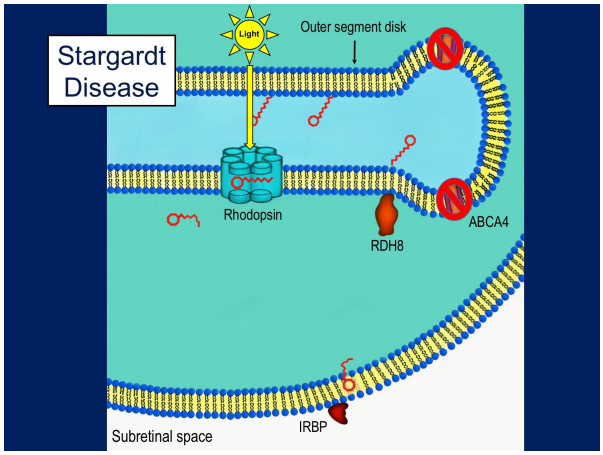
Light isomerizes 11-*cis* retinal to all-*trans*-retinal, which then binds to the disk membrane as N-ret PE.

ABCA4 flips the N-ret PE to the cytoplasmic leaflet of the disk membrane.

RDH8 reduces the 11-cis-retinal to all-trans-retinol.

IRBP carries all-trans-retinol to the retinal pigment epithelium where RPE65 re-isomerizes it to 11-cis-retinol and RHD5 oxidizes it to 11-cis-retinal.

IRBP then returns the 11-cis-retinal to the photoreceptor.



Autosomal recessive Stargardt disease-is caused by a deficiency of the ATP-binding cassette transporter A4, which as we have just seen usually flips N-retinylidene phosphatidyl ethanolamine from the inner to the outer leaflet of the outer segment disk membrane.

Because of the dysfunction of ABCA4, the N-ret-PE gradually accumulates on the inner leaflet until the concentration reaches a point that a second-retinylidene molecule binds to a single ethanolamine creating an irreversible and insoluble bis-retinoid known as A2PE.

A2-PE is directly toxic to the photoreceptors but also accumulates within and beneath the retinal pigment epithelium in the form of the ophthalmoscopically visible pisciform flecks that are characteristic of Stargardt disease.