CNS

BIOCHEMISTRY

1

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Sheet 1: Visual Transduction

There are different layers of information and each layer has also sublayers within it. There is a lot of information in the field, but the doctor tried to focus on important issues and summarized it.

References

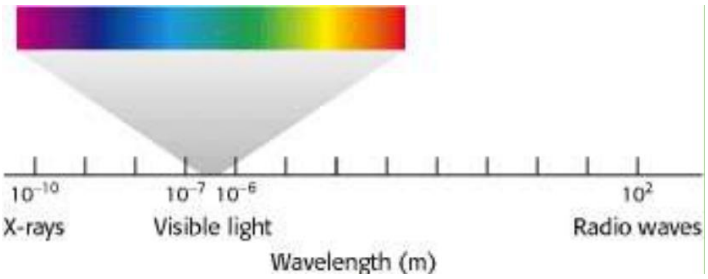
- Webvision: The Organization of the Retina and Visual System (https://www.ncbi.nlm.nih.gov/books/NBK52768/)
- The Molecular Design of Visual Transduction (https://www.biophysics.org/Portals/0/BPSAssets/Articles/Phototransduction.pdf)
- Adaptation of Rod Photoreceptors to Light and Dark (http://photobiology.info/Rozanowska2.html)

Lecture Outline

- **Visual transduction (dim vs. bright light)**
  - Components (cells and molecules)
  - Mechanisms of activation, amplification, and termination
- **Color blindness**

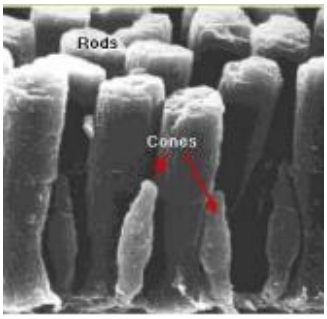
Basics of Human Vision

- 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.

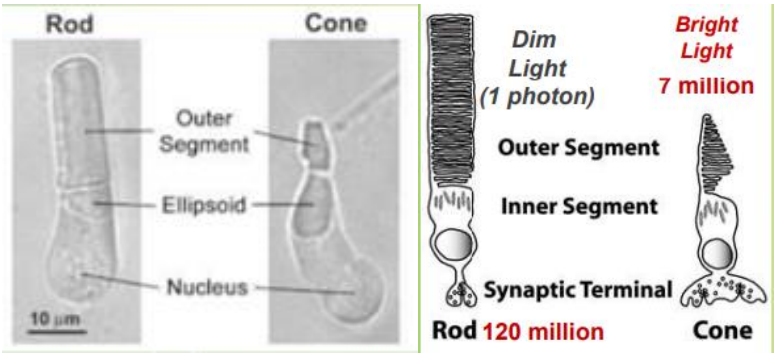


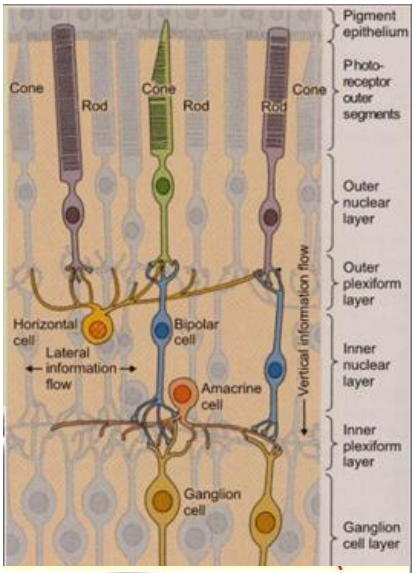
- Animals and insects can see UV light and some can also see in the infrared ranges as well. However, we only see the visible light.

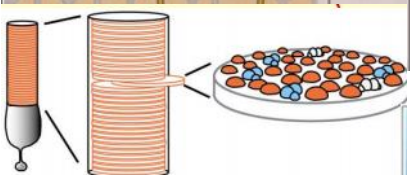
There are two types of cells that are responsible for vision; **Rods** and **Cones**. They were given these names due to how they look (their shape). Rods and Cones are also found embedded together and are both connected to nerves through which signals are transmitted to the brain where the seen image is processed.



Cell Type	Rod Cells	Cone Cells
Shape	Cylinder and tall	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 (many more than cones)	7 million





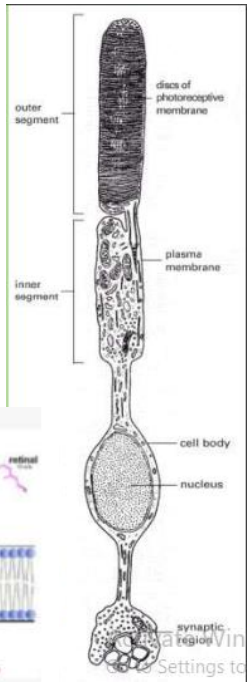


Other cells participating in vision: amacrine cells and the ganglion cells (we won't talk about their roles though).

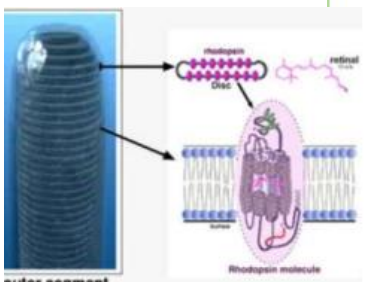
More on Rod cells

They can be divided into three sections:

1. **Outer segment:** A stack of membranes 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 packed into stacks of membranous vesicles ("disks").
2. **Inner segment:** Consists of the cell body where the nucleus is, and other cellular organelles which are found in other neurons. It is also the place where the synthesis of different proteins happens, including a synaptic terminal.
3. **Synaptic terminal:** Where the signal is transmitted to the nerves.



**The synaptic part can be considered as a part of the inner segment or as a separate segment depending on the source classification. We asked prof Mamoun about it and this was his answer: (It depends on the source you read, you can consider it part of the inner segment. I know I said the rod cells are divided into three parts, but this is just to distinguish the synaptic terminal from the rest of the cell).*



Rhodopsins are the molecules that can absorb light.

The Dark Current

There is a difference between rod cells and other neurons:

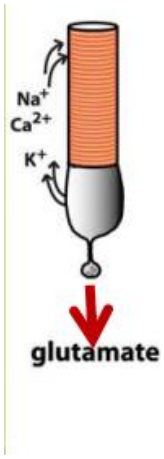
- Most neurons maintain a resting membrane potential (-60 to -70 mV). When excited, they open cation channels causing depolarization and opening of voltage-gated Ca^{+2} channels at the synapse. Ca^{+2} ions flow in and promote fusion of synaptic vesicles, which release neurotransmitters.
- Rods and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

1. **When it's dark,** 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.

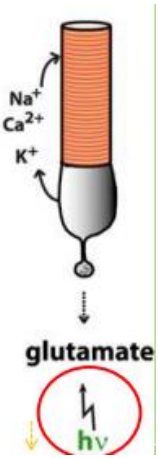
4. The neurotransmitter glutamate is released continuously and in huge amounts.



1. **When excited by light,** channels in the outer segment membrane close which results in less entry of these ions, so rod cells hyperpolarize.

2. The release of the neurotransmitter glutamate decreases.

So, we can say that the signal of vision is considered to be the decreased release of the Glutamate neurotransmitter.

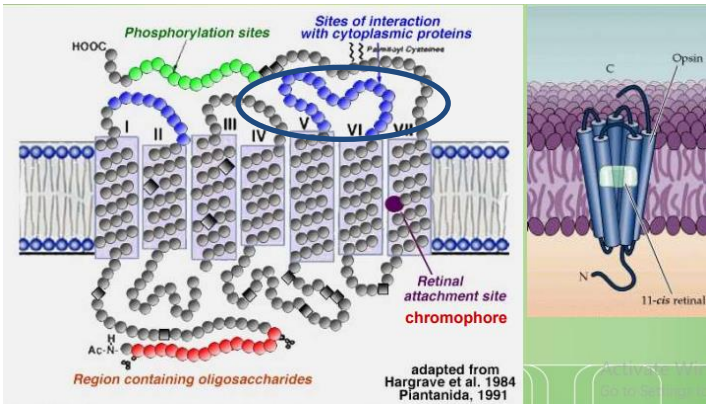


Generation of Vision Signals

What happens after absorption of light? What happens when light hits rods?

These are the different molecular players in signal transduction:

- **Rhodopsin:** a holo-protein receptor that absorbs light formed by an apo-protein called opsin and the pigment molecule that gets excited.
- **Transducin:** a G-protein.
- **Phosphodiesterase**.
- **Na+ gated channels**.
- **Regulatory proteins**.



Rhodopsin

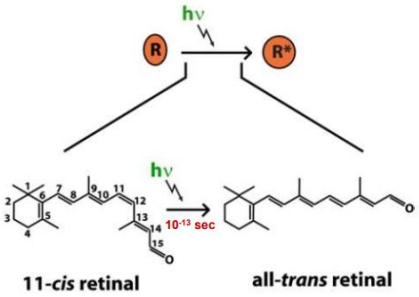
Opsin is a single polypeptide chain with seven helical segments that span the membrane (seven transmembrane domain protein) and in the last transmembrane domain **there is a retinal attachment site where the chromophore gets attached.**

You can see that there are different sequences which are important for regulation and function.

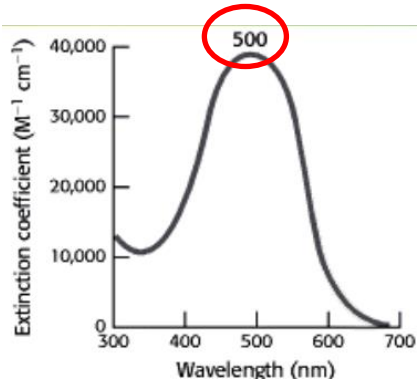
In the **cytosolic region** of the protein, the site of interaction with cytoplasmic proteins (**blue region** in the figure above) is important for transmitting the signals.

Seven transmembrane domain proteins are the second largest membrane proteins after single chain transmembrane domain protein.

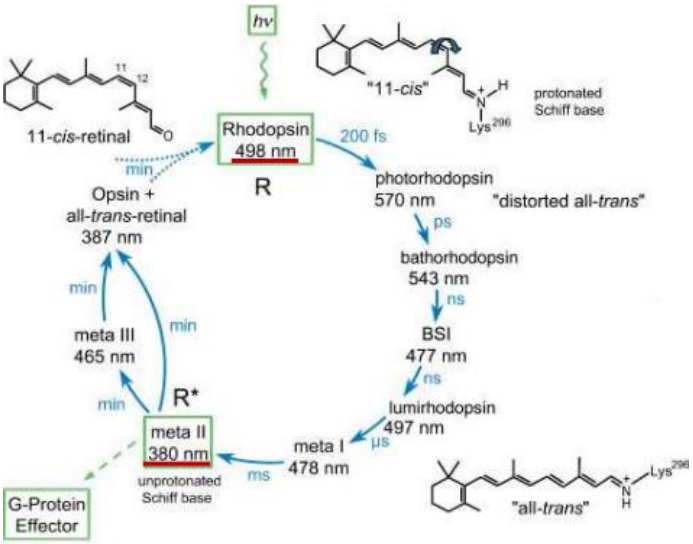
The chromophore is known as ***11-cis-retinal*** which is derived from vitamin A, thus vitamin A is important for vision. Vitamin A is derived from carotene (carrots) and that is why they say if you eat carrots, you will see better especially at night.



Notice how 11-cis retinal is kinky; the electrons in the cis bond of (11,12) get excited when they are hit by light and the structure of the whole molecule changes from **cis molecule to trans**. This change in the structure causes rhodopsin to get excited ***(activated, R*)*** through which transduction of the signal occurs. This change happens very fast, taking about 100 femtoseconds (10^{-13} secs). The scientist, George Wald, who discovered this mechanism got the noble prize.



So, when rhodopsin gets activated, it is able to absorb light at a wide range (350-750 nm) which is the bright light range. The maximum absorption is about 500 nanometers.



When Rhodopsin is activated, it goes under different conformations and each conformation can absorb light at a different wavelength. So, in fact, Rhodopsin changes different colors with

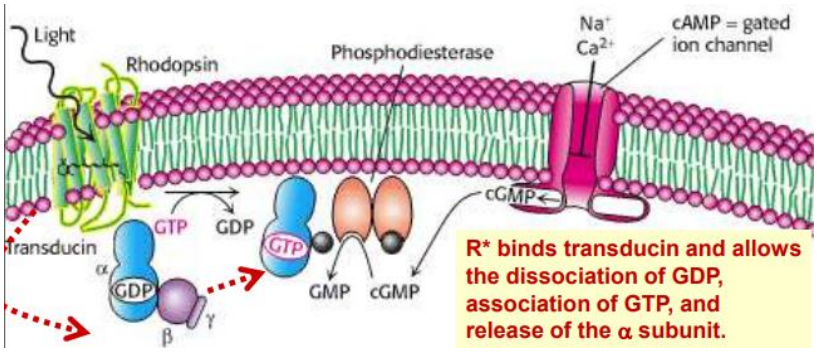
different wavelengths. Meta II is the activated form which can transmit the signal to G-protein.

By itself, 11-cis retinal absorbs near UV light (but when it is part of the **rhodopsin (opsin+ 11-cis retina)** structure, it can absorb up to 498 nm wavelength). But opsin perturbs the distribution of the electrons exciting its electrons with less energy (i.e., longer wavelength light).

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

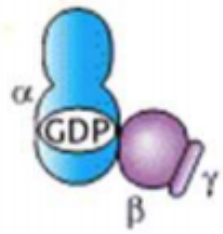
• Rearrangements in the surrounding opsin protein convert it into the active R* state, an intermediate known as metarhodopsin II.

Activation of Transducin



When Rhodopsin is activated, it **activates Transducin** by the **replacement of GDP with GTP**, which **releases the** α subunit of Transducin from β and γ. Then, the α-GTP bound subunit **interacts with phosphodiesterase** which converts **c-GMP into GMP**, thus **reduces the amount of c-GMP** in the cytosol. The importance of c-GMP 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.

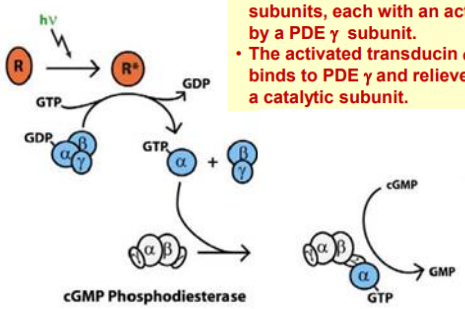


Transducin is a g-protein, which is heterotrimeric made of the 3 subunits α, β, and γ. In its inactive state, transducin α subunit has a GDP bound to it.

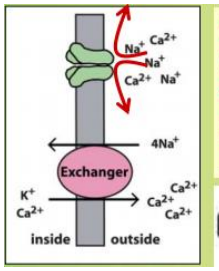
So, when G-α is bound to GTP, it gets released from the β and γ subunits. It then binds to the c-GMP dependent phosphodiesterase enzyme, activating it, and leading to the hydrolysis of c-GMP into GMP. Notice that the binding ratio is 1 GTP to 1 G-α subunit.

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

- 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.



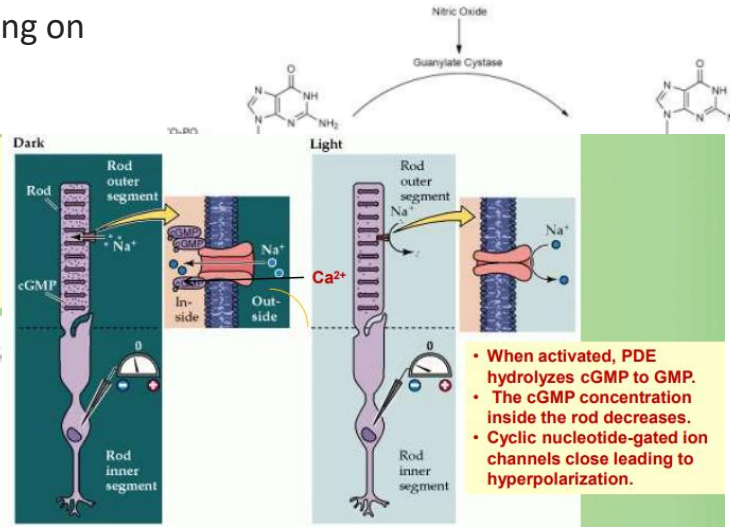
Reduction of the release of the glutamate neurotransmitter -> signal transduction to the brain -> formation of the image depending on which rod it gets its signal from.



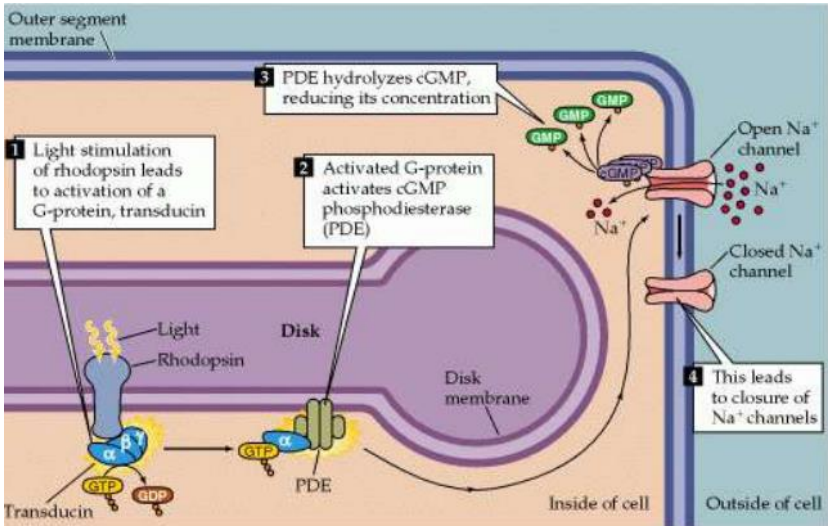
When the channels close, Ca^{2+} ceases to enter, but extrusion through the exchanger continues, so intracellular $[Ca^{2+}]$ falls.

$[cGMP]\downarrow \longrightarrow$ Ion channels closed $\longrightarrow [Ca^{2+}]\downarrow$ (500 nM → 50 nM)

So intracellular $[Ca^{+2}]$ falls from 500 nM to 50 nM or even less.

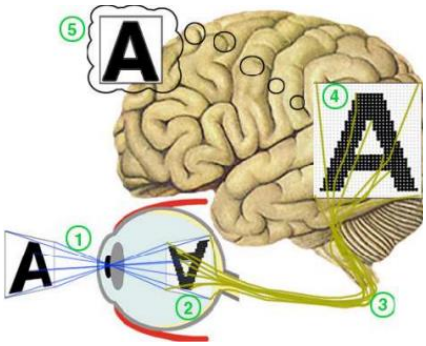


- When activated, PDE hydrolyzes cGMP to GMP.
- The cGMP concentration inside the rod decreases.
- Cyclic nucleotide-gated ion channels close leading to hyperpolarization.

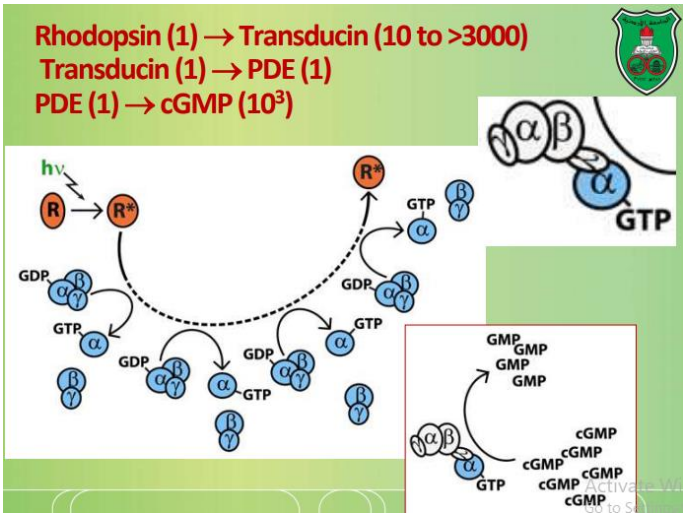


Creating an Image

- The large potential difference travels as an electrical impulse down the rod cell to the synaptic terminal and is then transferred to an adjoining nerve cell.
- The nerve cell carries this impulse all the way to the brain.
- The brain then determines where the nerve impulse originated and interprets the image.



Signal Amplification

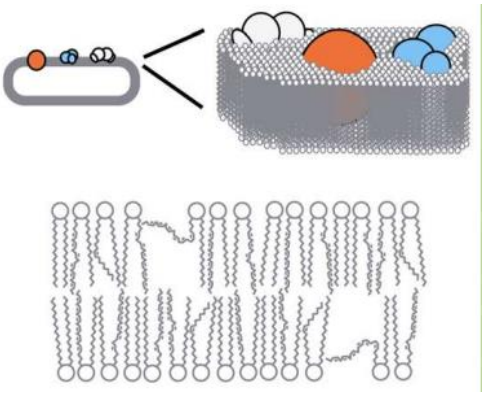


Since 1 photon is really little in terms of induction of rod cells, it does not really activate many rod cells. So, the signal has to be amplified.

When one Rhodopsin molecule is activated, it can activate 10 to >3000 molecules of Transducin, moreover an average of 500. (The range is based on the number of photons and experimental measurements that can be inaccurate….). One Transducin molecule can activate one phosphodiesterase molecule (a ratio of 1:1, no amplification happening here). Then, one phosphodiesterase activates/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

Factors that facilitate signal transduction:



- **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.
- 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.
- **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.

- 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.

Overall, a single photon closes about 200 channels and thereby prevents the entry of about million Na^+ ions into the rod cells (and that is a lot in terms of the prevention of entry of Na^+ and Ca^{+2}).

Signal Termination

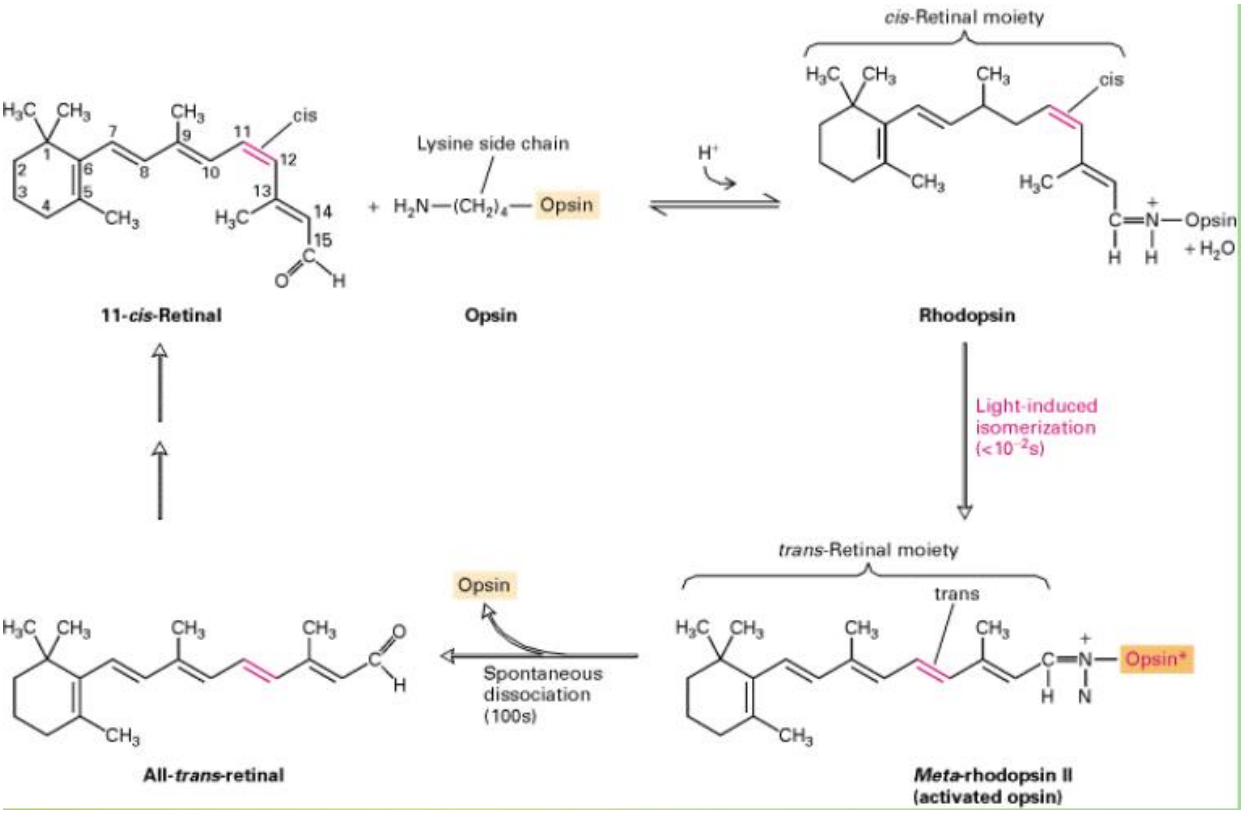
Signal termination is quite important. It allows us to see the smooth movement of a person. If the signal is not terminated, we would not see the whole movement of a person. For example, without signal termination you will only see the two images to the right, the individual with their left arm up then 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.

General Idea: without signal termination, you would only see interrupted images.

There are multiple mechanisms by which the signal is terminated:

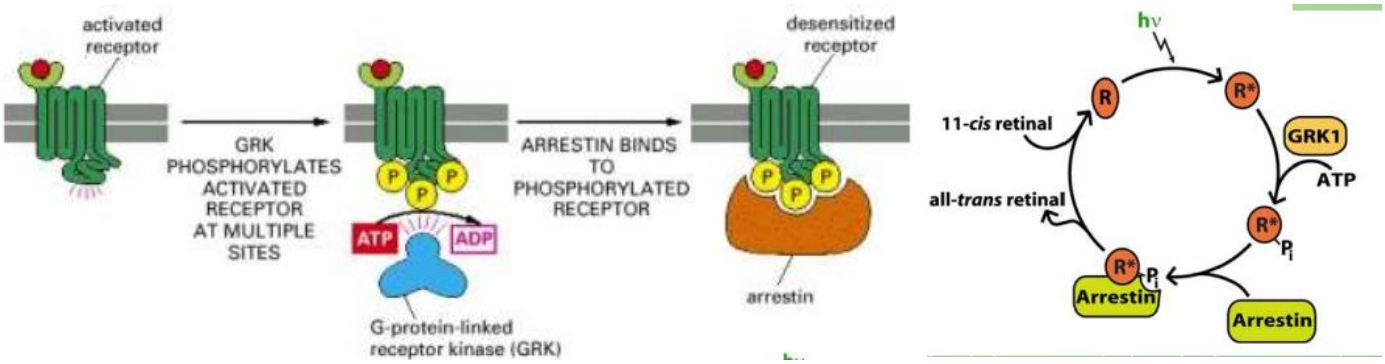
Mechanism 1 – Unstable All-Trans Rhodopsin Complex

- 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

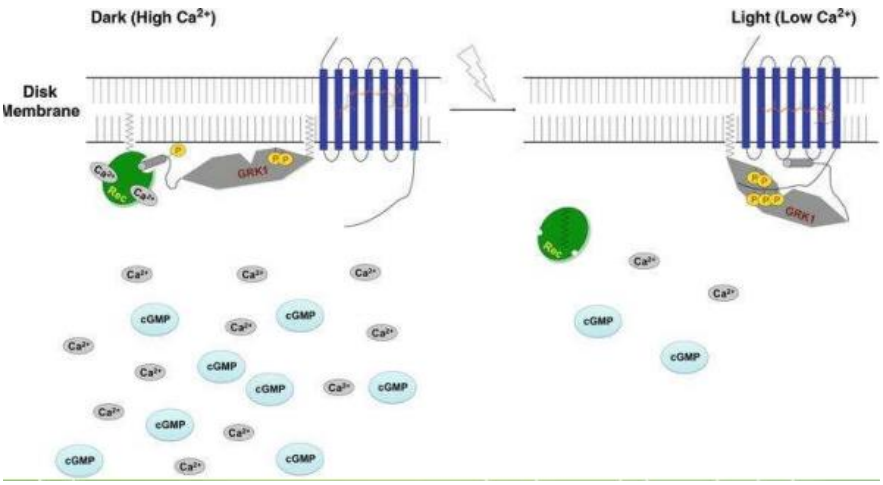
- **Rhodopsin kinase 1 (GRK1)** can phosphorylate the C-terminus of ***active*** Rhodopsin (=R* or meta-rhodopsin II). It does **not** phosphorylate the inactive form of rhodopsin.
- Phosphorylation of R* has two effects:
    - The ability of rhodopsin to activate Transducin decreases.
    - It facilitates binding of the protein **Arrestin** to rhodopsin, which completely quenches (stops) its activity (so no transducing can be activated). Additionally, binding of Arrestin leads to the release of all trans-retinal, regenerating rhodopsin.



Recall that when the ion channels close (in the presence of light), not only does the membrane hyperpolarizes, but there is also a reduction in the concentration of Ca^{2+}. It turns out that GRK1 is more active at low intracellular calcium ion concentration.

How does the enzyme become more active?

- In the dark, Ca^{2+} 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.
- In contrast, Ca^{2+}-free Recoverin does not bind to GRK1. Without this inhibition, the kinase is more active and can phosphorylate rhodopsin.

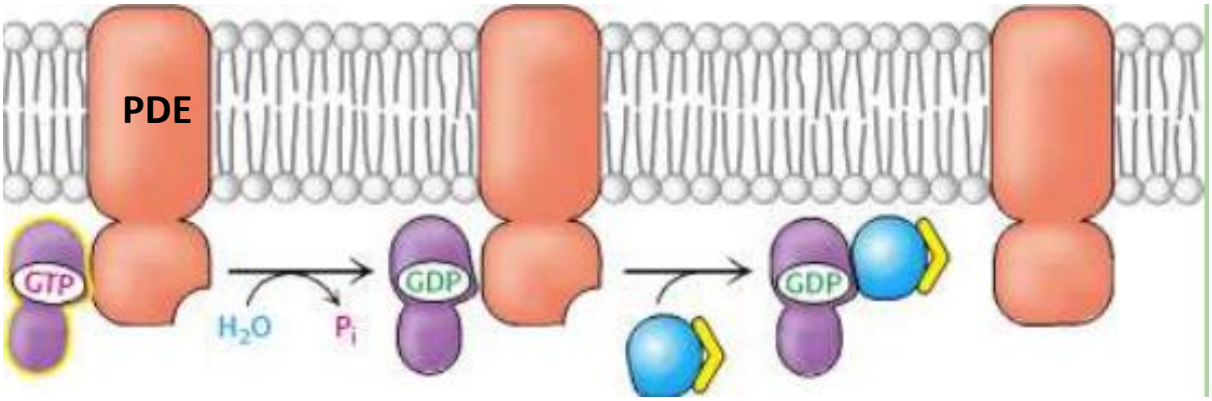


Additionally, **Calmodulin,** another protein, can also bind to Ca^{2+} when it is present in high concentrations. Ca^{2+}-Calmodulin can also bind to GRK1 and inhibit it.

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 Ca^{2+} ions.

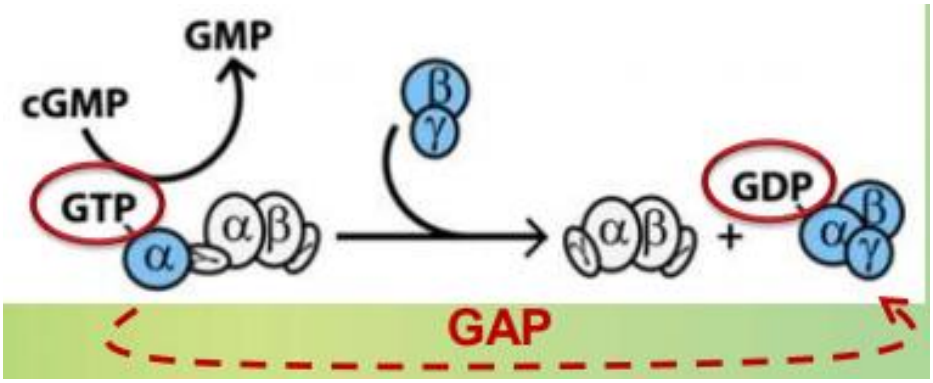
Mechanism III – Intrinsic GTPase activity of G protein

- Gα (of Transducin) has an intrinsic GTPase 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 is the subunit that inhibits the catalytic subunit of PDE. Then, Transducin α-GDP eventually combines with Transducin βγ.

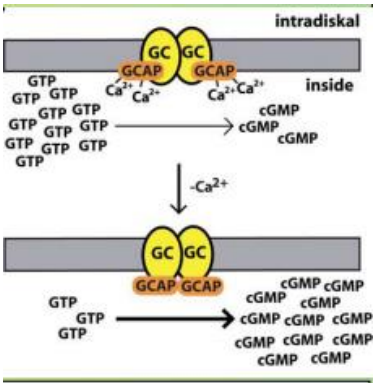


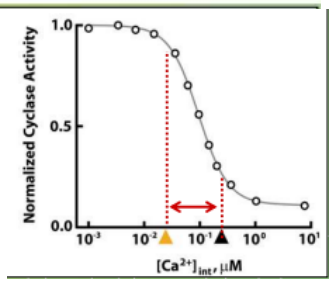
Mechanism IV – Facilitation of GTPase activity of G protein

- 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

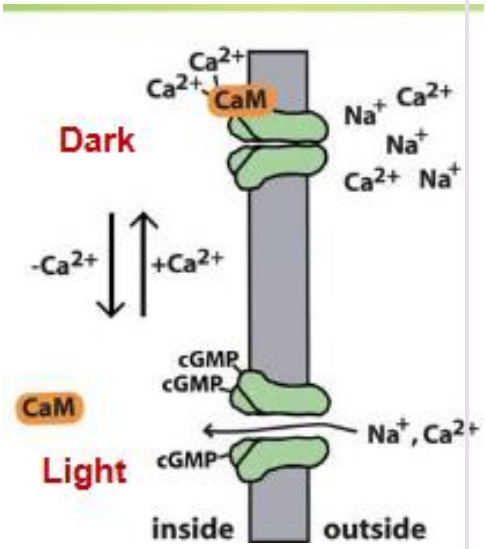




- When it's dark and there's high levels of Ca^{2+} ions, guanylate cyclase activating proteins (GCAPs) can bind to Ca^{2+} ions. This binding blocks their activation of guanylate cyclase.
- When the concentration of Ca^{2+} ions decrease, Ca^{2+} dissociates from GCAPs. The GCAPs can now activate guanylate 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. Any reduction in the concentration of calcium ions leads to high activity of guanylate cyclase.

Mechanism VI – Ca^{2+}-Calmodulin and cGMP-gated channels

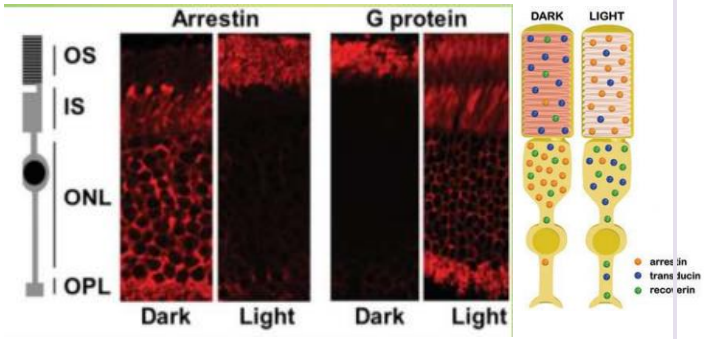
- In the dark, when the calcium concentration is high, they can bind to calmodulin to form Ca^{2+}-Calmodulin (CaM). CaM, in a sense, balances things out by keeping some of the channels closed.
- CaM binds to the channel and reduces its affinity to cGMP, closing the channel.
- During visual transduction, the decrease in intracellular Ca^{2+} 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.



Adaptation to Light/Dark Conditions

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.

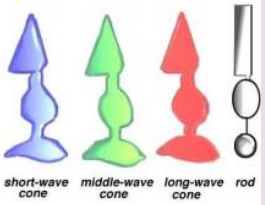
	Dark	Light
Arrestin	More in inner segment	More in outer segment
Transducin	More in outer segment	More in inner segment
Recoverin	More in outer segment	More in inner segment
Overall	Low inhibition, receptor ready to be activated.	High inhibition, receptor ready to be inactivated.

So why is this the case?

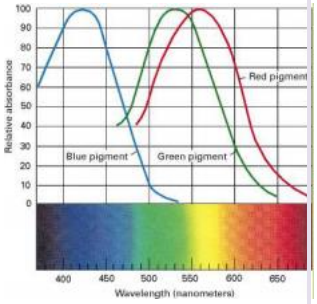
When it's dark, you want Arrestin to stay in the inner segment to lower inhibition (of rhodopsin) and have the rod cell very sensitive to any light. On the other hand, the G protein is localized in the outer segment waiting for any signal (any photon to hit rhodopsin) so it can be activated.

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 in order 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.

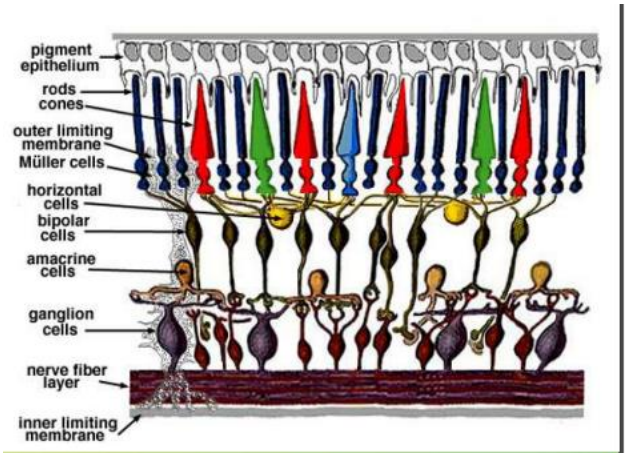
Color Vision



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



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

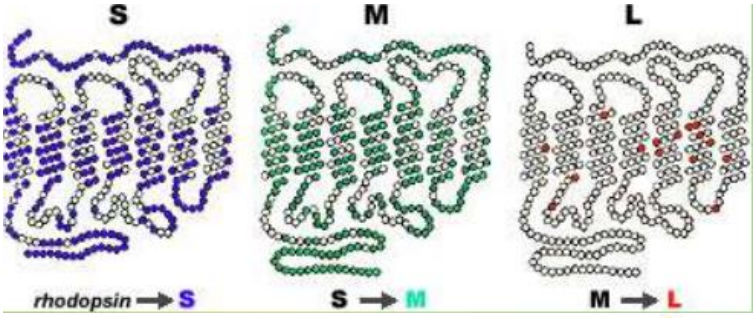


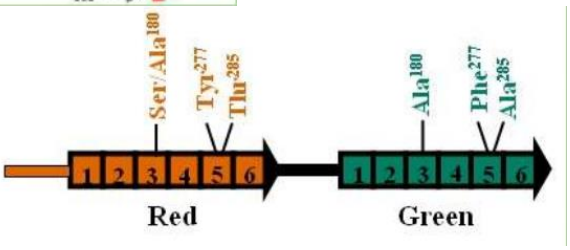
The combination of the three types of cones gives us color vision. Recall that the cone cells are embedded within a larger population of rod cells.

One difference that should be noted between cone and rod cells is that **multiple rod cells can connect to a single neuron**. On the other hand, **each cone cell is connected to a single neuron** by itself (or less cone cells in general are connected to a single neuron as compared to rod cells). This has important implications to be discussed later.

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 (λmax) that is absorbed (λ becomes longer=lower energy).

Rods vs Cones

We've already discussed the differences in regards to light absorption (the wavelength that is absorbed), number (there are more rod cells), structure, and photoreceptors (recall the homology). The chromophore is the same for rods and cones.

There are two more points to discuss: sharpness and sensitivity (amplification). 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.

1. 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.
2. 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

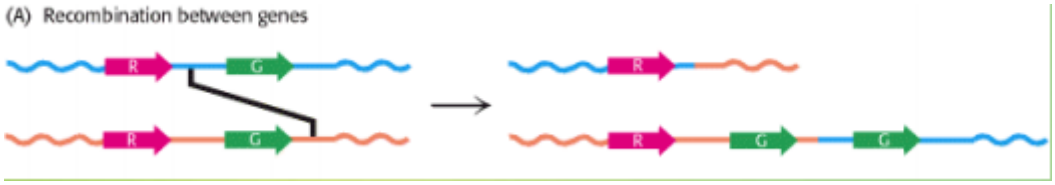
Chromosomal Locations

- **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.

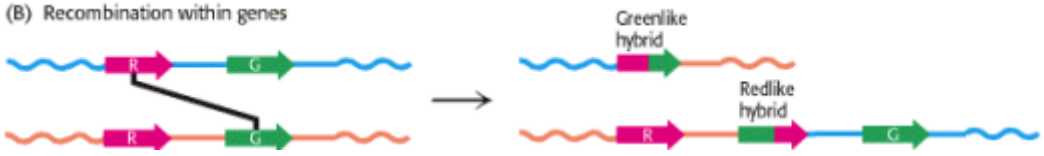
Red-Green Homologous Recombination

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:

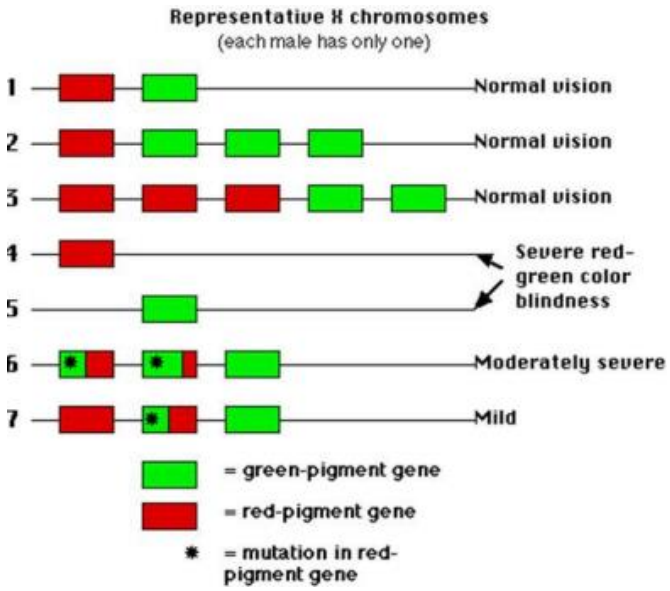
1. Inter-genic (recombination between transcribed regions of the gene).



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.



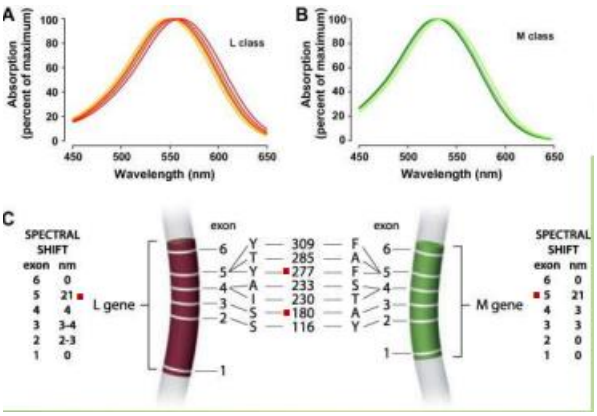
Genetic Probabilities



- 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.
- 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).
- If one (red or green) is totally missing, then that individual will have severe red-green color blindness. (more common than blue color blindness).
- 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

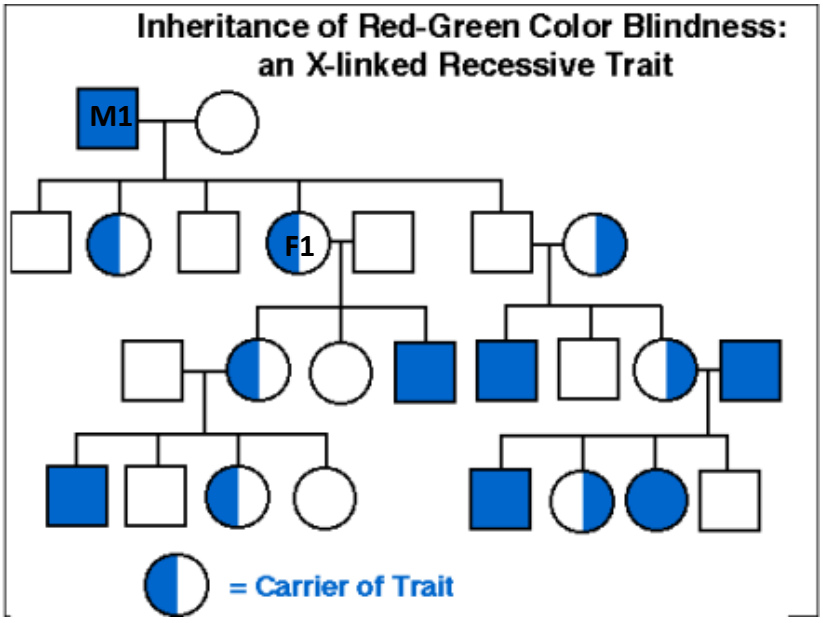
- Individuals are not equal in how they visualize color. So, some people will see red differently than how others do. The reason is genetic differences (polymorphisms).
- The substitutions at positions 277 and 285 account for about 20 nm of the difference in peak sensitivity.
- The presence of serine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



Pedigrees

As an X-linked recessive disorder, males are more affected due to the fact they only have one X chromosome.

The professor said he may give multiple pedigrees in the exam and ask which represents color blindness.



Let’s examine the pedigree:

- M1 is affected and will transmit his X-chromosome to his female daughters, who are now carriers.
- There’s a 50% chance for F1’s daughters to become carriers (they will receive a normal copy of the gene from their father). F1’s male son, however, will have color blindness if the mother gives her copy of the color blind gene.

The following images attempt to depict how color-blind individuals see. However, they do adapt and are able to recognize red or green, even if they don’t see them the same way non-colorblind individuals do.