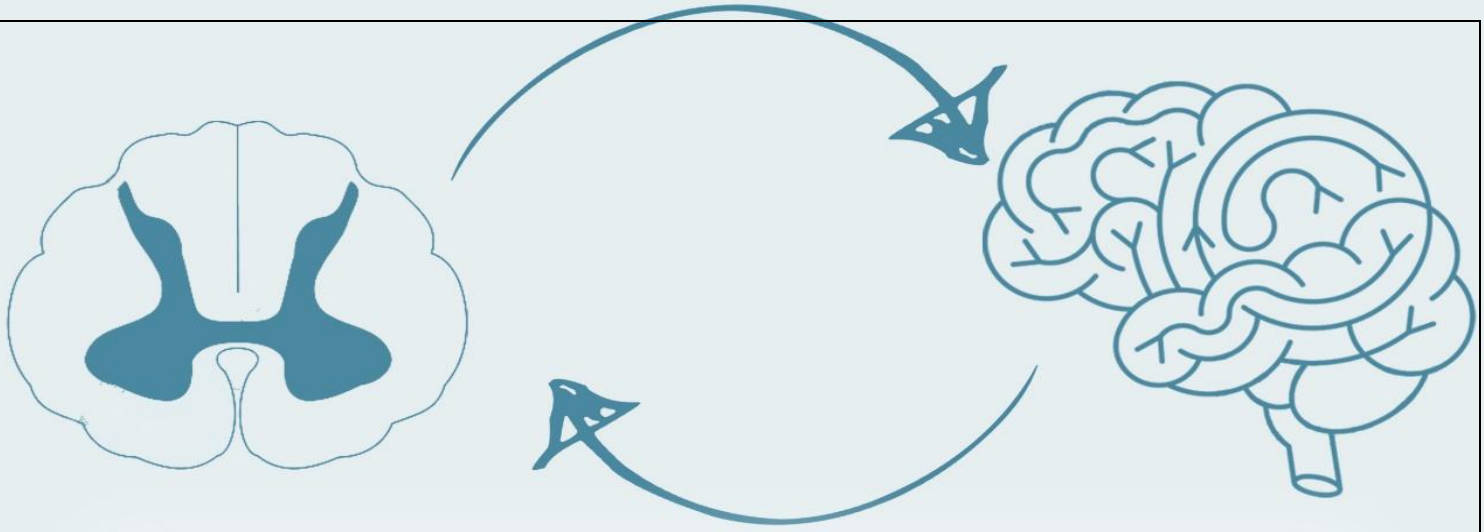

CNS

Biochemistry

0 slides sheets

number	3
Done by	Ammar Ramadan
Correction	Correction team
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In this lecture we are going to talk about:

- Visual transduction (transferring the signals starting from the photon which hits the photoreceptor until the processing of vision in the cortex).
  - Components (cells and molecules).
  - Mechanisms of activation, amplification, and termination.
- Color blindness.

- There are so many different wavelengths, but we as humans can see a very narrow range of wavelength (400-700 nm). Beyond this range, we will not see anything.

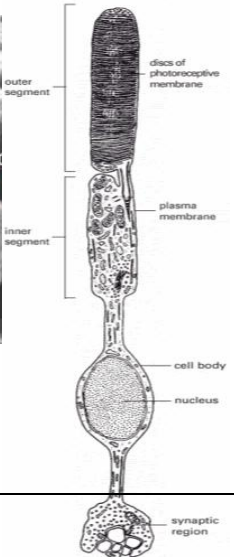
So which cell components are responsible for vision ?

There are two types of cells; “rod cells” and “cone cells” . Named according to their shape. Rods are slim and elongated while the cones are conical in shape.

	Rods	Cones
Sensitivity	High Night vision (in the dark)	Low Day vision (in bright light)
Acuity (image sharpness)	Lower visual acuity	Higher visual acuity
Coloured vision	No (narrow range of wavelengths)	Yes (all wavelengths of the visible light)
Number of receptors	Over hundred million	Seven million only
Connection to a nerve	More than one are connected to one nerve	cells connected to one or multiple nerves

- This is how the rod cells and the cone cells appear under scanning electron microscope. Rods are bigger than cones.

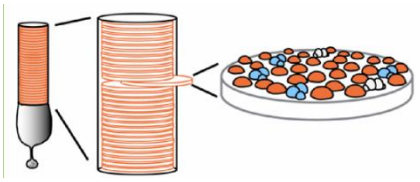
- This is the structure of the rod cell, it is composed of an inner segment and an outer segment. The inner segment is composed of cell body which contains the nucleus, ER,



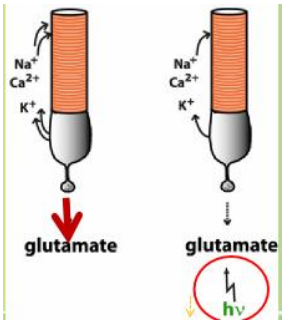
golgi, and other cellular organelles *found in other neurons, including a synaptic terminal*. The outer segment is composed of disks (*contains the biochemical machinery needed for visual transduction*.).

- Each disk has the receptors (channels) of the visual transduction. Synthesis of these receptors happen in the inner segment and then go to the outer segment (to the disks).

- In other words: "The components of the photo-transduction enzyme cascade are packed into stacks of membranous vesicles ("disks")"



- There is a difference between the visual nerve and the normal nerve in terms of action potential. The normal nerve maintains a resting membrane potential at rest and gets depolarized when activated. While the rod cells are the opposite (backward); they are depolarized in the dark and gets hyperpolarized when activated (in the light). In the dark, channels are open, but in the light the channel closes. In the dark there is a lot of release of the neurotransmitter **glutamate,** but in the light there is no release of glutamate. (I.e the signal (light) leads to sequester of the neurotransmitter and not causing its release).



Please refer to slide 7.

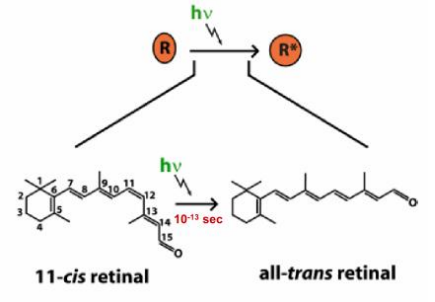
<u>Molecular mechanism of transduction</u>

The elements needed to generate the vision signals include:

- Rhodopsin (opsin + chromophore [which absorbs the light])(the protein in the rods).
- Transducin.
- Phosphodiesterase (enzyme).
- Na+ gated channels.
- Regulatory proteins

  - Rhodopsin: it is a 7 transmembrane domain protein (spaning the membrane 7 times). At the 7^{th} domain there is a chromophore.

- The chromophore is a **11-cis retinal.** Produced from vitamin A; and that's why this vitamin is good for vision. The retinal protein has two structures. The **11-cis retinal** is when the protein is **inactive (absence of light)**. When activation occurs the 11-cis double bond turns into trans double bond. This leads to transformation of the retinal protein from a structure which has a kink to a structure that is straight. (Minor change in the 1° structure leads to a change in the 3° structure. Remember the heme) AMAZING !!

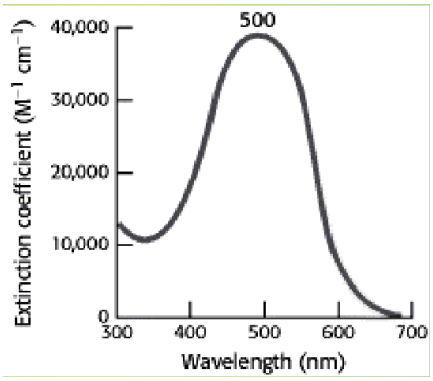


- This change takes 10^-13 sec to occur.

- This graph shows the wavelength that the Rhodopsin can absorb, from 350 to 650 nm, (wide range).

- That's why we are able to see the colors in the dark but not as perfect as we see them in the light (but we can see).

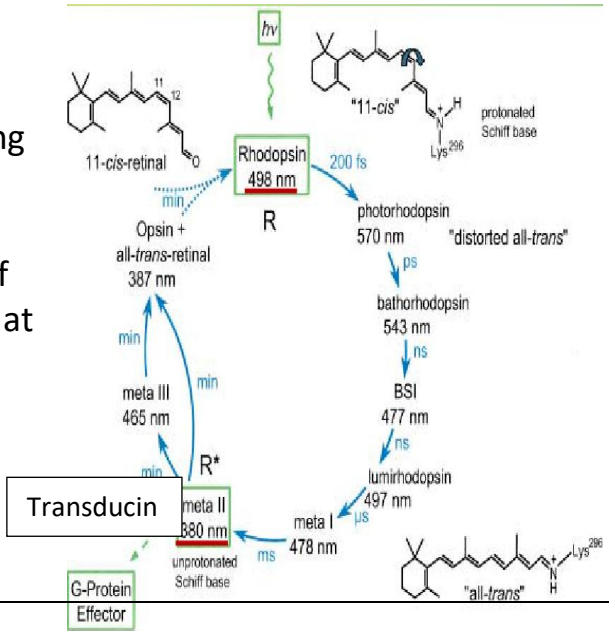
- The peak is around 500 nm which represents the yellow colour. That's why in the dark we see the yellow light better than other colours.



10:40 min

- Conversion of 11-cis retinal to all-trans retinal changes the structure of the whole rhodopsin molecule (conformational change). This reaction is not like the on/off switch; it takes different intermediate conformations and structures.

- Notice that in each step in the transformation, a structure forms which can absorb different wavelength, and the following structure absorbs another different wavelength...etc. That's why Rhodopsin can absorb different wavelengths. (i.e Each one of the rhodopsin intermediates can absorb light at a different wavelength).



- The activated form **metarhodopsin 2** -designated **R*** in the figure- will activate the **transducin.** When this structure changes there will be release of the **all trans retinal** and a new 11-cis retinal will join opsin to repeat the cycle.

Important points to remember:

1- Rhodopsin takes different structures.
2- These different structures can absorb different wavelengths (there is wide range).
3- There will be release of the all trans retinal so that a new 11-cis retinal can take its place. Then the molecule can reactivated again when another photon enters.
4- The structure which activates the transducin is the **metarhodopsin 2**

Remember:

G Protein is A trimetric protein .The 3 subunits are α,β and γ . In its inactive form , the α subunit is bound to GDP and is in complex with the β and γ subunits (Inhibitors for α subunit). When activated the GDP is replaced with GTP enabling the α subunit to dissociate from the β and γ subunits and do its assumed function (activating phosphodiesterase here).

- After that the transducin is activated, (Transducin is a G-protein).

- After transducin gets activated the GTP will replace the GDP which results in separation of the α subunit from the β and γ subunits.

- The α subunit then activates phosphodiesterase***.

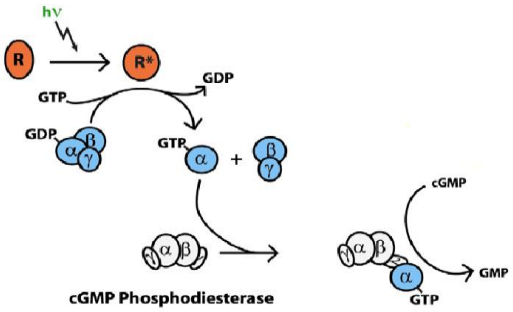
- Inactivation of the α subunit occurs when the GTP gets hydrolysed into GDP. Then the α subunit can join the 2 inhibitory subunits.

- Phosphodiesterase converts cGMP** to GMP. So the amount of cGMP in the Rod cell decreases and the Na+ gated channels(allow entry of Na and Ca) close and there will be no entry of Na+ and Ca+2 in the cell. And the amount of the neurotransmitter released will also decrease. See slide 17 (figure)

**cGMP is important because it keeps the Na+ gated channels open.

***Phosphodiesterase is a heterotetramer that is composed of alpha, beta, and 2 Gama subunits. Gama subunits are inhibitory to PDE.

From the slides: The activated transducin alpha subunit-GTP binds to PDE gama and relieves the inhibition on the catalytic subunit.



Signal amplification

- Although retinal cells absorb 1 photon of light, they are able to activate rod cells. The photon is enough to activate them because they are very sensitive.

- What makes them so sensitive like that ? Signal amplification; if you activated 1 Rhodopsin it will activate 500 transducin molecules. And each one of these transducin molecules will activate one phosphodiesterase (PDE) molecule. Each PDE will transform 1000 cGMPs into GMPs. AMAZING !!

Remember that enzyme/substrate interaction is a random process. To facilitate this interaction and make it easier to occur we put the enzymes and the substrates in smaller compartment.

What facilitated this amplification to happen?

1- **These components are not in the cytoplasm**; Instead they are in the membrane. So they move in two dimensions instead of 3 Which increases the chance for a successive collisions.

2- **The membrane of the rods is so viscous;** like the olive **oil.** Thick but fluidic because it contains **low cholesterol and high levels of unsaturated fatty acids.** This makes the movement of the molecules very fast.

3- **Cooperation;** when cGMP binds to the channel, it makes it easier for the 2nd cGMP and the 2nd makes it easier for the 3rd one and so on. Removal of one cGMP reduces the activity of the channel and the channel closes.

From the slides: 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.

From the slides: Overall, a single photon closes about 200 channels and thereby prevents the entry of about million Na+ ions into the rod cells. *21:42 mins*

Signal termination

For us to be able to see a continuous image and not a separated images, there has to be a rapid activation followed by a rapid termination.

Signal termination mechanisms:

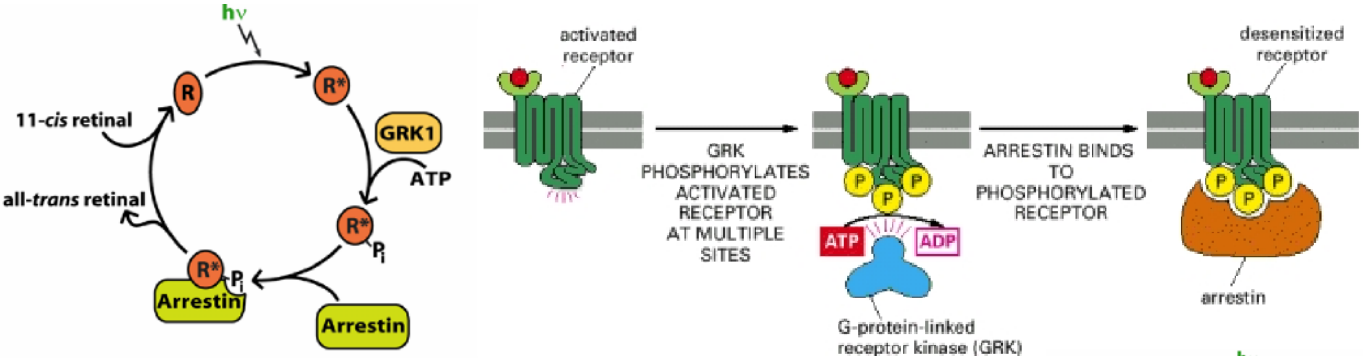
1- As soon as the Rhodopsin gets activated, a **kinase will phosphorylate the Rhodopsin** which inactivates the Rhodopsin. Then **Arrestin** will stop the activity of Rhodopsin (binds to the phosphorylated Rhodopsin).

From the slides:

" • Rhodopsin kinase (GRK1) phosphorylates the C terminus of R."*

"• Phosphorylation of R decreases transducin activation and facilitates binding to arrestin, which completely quenches its activity, and releases of the all trans-retinal regenerating rhodopsin."*

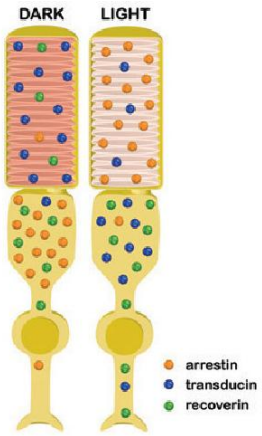
Look at the figures below



2- Arrestin/transducin distribution;

In the **dark** the **transducin** is situated in the **outer segment**, but the **arrestin** is situated in the **inner segment** away from Rhodopsin. Because the transducin is waiting the Rhodopsin to be activated so that it can inhibit it.

While in the light (When there's an activation for the Rhodopsin) **transducin** gets activated and then it will shift to **inner segment**, then the **arrestin** will shift to the outer segment. So there will be a fast inactivation for the Rhodopsin.



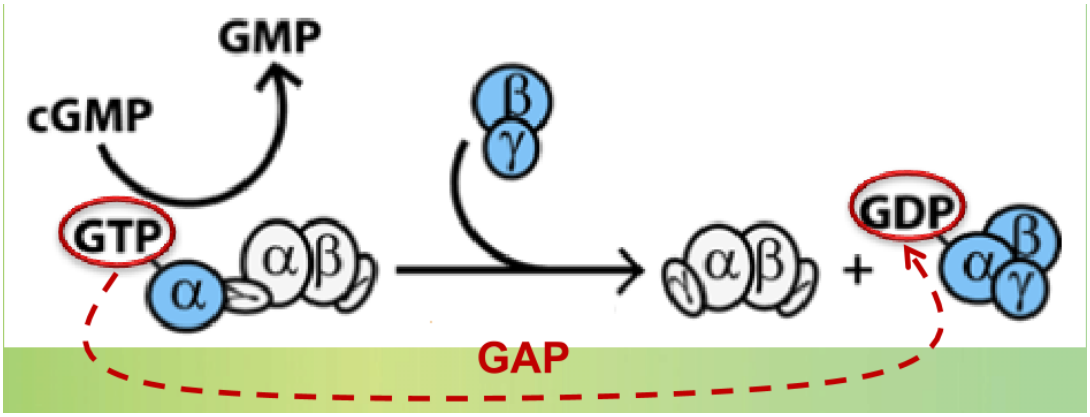
From the slides :

• In dark, the outer segment contains high levels of transducin and low levels of arrestin (low inhibition; ready to be activated).

• In light, it is the opposite (high inhibition; ready to be inactivated.

3- **Intrinsic GTPase activity of G protein (transducin**); will Hydrolyse the GTP in the alpha subunit into GDP as soon as alpha subunit activates the PDE. Then the α subunit can join the 2 inhibitory subunits.

4- Facilitation of GTPase activity of G protein; GTP hydrolysis is slow intrinsically, but it is accelerated by the GAP (GTPase Activating Protein) complex. These GAPs ONLY activate the GTPase activity **after** transducin a-GTP binds PDE-g.
look at the figure below.

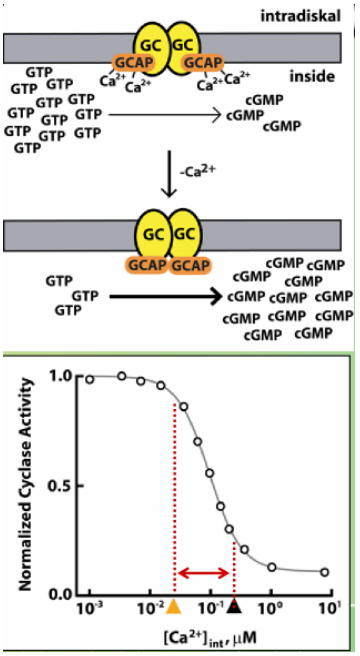


5- Unstable all-trans rhodopsin complex; when the 11-cis retinal transform into all trans retinal it will change the structure of Rhodopsin. As soon as the 11-cis retinal transform into all trans retinal, the Rhodopsin will release the all trans retinal and the signal will be terminated because the interaction is unstable between Rhodopsin and all trans retinal.

6- Feedback regulation by calcium ions; remember that in the presence of light there will be a lesser amount of cGMP; which was keeping the Na+ channels open. As a result, the channels will close and no more Na+ or Ca+2 will enter the cell. The Ca+2 levels inside these cells will drop markedly from 500nM to 50nM ; because **there is no entry for Ca+2**, and because **there are some transporters that expel the Ca+2 outside the cell**. The

reduction in the amount of Ca+2 is what causes the feedback regulation. How?
This reduction will activate the **guanylate cyclase** which will produce cGMP from GTP. When cGMP increases, the channels will open. So the feedback mechanism is the reduction in Ca levels.

- Remember that the Ca+2 levels decrease in the light.
- Note: the activity of guanylate cyclase in the dark is low, but in the light the activity is very high.
- Notice that any change on the level of Ca+2 will have a huge effect on the activity of guanylate cyclase.



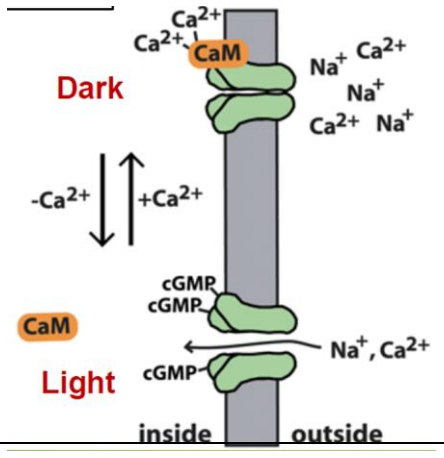
- How will the reduction in Ca+2 cause activation for guanylate cyclase?
The guanylate cyclase is connected to **guanylate cyclase activating protein (GCAP)**. If the level of Ca+2 is high (normally; in the dark), the Ca+2 will bind to GCAP and GCAP will be inactive. Thus, the guanylate cyclase will not be very active and there will be a little production of cGMP. Look at the figure.

- However, A decrease in intracellular [Ca2+] causes Ca2+ to dissociate from GCAPs leading to full activation of guanylate cyclase subunits, and an increase in the rate of cGMP synthesis.; cGMP increases allowing for the opening of the Na+ channels.

- Notice that a small reduction in the amount of calcium causes a tremendous change in the activity of guanylate cyclase. Look at the figure.

7- **Ca-calmodulin (also calcium feedback)**

- Ca-Calmodulin (CaM) binds the cannels in the dark (high levels of Ca+2) and keeps some of them closed, so not all channels are open in the dark. During visual transduction, when the level of



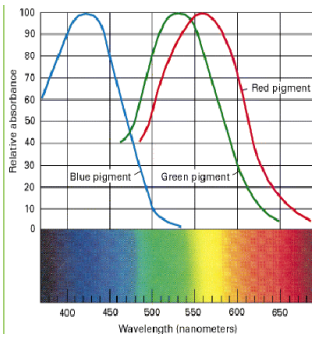
calcium is down, CaM is released and channels open up to allow sodium ions to get into the cell again.

- In other words: During visual transduction, the decrease in intracellular [Ca2+] causes CaM to be released, and the channel reopens at lower levels of cGMP.

36:00 mins

Colour vision

- Cone cells are responsible for the colored vision. Cone cells are similar to rod cells that both contain the same chromophore (11-cis retinal) but the effect is different because cone opsins have similar structures as rhodopsin, but with **different amino acid residues** surrounding the bound 11-cis retinal; thus they cause the chromophore's absorption to different wavelengths.



- We got 3 different types of cone cells. Each is responsible for a certain range of wavelengths (we have 3 ranges : Red, green, blue).

- The wavelengths for the red and green are very close to each other (*The green vs. red photoreceptors > 95% identical.*).

- If you compare the primary structure (amino acid sequence) of the opsin in cones vs rods: similarity~40%.

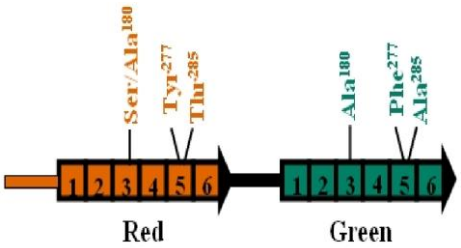
- If you compare red and green vs blue ~40% homology.

- Red vs green > 95% homology and minimal differences. This has important implications.

- Notice that the sequence of the amino acids is what determines the wavelengths that the protein can absorb.

- If you looked at the sequence of amino acids of the red and green:

the differences in these amino acids is what makes one of the opsins absorbs red light (wavelength) and the other absorbs the green light (wavelength).



Three amino acids:

• In the red they are serine, tyrosine and threonine: these are polar amino acids with **hydroxyl side group** which **determines which wavelength will be absorbed**.

• In the green: Alanine, phenylalanine, and Alanine; these are **Non polar** amino acids.

From slides: A hydroxyl group has been added to each amino acid in the red pigment causing a "lambda max" shift of about 10 nm to longer wavelengths (lower energy).

Image sharpness and sensitivity

In the beginning of the lecture we said that a group of rod cells are connected to one neuron. And one cone cell is connected to one or multiple neurons**.**

• Few cones are connected to a single neuron. This arrangement is the basis for the high sharpness(acuity) and low sensitivity of the cones.

• Many rods are connected to a single neuron. So the signal will come from multiple rod cells to one neuron. As a result, the image would be less sharp in the rods than in the cones. However, there will be a greater sensitivity in the rods because more rods = amplification of the signal and so more intense signal to the brain.

"To understand the sharpness; imagine that multiple rod cells form one pixel in a picture, but one cone forms one pixel. For the same amount of cones and rods, there will be much higher pixels from cones compared to rods and thus more sharp image"

<u>Color blindness</u>

- The blue opsin gene is on chromosome 7.
- Red and green opsins genes are on the X chromosome. So males are more affected in red green color blindness than females.

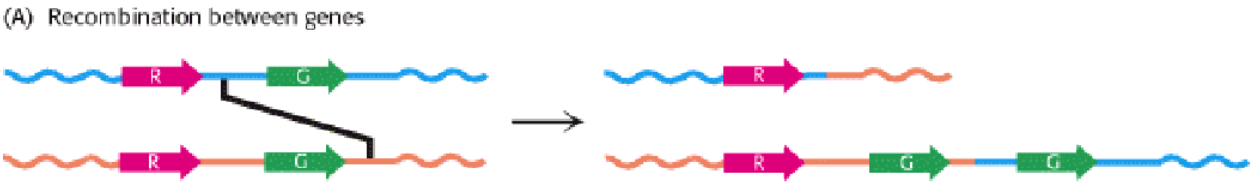
From the slides:

- The X chromosome normally carries a cluster of from 2 to 9 opsin genes.

- Multiple copies of these genes are fine.

- We said that the green and red opsins have very similar amino acid sequence and they are on the X chromosome. So there will be a higher possibility for **gene recombination**.
- Genetic recombination can occur between or within genes (inter vs intra genetic recombination).

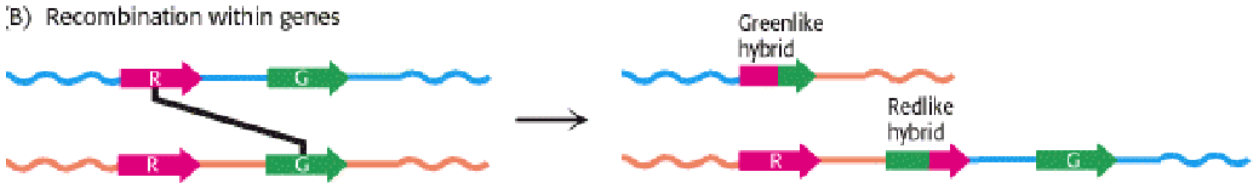
• Inter-genic recombination (Between transcribed regions of the gene):



-You can have loss of a whole gene (X chromosome missing the gene). There will be a color blindness. If the green gene was removed, the person will not see the green color.

-Or you can have multiple genes in one chromosome (which would make you no extra good; if you had two copies of the green you'll not see the green better than normal person).

● Intra-genic recombination (Within transcribed regions of the gene):

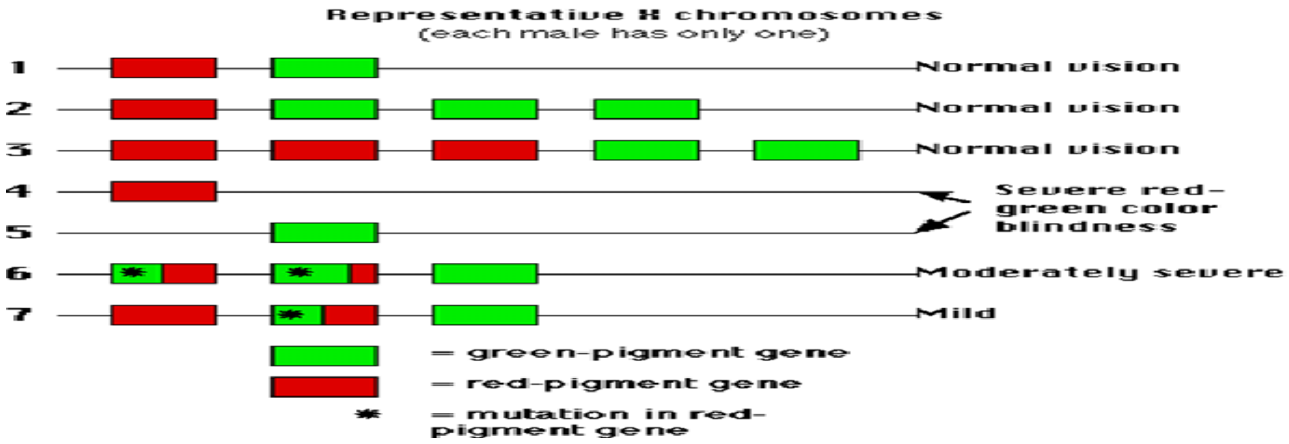


-Hybrid genes will form

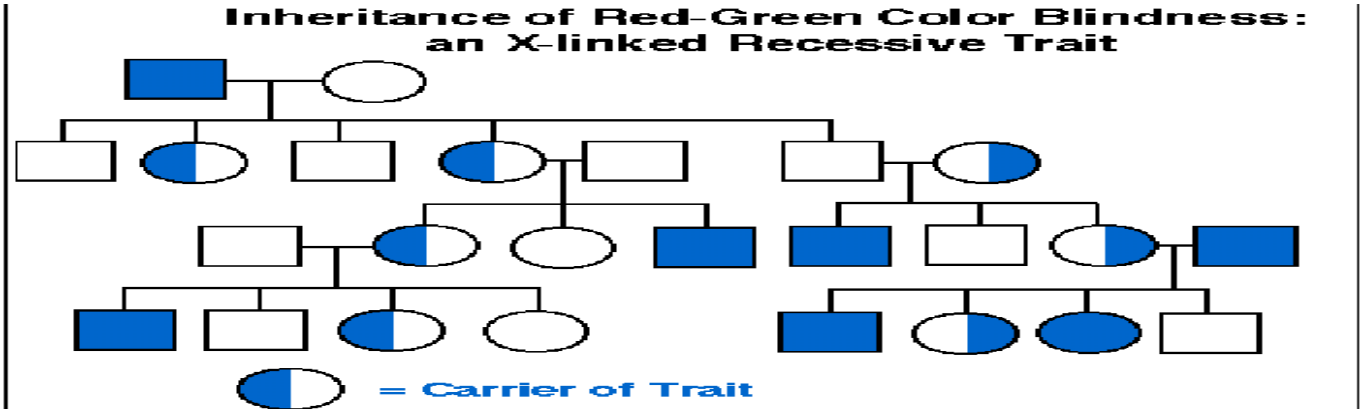
-Severity depends on the amount of the genetic loss; if you are missing a lot of the red, you will see the red but not as good as normal person.

Note: Genetic polymorphism plays a role in how we see colors (we can have a single nucleotide polymorphism), so we do not see colors in the same exact way. These polymorphisms affect the absorption of light (Red and Green).

Please have a look on this figure.



Have a look on this pedigree



please refer to the slides.

"Winners NEVER Quit

And

Quitters NEVER Win"