CNS

Biochemistry

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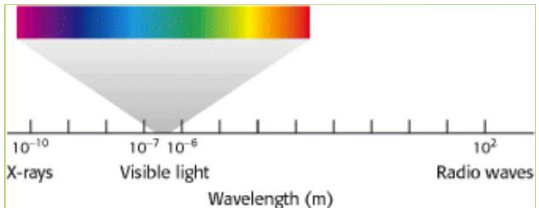
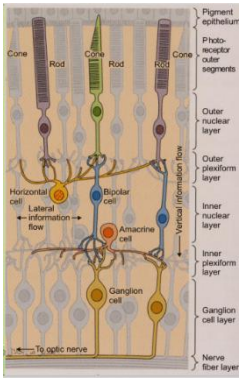
BASICS OF HUMAN VISION :

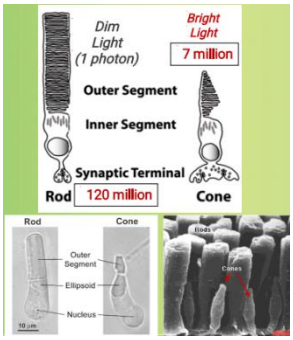
There is a large spectrum of wavelengths, but we see a small fraction of this spectrum. We only see colors in the visible light spectrum and there are thousands of different ranges of colors within this spectrum.

Some Animals can see UV light, and some can also see in the infrared ranges as well. However, we only see the visible light.

There are two types of cells that are responsible for vision; Rods and Cones. They were given these names due to how they look (their shape). they are found intermixed together and are both connected to Bipolar nerves, amacrine & ganglion cells that regulate the transduction process to the brain.







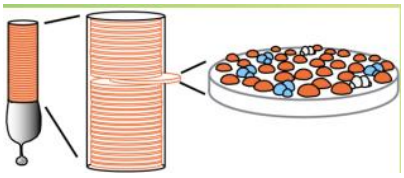
Cell Type	Rods	Cons
Shape	Thin, Tall & Cylindrical	Cone-shape
Vision Type	Responsible for vision in dim light (they can absorb as little as 1 photon)	Responsible for colored vision in bright light
Number	120 Million	7 Million

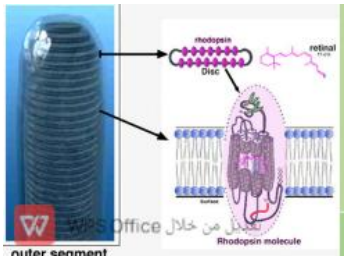
Rods Structure & Function

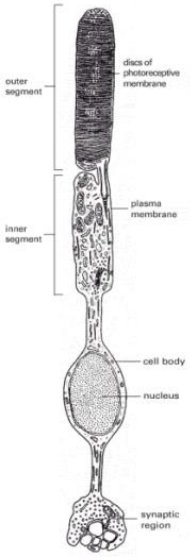
STRUCTURE

A Rod can be divided into 3 Areas:

1- Outer segment: A stack of membranes "Discs" where the absorption of the light happens. It contains the biochemical machinery needed for visual transduction. The components of the photo transduction enzyme cascade are **imbedded in the membrane** of those Discs.
2- Inner Segment: It's where the nucleus and other organelles are contained.
3- Synaptic terminal: where the signal is transmitted to the nerves.





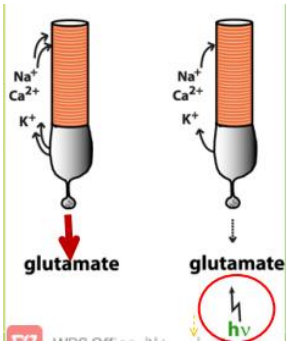


FUNCTION

- Most neurons maintain a resting membrane potential (-60 to -70 mV). When excited, they open cation channels causing depolarization and opening of voltage-gated Ca^{2+} channels at the synapse. Ca^{2+} ions flow in and promote fusion of synaptic vesicles, which release neurotransmitter.
- Rods and cones work "backwards". At rest (in darkness), rods and cones are depolarized to -35 to -45 mV.

A) At Dark:
   1- Na^{+} and, to a lesser amount, Ca^{2+} enter through cyclic nucleotide gated channels in the outer segment membrane.
   2- K^{+} is released through voltage-gated channels in the inner segment.
   3- Rod cells are depolarized.
   4- The neurotransmitter glutamate is released continuously.

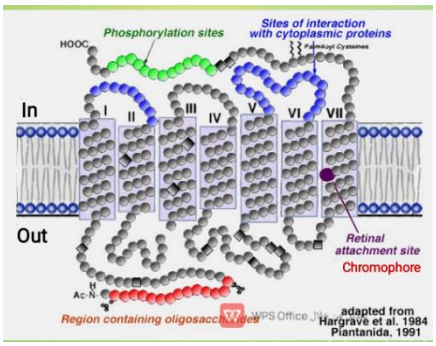
B) When Excited:
   1- Channels in the outer segment membrane close, neither Na^{+} nor Ca^{2+} get in, also Ca^{2+} gets out, which cause Rod cells to hyperpolarize
   2- Glutamate release decreases, which is the Signal of vision.



WHAT HAPPENS WHEN LIGHT HITS RODS?

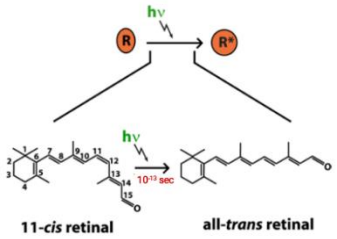
There are different molecular players in signal transduction:

1- Rhodopsin: a **holo-protein** receptor that absorbs light formed by an apo-protein called **opsin** and the pigment molecule **(Retinal** Molecule **)** that gets excited.
2- Transducin: G-Protein which is heterotrimeric made of the 3 subunits a , b, and g
3- Phosphodiesterase.
4- Regulatory proteins.



A) Rhodopsin

Opsin is a single polypeptide with 7 helical trans-membrane domains, the last domain contains the retinal attachment site where the **chromophore** gets attached.

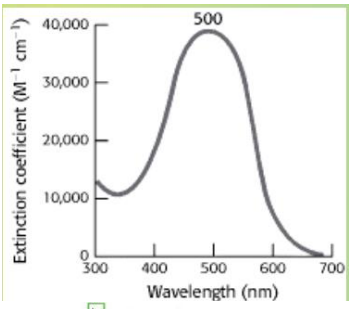


The chromophore is known as 11-cis-retinal which is derived from vitamin A; thus vitamin A is important for vision. Vitamin A is derived from carotene (carrots) and that is why they say if you eat carrots, you will see better especially at night.

The chromophore coverts the absorbed energy of a photon into chemical energy as a result of the conformational change in the protein structure of opsin.

The 11-cis-retinal molecule changes to All-trans when it's hit by light. This change in the structure causes rhodopsin to get activated, through which transduction of the signal occurs. This change happens very fast, taking about 100 femtoseconds (10^{-13} secs).

When rhodopsin gets activated, it can absorb light at a wide range (350-750 nm) which is the visible light range. The maximum absorption is about 500 nanometers (yellow-green color).

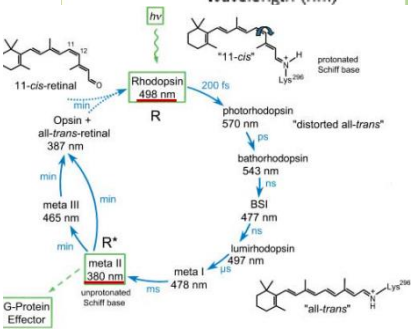


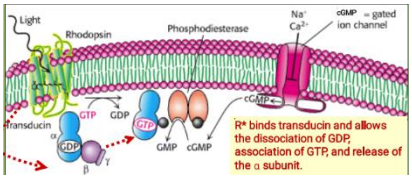
When Rhodopsin is activated, it goes under different conformations and each conformation can absorb light at a different wavelength. **Meta II is the activated form**(very important) which can transmit the signal to G-protein.

B) Transducin

When Rhodopsin is activated, it

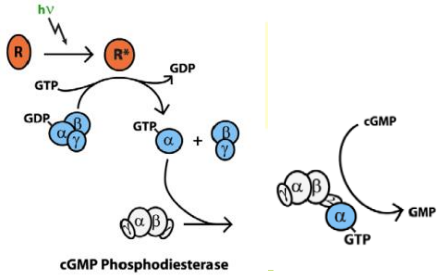
1- activates Transducin by the replacement of GDP with GTP.
2- which releases the a subunit of Transducin from β and γ .
3- Then, the α-GTP bound subunit interacts with phosphodiesterase (PDE) which converts cGMP into GMP, thus reduces the amount of cGMP in the cytosol.





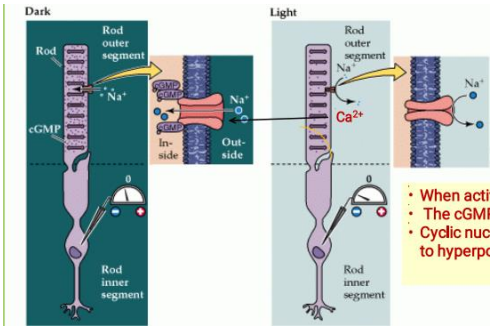
PDE is a heterotetramer that consists of a dimer of two catalytic subunits, α and β subunits, each with an active site inhibited by a PDE γ subunit. The activated Transducin α subunit-GTP binds to PDE γ and relieves the inhibition on a catalytic subunit.

The importance of **cGMP lays in it keeping the ion gated channel open** by binding to it. So, when it decreases, it causes the closure of these channels inhibiting the inflow of the Na^{+} and Ca^{+2} channels which decreases the release of glutamate.



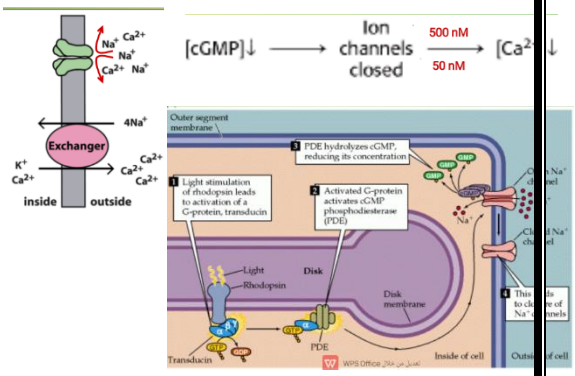
No c-GMP binding to the channels -> no entry of Na+ -> cell hyperpolarization and reduction of the release of glutamate -> signal transduction

c-GMP is formed when Guanylyl Cyclase converts GTP into c-GMP. c-GMP can then bind to cGMP-dependent protein kinases leading to multiple effects. When c-GMP gets hydrolyzed by phosphodiesterase, it loses its effect.



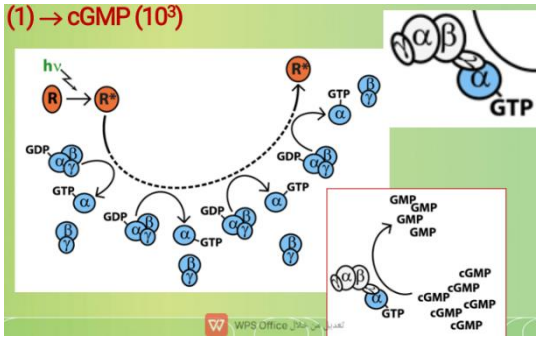
When the channels close, Ca 2+ ceases to enter, but extrusion through the exchanger continues, so intracellular [Ca 2+] falls from 500nM to 50nM.

The nerve cell carries this impulse all the way to the brain that determines where the nerve impulse originate and interpret the image.



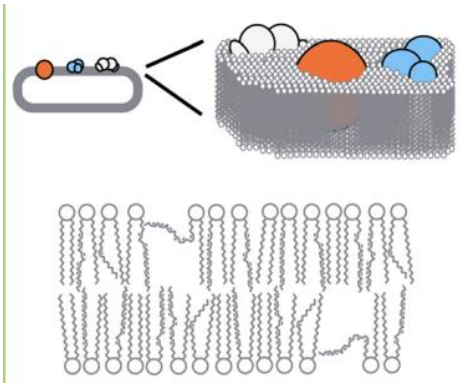
SIGNAL AMPLIFICATION

A) When one Rhodopsin molecule is activated, it can activate 10 to >3000 molecules of Transducin, (The range is based on the number of photons and experimental measurements).
B) One Transducin molecule can activate one phosphodiesterase molecule (a ratio of 1:1, no amplification happening here).
C) Then, one phosphodiesterase converts one thousand molecules of c-GMP into GMP molecules and these would affect many other c-GMP gated channels (another amplification).



FACILITATION OF TRANSDUCTION

A) 2D surface membrane: **Compartmentalization** is important in speeding up reactions through placing the enzymes and substrates in a small area. Since enzymatic reactions depend on a random collision, when they are placed in a small place like lysosomes, the chance of collision is higher resulting in catalysis of reactions. The same thing happens when you place all of these components in a plasma membrane. So, instead of having enzymes and substrates finding each other in a 3D space, they move in a 2D space so they can find each other faster and that facilitates the transmission of the signal.



B) The membrane of the outer segments in rods cells **is low in cholesterol** and has **high content of unsaturated fatty acids**; meaning that the membrane is quite **viscous** which means that it is easy for these proteins to move through this 2D space. So, the membrane is not rigid but quite flexible Because of that **Omega 3 FA deficiency can lead to progressive retinal dystrophy.**
C) **Cooperativity** of binding: The binding of one c-GMP enhances additional c-GMP binding and channel opening (n=~3); so one c-GMP binding makes it easier for another c-GMP to bind, just like the heme effect. And this effect gives us a **sigmoidal** type of plot where we have increased successive binding/release and therefore increase in closure or opening of c-GMP gated channels. So, the release of one c-GMP makes it easier for the channel to close; **whereby release of one c-GMP makes it easier for the release of another one and, thus, easier closure of the channel**.
D) Since multiple cGMP molecules are required to open the channel, it will close when only one or two cGMP molecules leave the channel, making it easily shut down by absorption of light.

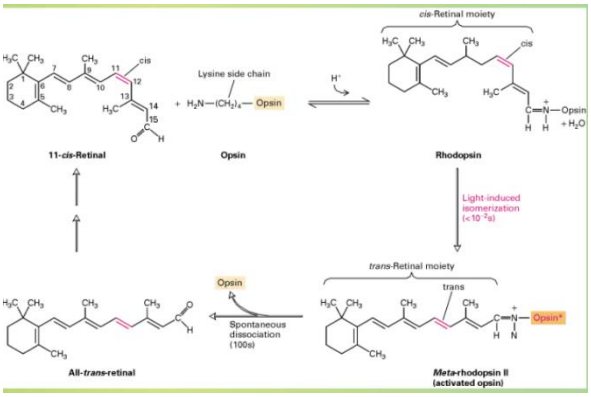
Signal Termination

Signal termination is quite important. It allows us to see the smooth movement of a person. If the signal is not terminated, you would only see interrupted images. For example, without signal termination you will only see the two images to the right, the individual with their left arm up then their right arm up. But you would not be able to see the smooth movements of the arm as it moves up or down. You would only see their final positions.

TERMINATION MECHANISMS

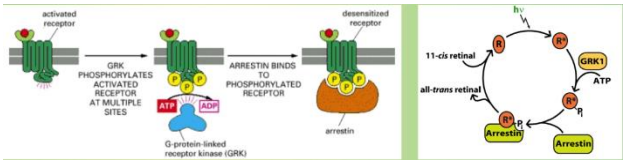
Mechanism I : Unstable All-Trans Rhodopsin Complex

- After the 11-cis-retinal absorbs light and becomes an all-trans molecule.This also results in the changing of rhodopsin into meta-rhodopsin II.
- Interaction of the trans-retinal molecule to rhodopsin becomes unstable, resulting in the release of the all-trans molecule.
- Therefore, the rhodopsin molecule becomes opsin and goes back to its inactive confirmation and cannot activate Transducins anymore.
- All-trans-retinal becomes 11-cis-retinal which can bind to opsin to form rhodopsin once again (see the following figure next page).



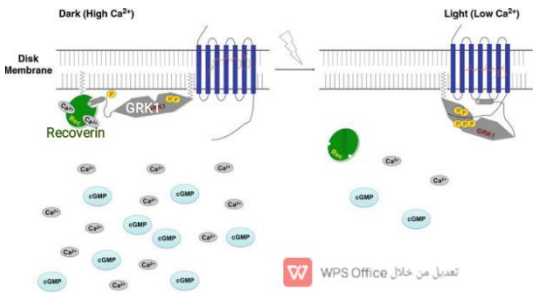
Mechanism II : Arrestin Binding

Rhodopsin kinase 1 (GRK1) can phosphorylate the C-terminus of active Rhodopsin (=R* or Metarhodopsin II). It does not phosphorylate the inactive form of rhodopsin.



Phosphorylation of R* has two effects:

1- The ability of rhodopsin to activate Transducin decreases. So It facilitates binding of the protein **Arrestin** to rhodopsin, which completely stops its activity (so no transducing can be activated).
2- Additionally, binding of Arrestin leads to the release of all trans-retinal, **regenerating rhodopsin**.



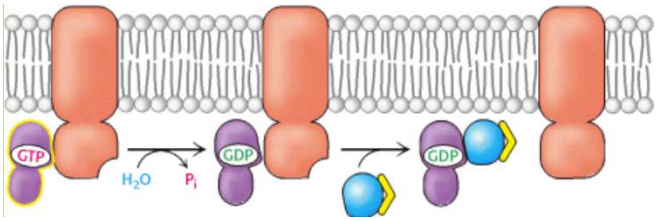
- **GRK1 is more active at low intracellular calcium ion concentration (in the presence of light), How?**
  A) In the dark, Ca2+ ions bind to a protein called Recoverin, allowing Recoverin to anchor to the membrane, bind to GRK1 (at the N terminus helix), and inhibit it.
  B) In contrast, Ca2+-free Recoverin does not bind to GRK1. Without this inhibition, the kinase is more active and can phosphorylate rhodopsin.

Additionally, **Calmodulin**, another protein, can also bind to Ca^{2+} when it is present in high concentrations. Ca^{2+} - Calmodulin complex can also bind to GRK1 and inhibit it.

Basically: There are two proteins (Recoverin and Calmodulin) that can bind to the kinase GRK1 and inhibit it. This binding depends on the concentration of Ca2+ ions.

Mechanism III : Intrinsic GTPase activity of G protein

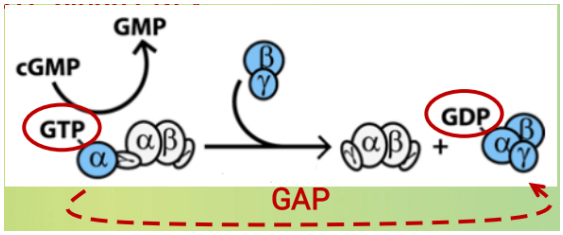
Gα (of Transducin) has an intrinsic Tase activity that hydrolyzes GTP to GDP.
Therefore, the protein inactivates itself.



Once the α subunit is bound to GDP (diphosphate form), it can bind to the β and γ subunits. Transducin is now inactivated and can no longer interact with the phosphodiesterase (PDE).

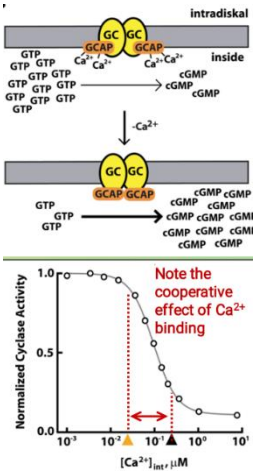
What happens is that the GDP α subunit releases the PDE γ subunit, which inhibits the catalytic subunit of PDE. Then, Transducin α-GDP eventually combines with Transducin βγ.

Mechanism IV : Facilitation of GTPase activity of G protein



- GTP hydrolysis is slow intrinsically, but it is accelerated when it binds to the GAP (GTPase Activating Protein) complex.
- To ensure that Transducin does not shut off before activating PDE, Transducin and the GAP complex have a low affinity for each other (i.e., they do not bind to each other) until Transducin α-GTP binds PDEγ.
- So, there is a period in which Transducin α-GTP is allowed to bind to the phosphodiesterase (so it can do its function) and then the GAP complex can bind to the alpha subunit and activate its intrinsic GTPase activity.
- The inhibition of the Gα subunit by GTP hydrolysis and, hence, dissociation from PDE is the rate limiting step in the recovery of rod response to light.

Mechanism V : Guanylate Cyclase

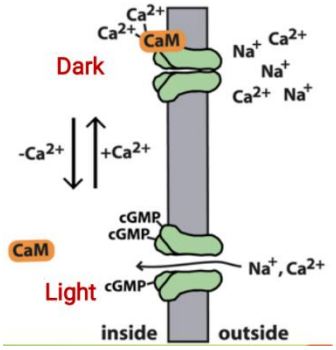


When it's dark and there's high levels of Ca^{2+} ions, guanylate cyclase activating proteins (GAPs) can bind to Ca^{2+} ions. This binding blocks their activation of guanylate cyclase.

When the concentration of Ca^{2+} ions decrease, Ca^{2+} dissociates from CAPs. The CAPs can now activate guanylate cyclase resulting in the conversion of GTP to cGMP. There are now high levels of cGMP resulting in opening of the channels.

As you can see in the graph, the activity of guanylate cyclase is highly sensitive to the level of calcium ions (notice the sigmoidal curve). Any reduction in the concentration of calcium ions leads to high activity of guanylate cyclase.

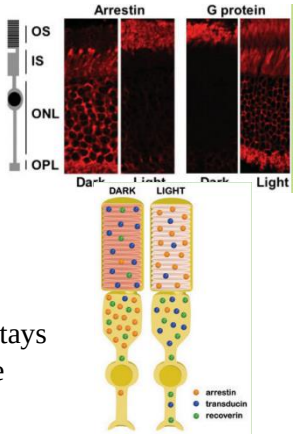
Mechanism VI : Ca2+-Calmodulin and cGMP-gated channels



- In the dark, when the calcium concentration is high, they can bind to calmodulin to form Ca^{2+}-Calmodulin (CaM). CaM, in a sense, balances things out by keeping some of the channels closed.
- CaM binds to the channel and reduces its affinity to cGMP, closing the channel and keeping some control.
- During visual transduction, the decrease in intracellular Ca2+ concentration causes CaM to be released. This allows the channel's affinity towards cGMP to increase and the channel can reopen in response to the slightest increase to cGMP.
- This is considered an amplification step as well.

Note: Notice that termination is done on different levels which provide very tight control over the activity

Adaptation



You've probably noticed that if you move from a well-lit room into a dark room, you can't see anything at first. It takes some time for you to be able to see something. The opposite is true as well, if you move from a dark room to a well-lit room, your eyes are very sensitive at first, and it takes some time for you to be able to see clearly and for there to be less strain on your eyes. This is how our eyes adapt to changing light/dark conditions.

As we can see in the image, the proteins Arrestin and G protein (Transducin) were labelled in a rod cell for an experiment. What was found is that in the dark Arrestin stays in the inner segment of the rod cell. On the other hand, with light it is localized in the outer segment.

Transducin and Recoverin have the opposite behavior. In the dark they are localized in the outer segment and in light it is mainly localized in the inner segment.

	Dark	Light
Arrestin	More in inner segment	More in outer segment
Transducin	More in outer segment	More in inner segment
Recoverin	More in outer segment	More in inner segment
Conc.	Low inhibition, receptor ready to be activated	High inhibition, receptor ready to be inactivated

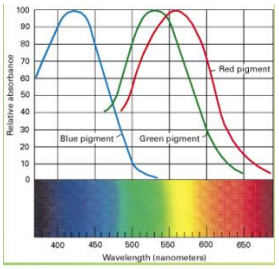
When it's dark, you want Arrestin to stay in the inner segment to lower inhibition (of rhodopsin) and have the rod cell very sensitive to any light. On the other hand, the G protein is localized in the outer segment waiting for any signal (any photon to hit rhodopsin) so it can be activated.

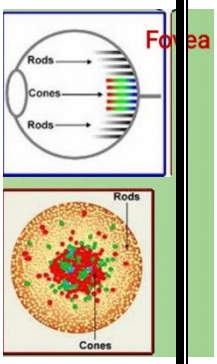
This is also why adaption takes time. In the case of adapting to light, Arrestin will slowly move from the inner segment to the outer segment to terminate the signal.
The G protein will also have to move from the outer segment to the inner segment so that the signaling in rod cells is terminated.

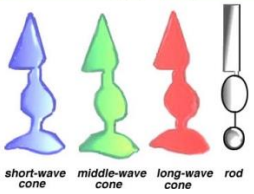
Color Vision

Cone cells are responsible for vision in bright light. There are three types of cone cells, each responsible for vision of a certain wavelength.

1- Short-Wave (Blue) Cone - Registers the shorter wave-lengths and has a peak for blue color vision.
2- Middle-Wave (Green) Cone - Responsible for visualizing the color green.
3- Long-Wave (Red) Cone - Responsible for visualizing the color red.

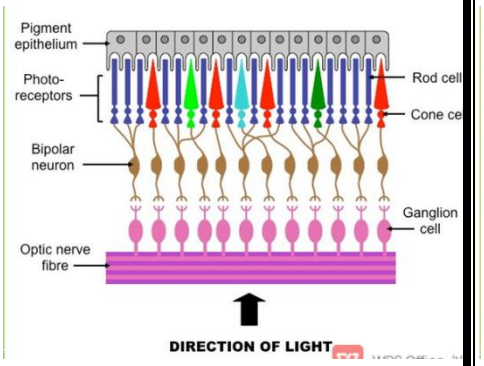






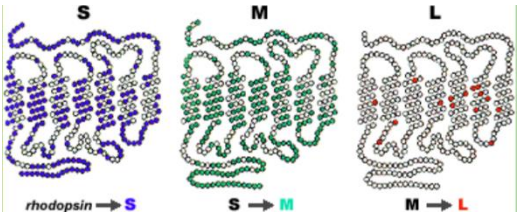
Notice that the range of wavelength absorption isn't as wide as Rods, and notice how close the activation wavelength for Red and green Cones.

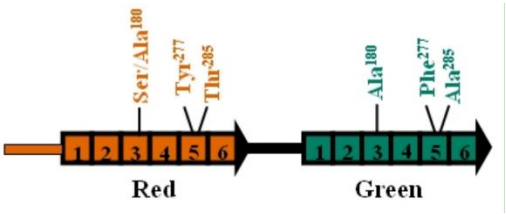
The combination of the three types of cones gives us color vision. Rod cells are distributed all over the retina, whereas Cons are concentrated in the **Fovea** **multiple rod cells can connect to a single neuron. On the other hand, each cone cell is connected to a single neuron** This has important implications to be discussed



- **HOW DO THEIR STRUCTURES DIFFER?**
  The chromophore (11-cis retinal) is the same in rod cells and the three types of cone cells, **what differs is the protein receptor.**
- Cone opsins have similar structures as rhodopsin, but with **different amino acid residues** surrounding the bound 11-cis retinal. So, it's actually the amino acids that determine what wavelength the chromophore will absorb.

- Each of the cone photoreceptors vs. rhodopsin = **~ 40% identical**.
- In figure S below, the homology between rhodopsin and short-wave protein shows 40% identical amino acids.
- The blue color represents amino acids specific to the short-wave protein while the amino acids in white are shared between the two.
- The blue photoreceptor vs. green and red photoreceptors = ~ 40% identical.
- The green vs. red photoreceptors are more than **95% identical**. This also has important implications.





  - There are three important amino acids that differ between the red and green photoreceptors. They are in positions **180, 277, and 285** (note the amino acids in the figure).

  - The amino acids for the red cone have hydroxyl groups, while the amino acids in the green are nonpolar. The added hydroxyl group in the red pigment causes a shift of about 10 nm in the wavelength (λ max) that is absorbed (λ becomes longer=lower energy).

SHARPNESS AND SENSITIVITY

Sharpness and sensitivity of viewing images depends on the brain determining the number and location of the photoreceptor cell(s) that passes an impulse to any given fiber.

A) Image Sharpness - As expected, we can see much better in bright light than in the dark. As in, the image is much sharper in bright light. The reason why the image is not as sharp in the dark is because multiple rod cells are connected to one neuron.
Therefore, when the signal reaches the brain, the brain doesn't know exactly which rod cell the image came from. The brain tries to form an image to the best of its ability, but it won't be very sharp. On the other hand, since each cone cell is connected to a nerve, the brain will know exactly where the image is coming from.

B) Sensitivity - We see better in terms of sensitivity in dim light than in bright light.
This is because there are many more rod cells than cone cells, so they can transmit a lot of signals to the brain, making a more sensitive effect in dim light. Additionally, the molecular machinery (the molecules responsible for vision) are more in number in rod cells than in cone cells. Therefore, the signal will be amplified much more in rod cells.

Color Blindness

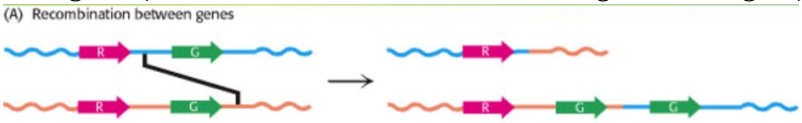
CHROMOSOMAL LOCATIONS:

- The blue opsin gene is located on **chromosome 7**.
- The red and green opsin genes are located on the **X chromosome**.
- The X chromosome normally carries a cluster of **2 to 9 opsin genes**.
- Multiple copies of these genes are fine, it won't make an individual better at seeing that color.

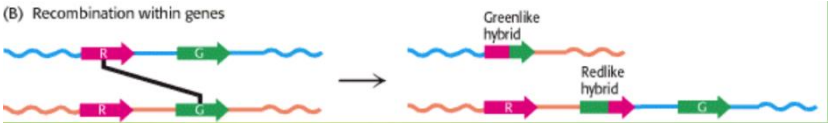
Red-Green Homologous Recombination

Recombination occurs in metaphase I of meiosis I, where exchange of genetic material may occur between the two chromosomes. The transfer of genes from one chromosome to another may be unequal. There are two methods:

1- Inter-genic (recombination **between** transcribed regions of the gene)



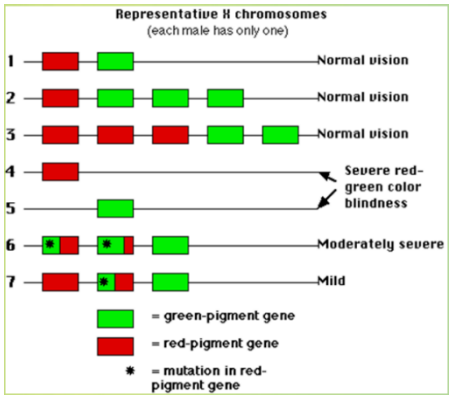
2- Intra-genic (recombination **within** transcribed regions of the gene) ´ The individual may end up with a gene with some of the green photoreceptor and some of the red.



Genetic Probabilities

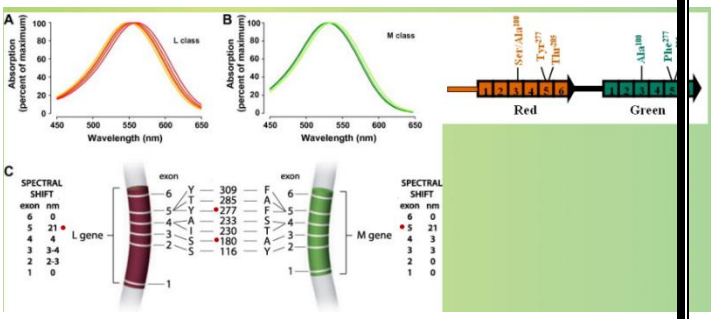
The figure to the right illustrates the different genetic probabilities. If it was a male, then they would only have one X-chromosome, so each scenario would give the effect as written.

1- Scenarios 1-3 give normal vision as both red and green photoreceptors are present (multiple copies of the gene is fine and gives no advantage).
2- If one (red or green) is totally missing, then that individual will have severe red-green color blindness. (more common than blue color blindness).
3- Someone with combinations of red and green, with most of the red gone as in scenario 6, this could lead to moderately severe colorblindness.



Spectral Tuning

1- Individuals are not equal in how they visualize color. So, some people will see red differently than how others do. The reason is genetic differences (polymorphisms).
2- The substitutions at positions **277 and 285 account for about 20 nm** of the difference in peak sensitivity.
3- The presence of serine vs alanine at position 180 produces a measurable shift in the spectrum.



PEDIGREES

Pedigrees As an X-linked recessive disorder, males are more affected due to the fact they only have one **X chromosome, This might be an exam question**.