# Biochemistry - CNS


Done By

Heba Altahat

Corrected By

Samah Freihat



النادي الطلابي

كلية الطب

The picture can't be displayed.

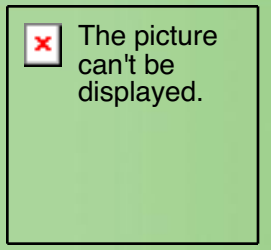
# Visual transduction

**Prof. Mamoun Ahram**
**Neuroscience, Biochemistry**
**Third year, 2022**

# 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

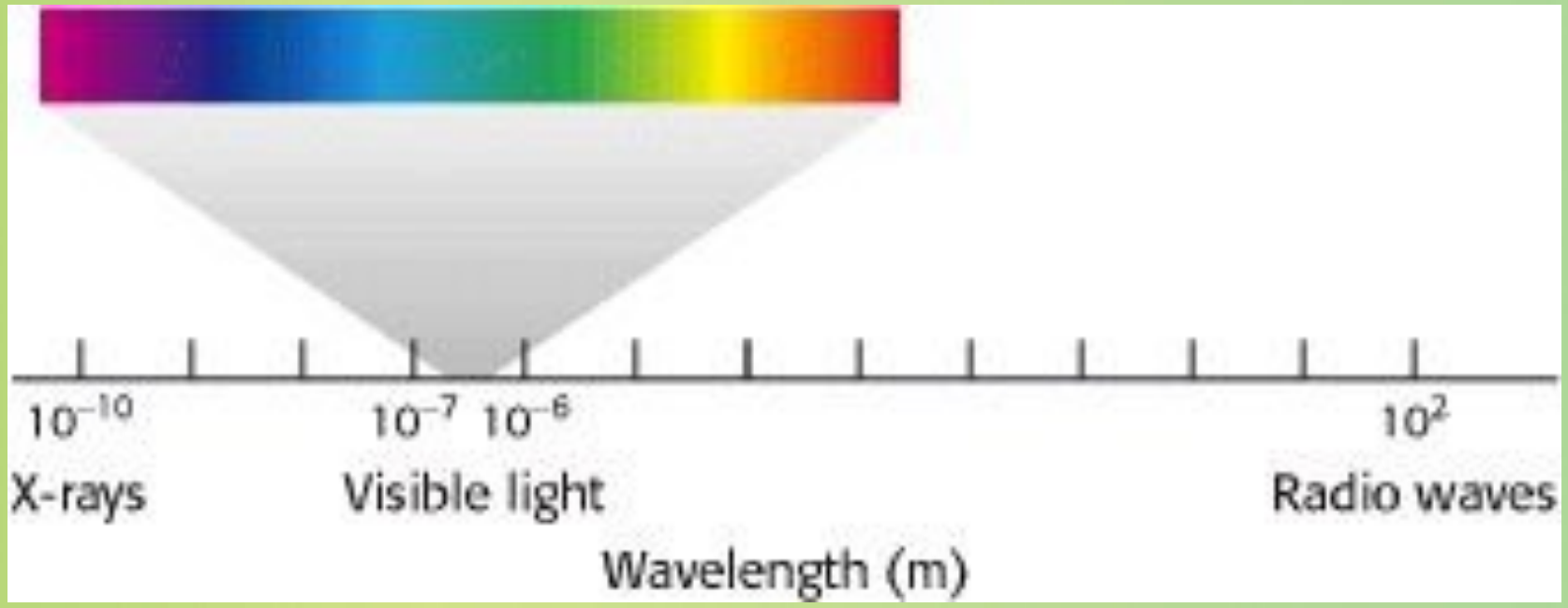



- Visual transduction (dim vs. bright light)
  - Components (cells and molecules)
  - Mechanisms of activation, amplification, and termination
- Color blindness

# Basics of human vision

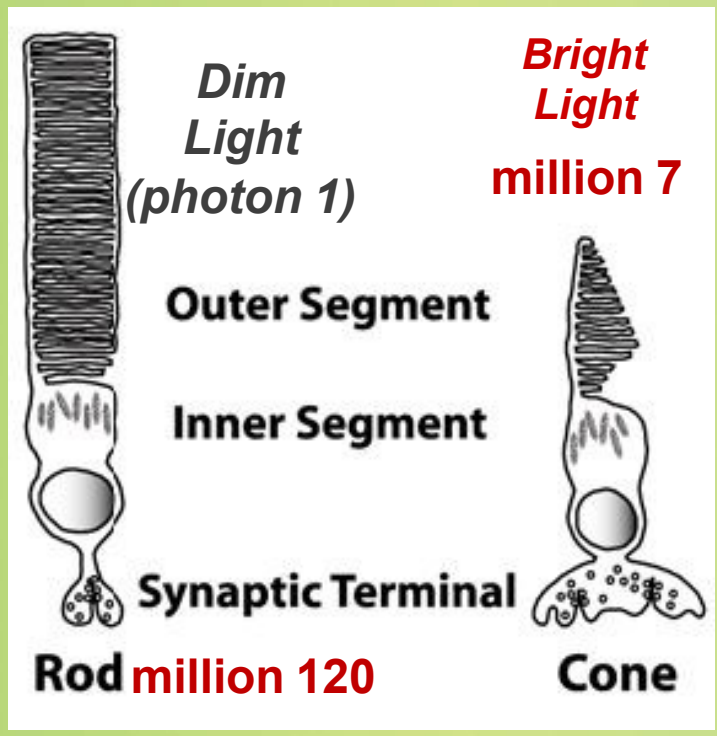
The picture can't be displayed.

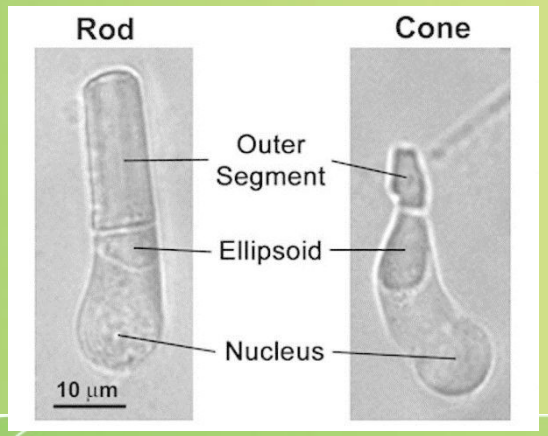
There is a large spectrum of wave length, we only see a small fraction of this spectrum, we only see the colors shown below.

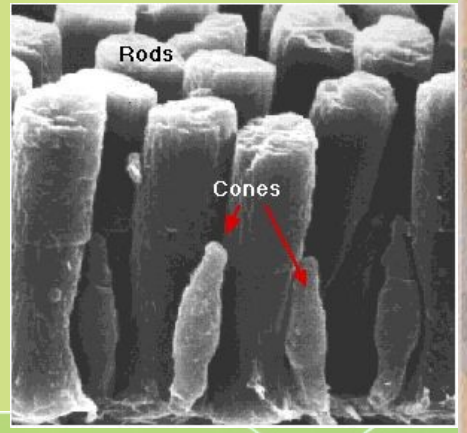


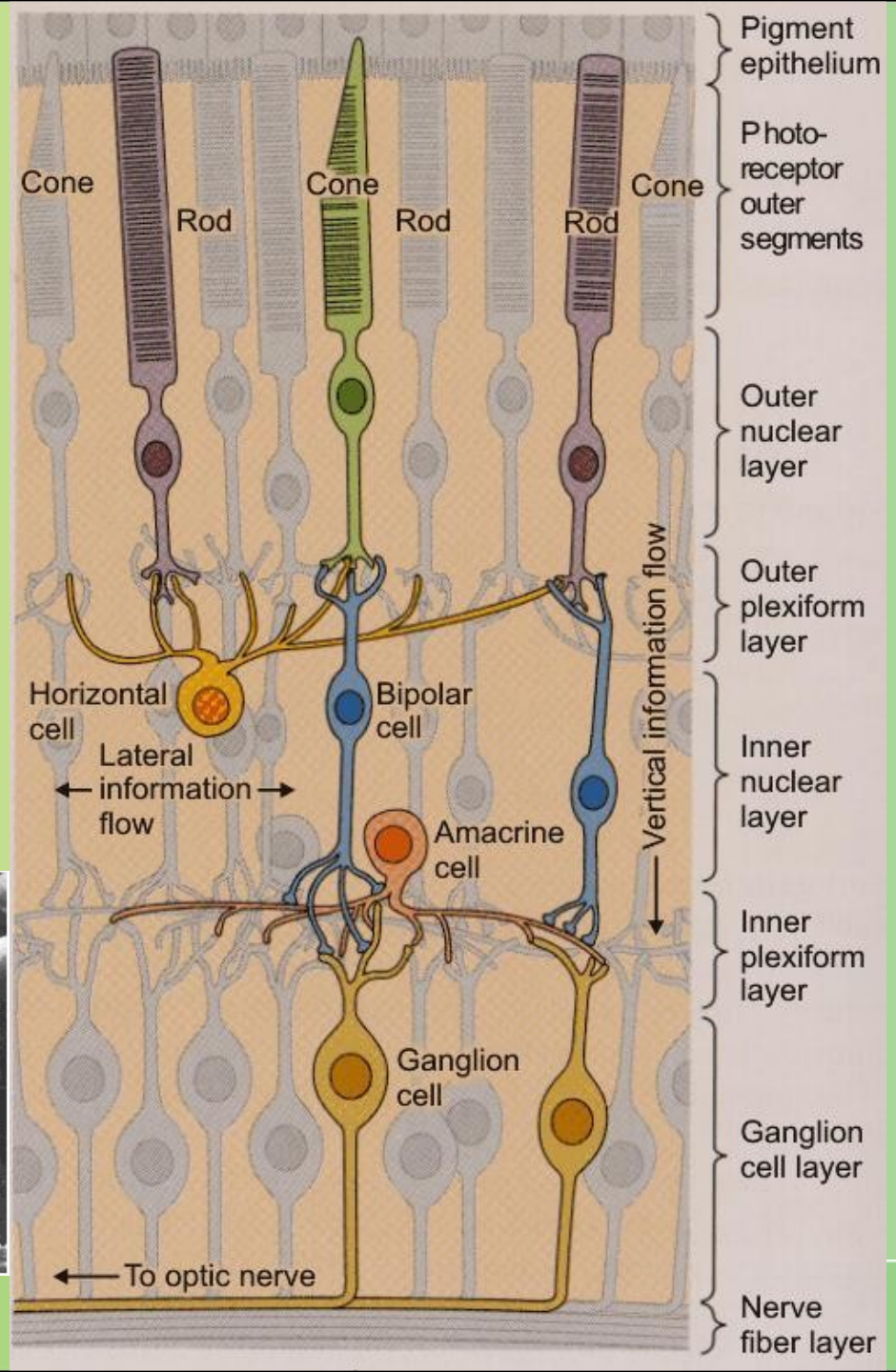

There are animals and insects that can see in UV light and some can see in the infra-red range as well

# Rods and cones




The picture can't be displayed.

There are two types of cells that are responsible for vision, and these are known as rod and cone cells.
They are imbedded between each other.
Rod cells are responsible for vision in dim light and amazingly they can absorb as little as one photon.
Cone cells are responsible for color vision in bright light.

If you look at these cells and how they are organized, they can be divided into three section; we have the outer segment, the inner segment and then we have the synaptic terminal, which is where the signal is transmitted to the nerves.
The outer segment is made up of a stack of membranes and this is the part of the cell where you have absorption of light, in the inner segment we have the synthesis of different proteins and where the nucleus is

Cone and rod cells are embedded and connected to nerves and the signals are transmitted from these cells to the nerves and then all the way to the brain, where we have processing of the image.
There are other cells that participate in vision such as the amacrine cells and the ganglion cells.

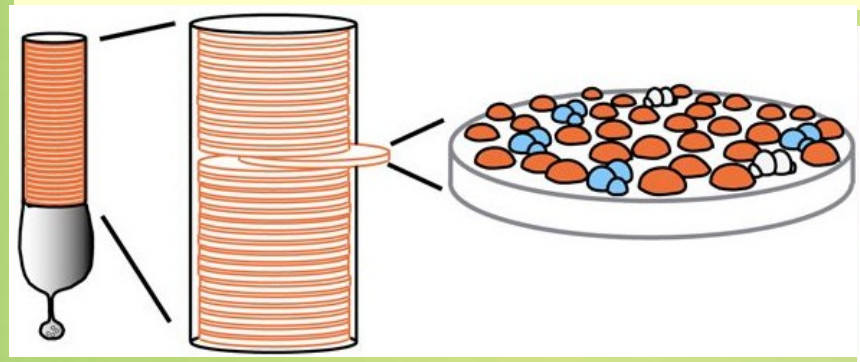
One important difference between rod and cone cells is that there is a lot more rod cells than cone cells; we have around 120 million rod cells compared to 7 million cone cells.

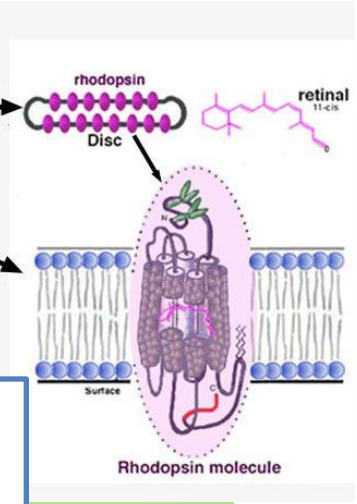
The picture can't be displayed.

# More on rod cells

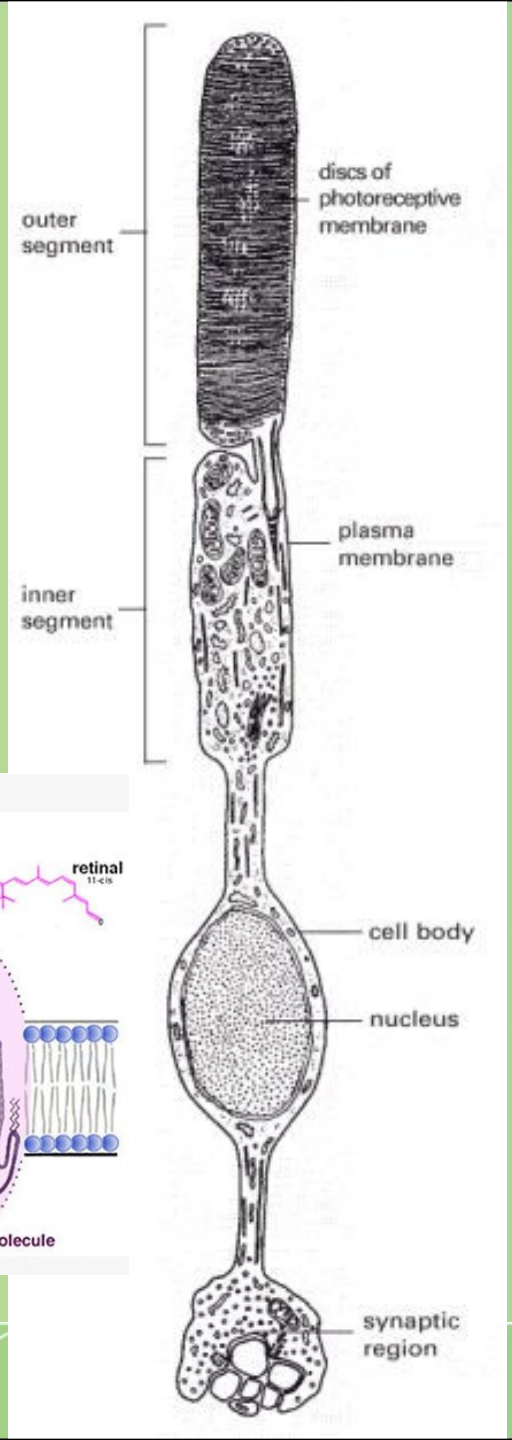
1. Rod cells consist of four regions: The inner segment, the cell body (contains the cellular organelles and is where protein synthesis occurs), and the synaptic region.
2. The outer segment contains the biochemical machinery needed for visual transduction.

   The components of the phototransduction enzyme cascade are packed into stacks of membranous vesicles ("disks").






the stacks of membranes in the outer segment contain (they are packed with) the components of the visual transduction, the signaling molecules.
These are the different molecules that can absorb light, such as rhodopsin.



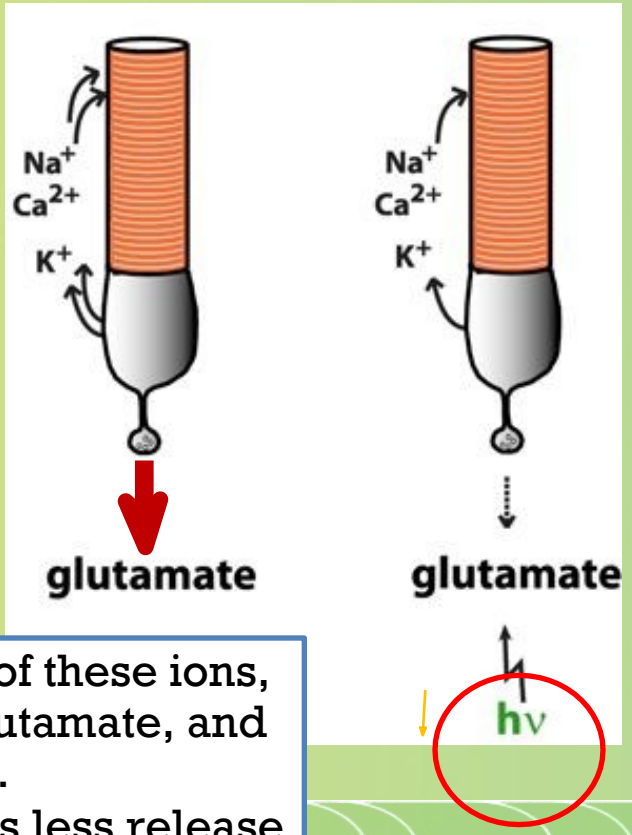

The picture can't be displayed.

# The dark current

The picture can't be displayed.

1. 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.
2. **Rods and cones work "backwards". At rest (in darkness), rods and cones are depolarized to -35 to -45 mV.**

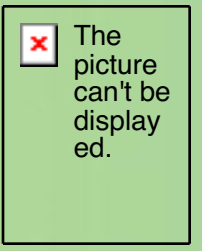
1. **At dark, $Na^+$ and, to a lesser amount, $Ca^{2+}$ enter through cyclic nucleotide-gated channels in the outer segment membrane.**
2. **$K^+$ is released through voltage-gated channels in the inner segment.**
3. **Rod cells are depolarized.**
4. **The neurotransmitter glutamate is released continuously.**




**When excited:**

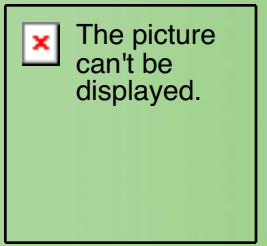
1. **channels in the outer segment membrane close**
2. **2. rod cells hyperpolarize, and**
3. **Glutamate release decreases.**

In the dark when we have entry of these ions, we have the release of a lot of glutamate, and this is known as the dark current.
When the channels close, there is less release of glutamate and this is the signal.




# *Generation of vision signals*

# The players

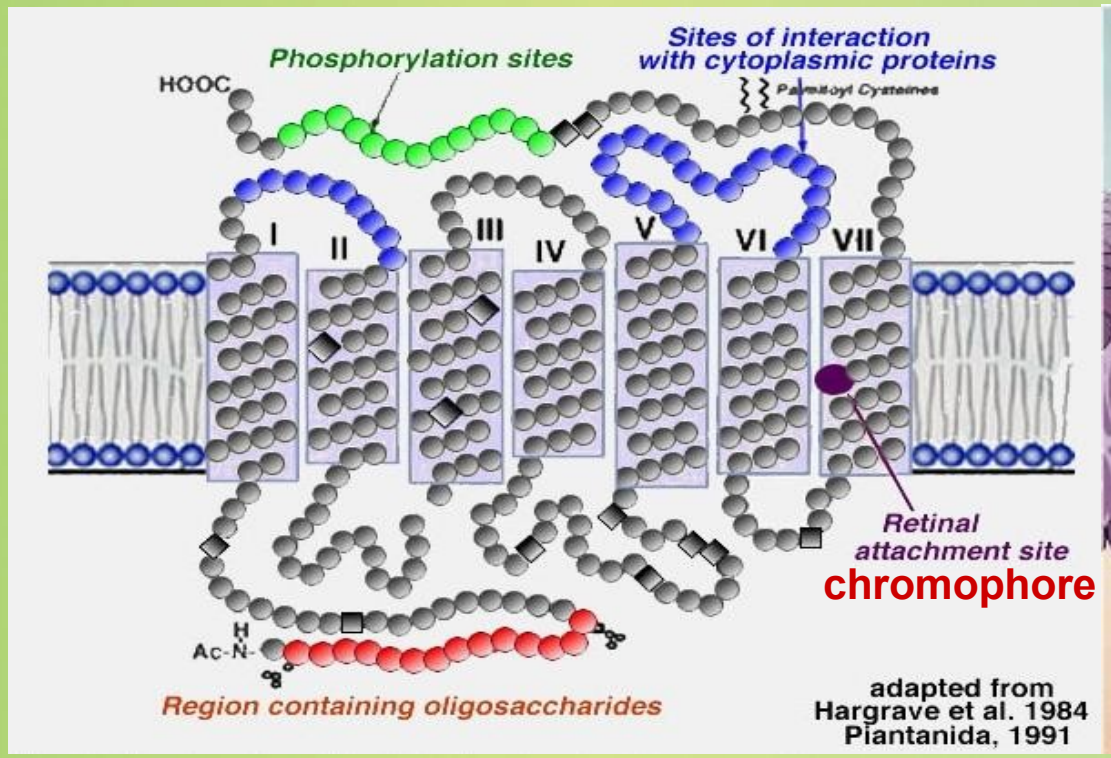


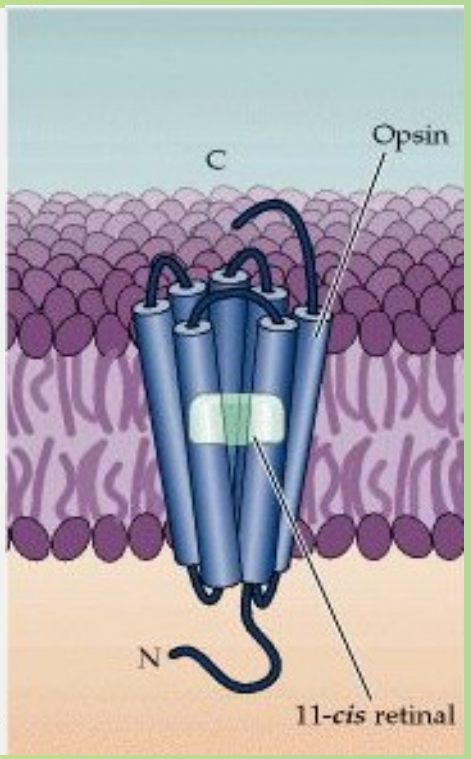

- Rhodopsin (opsin + pigment molecule), the receptor that absorbs light, and it is a holoprotein composed of a protein which is opsin and a chromophore (pigment) that gets excited.
- Transducin G protein
- Phosphodiesterase, which amplifies the signal
- $Na^+$-gated channels
- Regulatory proteins

# Rhodopsin

The picture can't be displayed.

**Opsin is a single polypeptide chain with seven helical segments that span the membrane.**

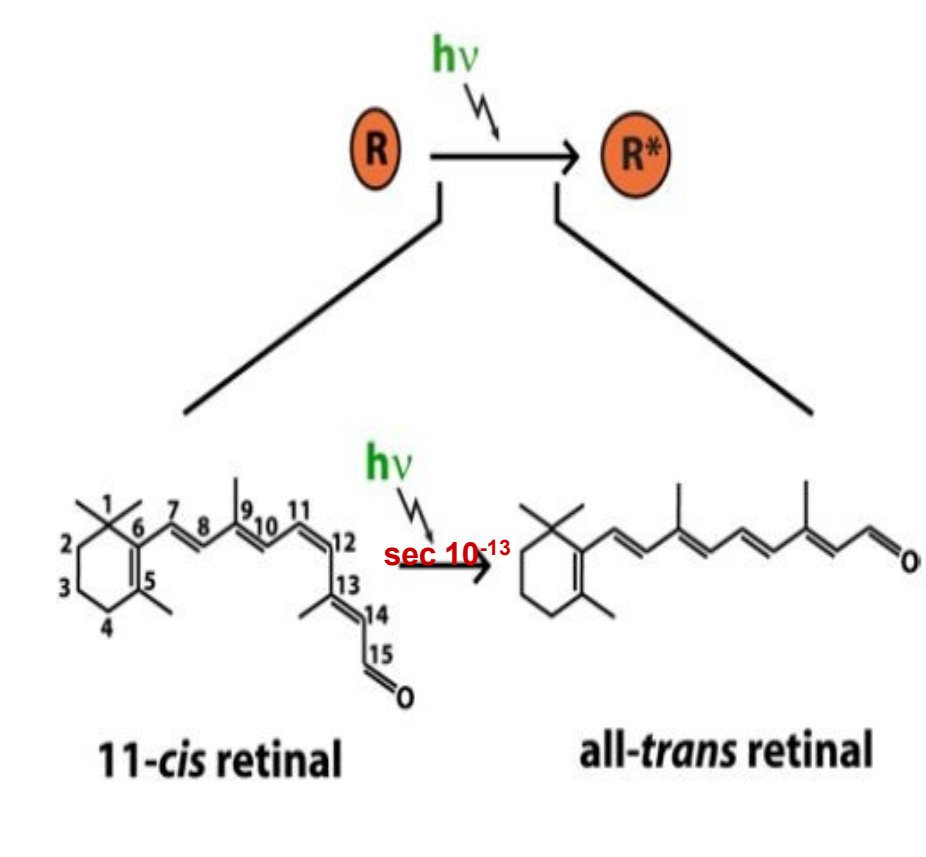



Seven transmembrane domain proteins are the second largest type of membrane proteins. In the very last transmembrane domain we have the attachment of the chromophore. In this protein , there are different sequences that are important for regulation and function.
In the cytosolic region of the protein , the region containing oligosaccharides is important for transmitting the signal

# The chromophore (11-cis-retinal)

The picture can't be displayed.



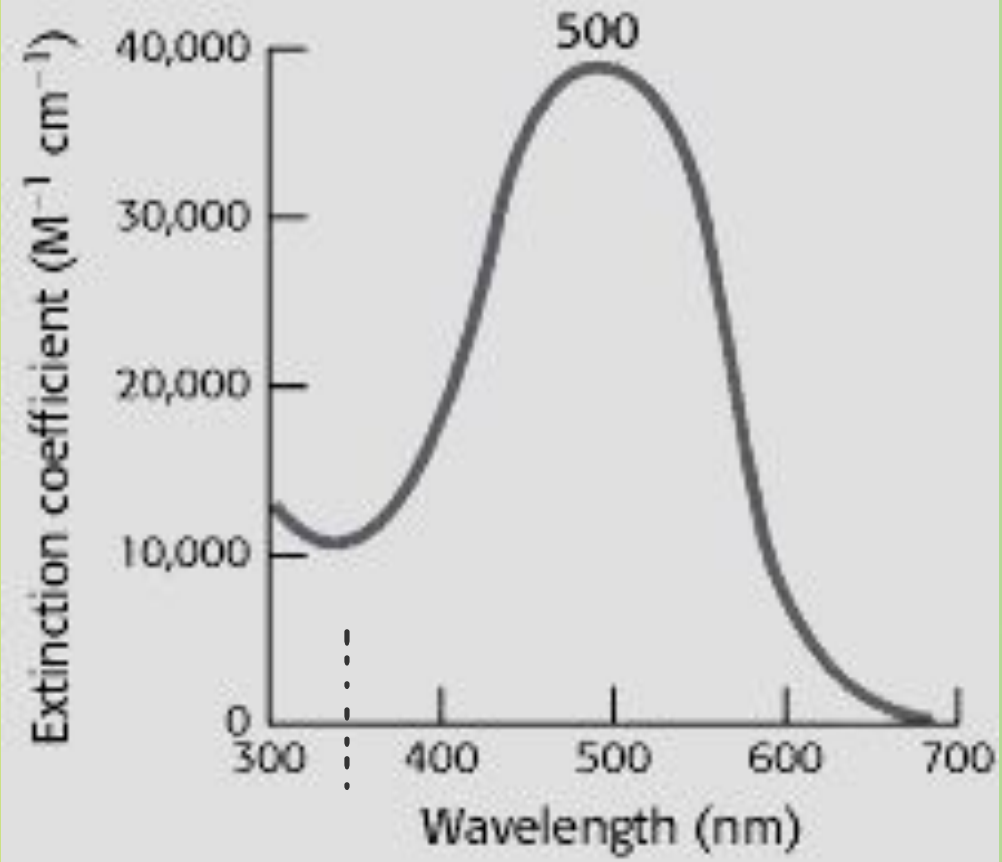

It is derived from vitamin A, and that is why vitamin A is important for vision, and vitamin A is derived from carotene (carrots).

11- cis retinal is kinked, the electrons in the cis bond get excited when they are hit by light, and this causes the conformation of the whole molecule to change from a cis conformation into a trans conformation.
This little change in the structure of the molecule causes the transmission of the signal and rhodopsin becomes excited (activated) and it is labelled with a star

The chemical change in the conformation of 11- cis retinal is very fast, it occurs in about 100 femtoseconds (=10^-13).
The idea here is that this change is really quick.

# Light absorption by rhodopsin

The picture can't be displayed.

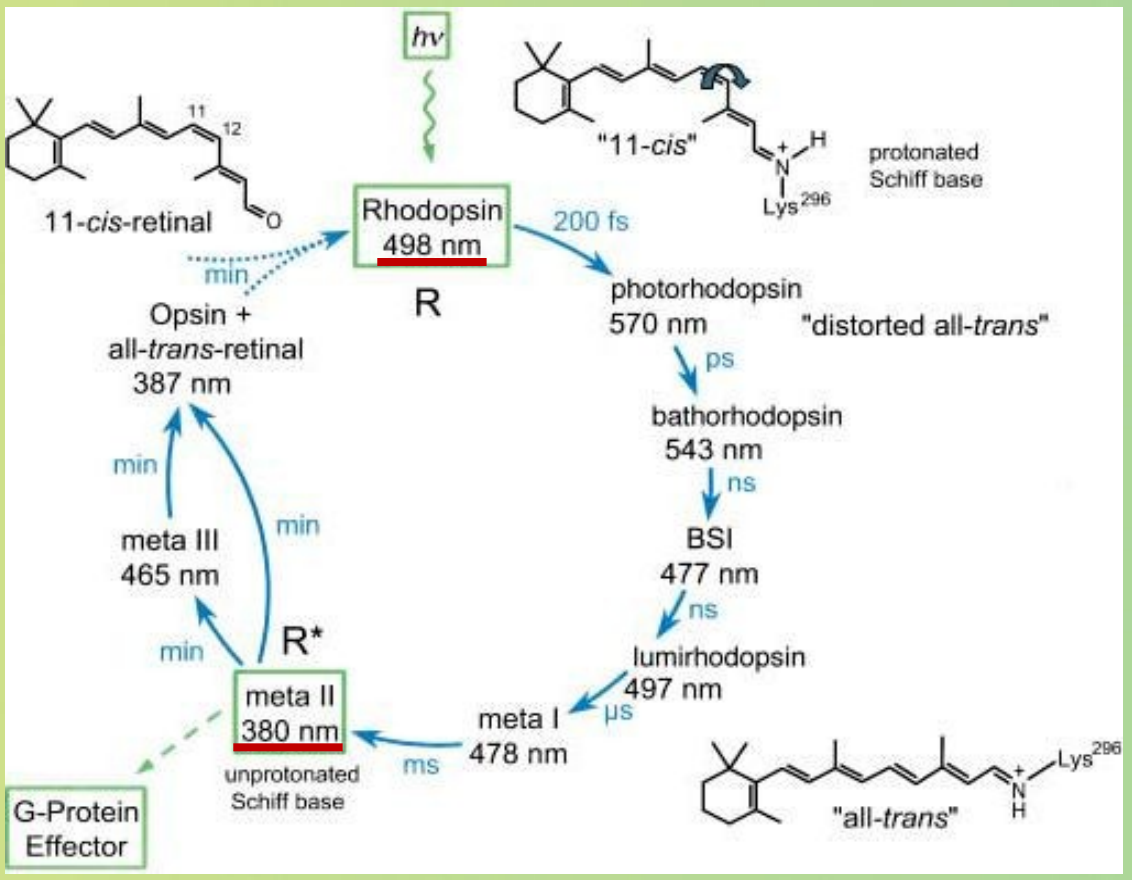



When rhodopsin is activated, it is able to absorb light at a wide range.
The maximum absorption is at 500nm.

# Rhodopsin intermediates

The picture can't be displayed.

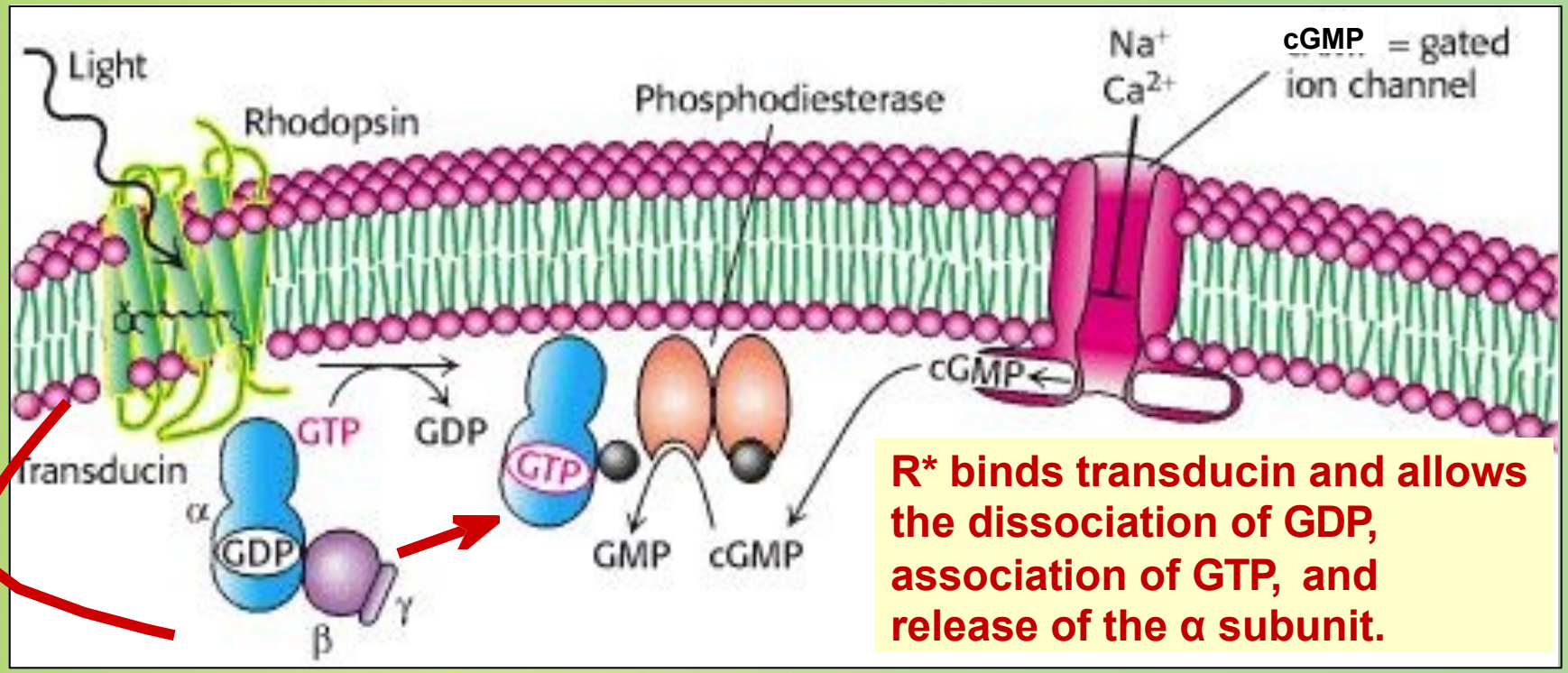
- By itself, 11-cis retinal absorbs near UV light. But opsin perturbs the distribution of the electrons exciting its electrons with less energy (i.e., longer wavelength light).
- The chromophore converts the energy of a photon into a conformational change in protein structure.
- Rearrangements in the surrounding opsin protein convert it into the active R* state, an intermediate known as metarhodopsin II.



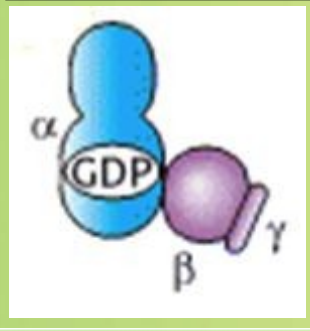

What happens when this protein is activated, it undergoes different confirmation. The whole protein does not only the chromophore.
It has different conformations and each conformation can absorb light at a different wavelength than another one, in fact it *does* change colors.
Eventually you have this activated form known as the meta rhodopsin II; this is the form of rhodopsin that can transmit the signal to the G protein.

# Transducin → Phosphodiesterase (PDE)

The picture can't be displayed.

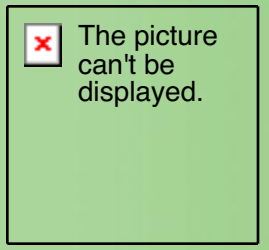



R* binds transducin and allows the dissociation of GDP, association of GTP, and release of the α subunit.




G proteins are heterotrimeric, consisting of α, β, and γ subunits. In its inactive state, transducin's α subunit has a GDP bound to it.

After this we have the activation of transducing, which as we mentioned before is a G protein.
G proteins are composed of three polypeptides, three subunits, and these are known as the alpha, beta, and gamma subunits.
The beta and gamma subunits are inhibitory of alpha subunits, when alpha is bound to GDP it is inactive.



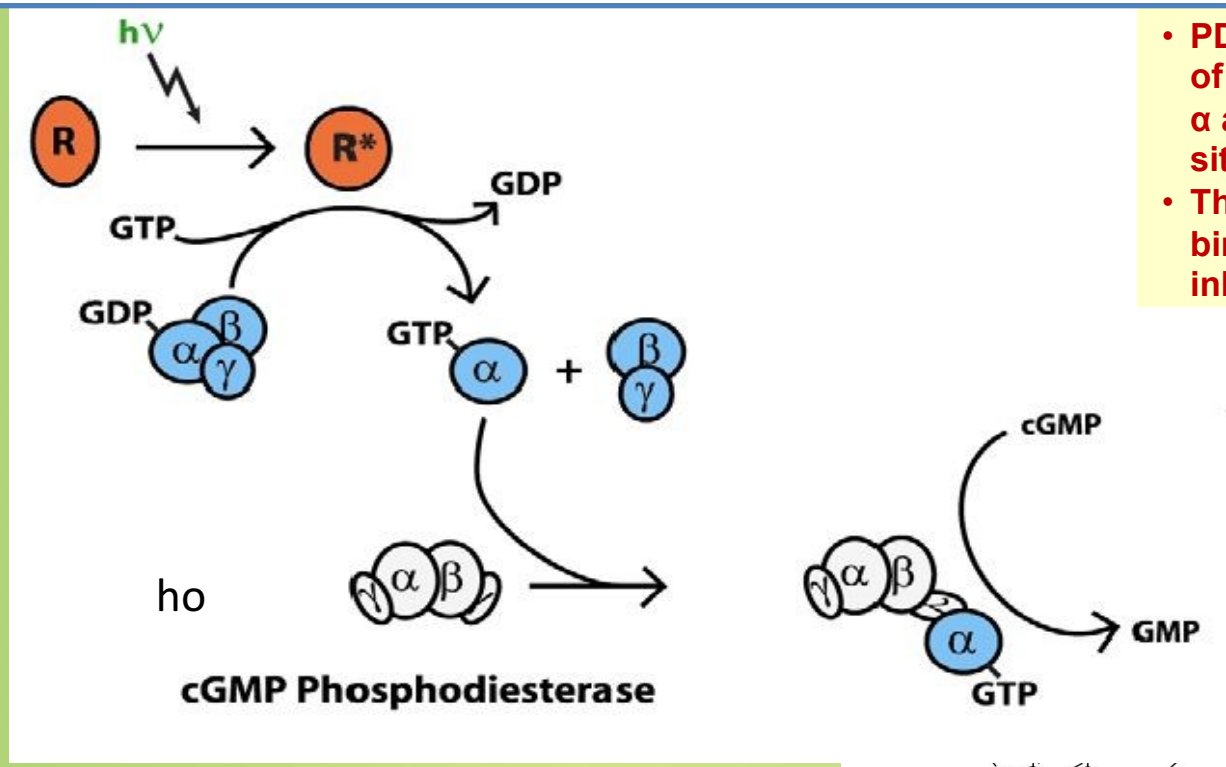

What happens when rhodopsin gets activated, it interacts with the G protein and allows for the displacement of GDP, and it is replaced by GTP which binds to the alpha subunit, this leads to the release of the beta-gamma subunit.
Then the activated alpha subunit interacts with phosphodiesterase which converts cGMP to GMP. That reduces the amount of cGMP in the cytosol.
cGMP is important because it binds to cGMP gated channels and when it binds to them, it keeps them open, but when you have reduction of the level of cGMP in the cytosol, you have release and less of cGMP bound to these channels and so they close.

These channels are responsible for the entry of sodium ions as well as some calcium ions, so less are entering the cells and that results in decrease of the release of the glutamate, which is the neurotransmitter

# Activation of phosphodiesterase

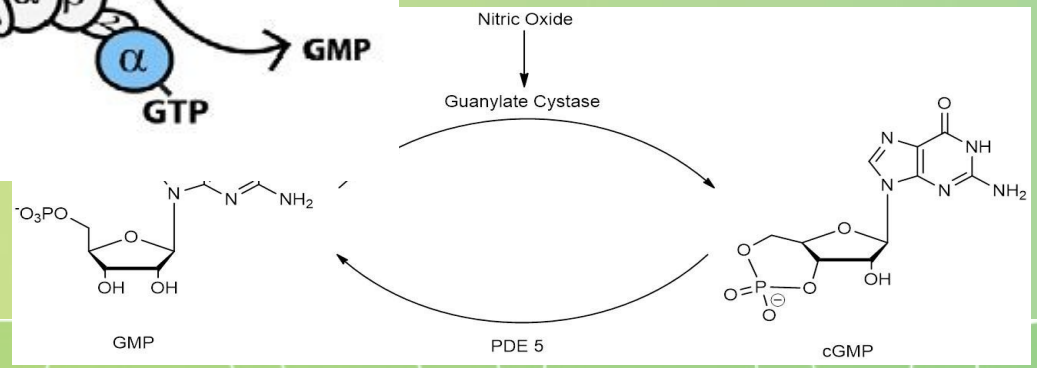
The picture can't be displayed.

**So what happens is that when G-alpha is bound to GTP and is released from the inhibitory beta-gamma subunit, it binds to the cGMP phosphodiesterase enzyme activating it and now the PDE can hydrolyze the CGMP converting it to GMP.**
**GTP is converted to cGMP by guanalyl cyclase and cGMP can be bound to kinases which then cause a cellular effect, cGMP also binds to PDE converting cGMP into GMP and cancelling the effect of the kinases.**
**The interaction between the alpha subunit and the PDE is at a ratio of 1:1.**



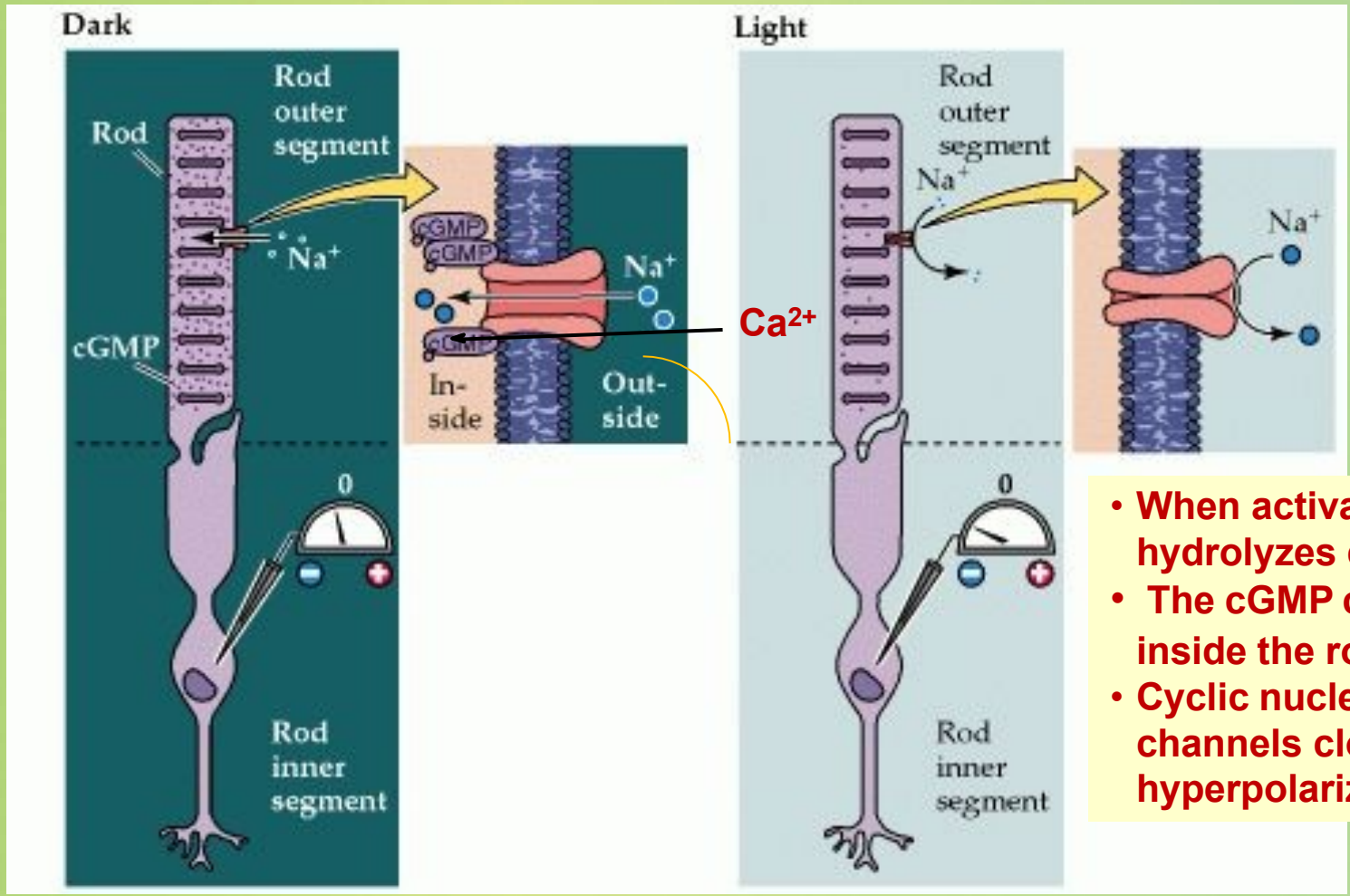

- PDE is a heterotetramer that consists of a dimer of two catalytic subunits, α and β subunits, each with an active site inhibited by a PDE γ subunit.
- The activated transducin α subunit-GTP binds to PDE γ and relieves the inhibition on a catalytic subunit.



# cGMP-gated channels

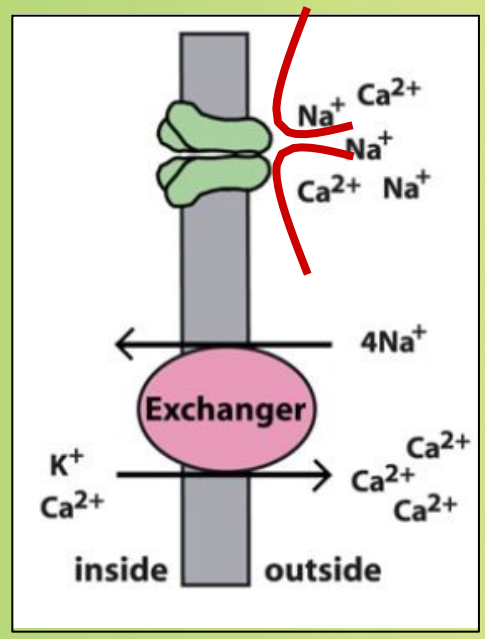
The picture can't be displayed.




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

# Levels of calcium ions are reduced, too.

The picture can't be displayed.



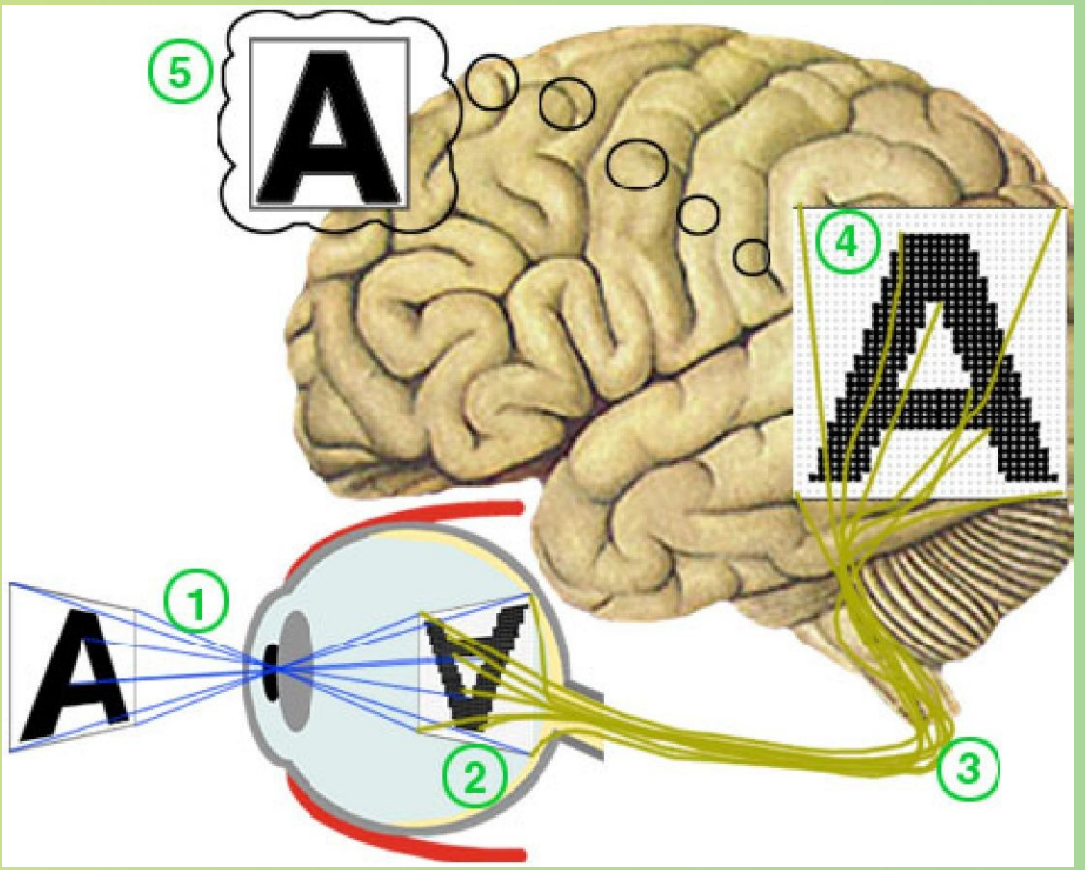

**When the channels close, $Ca^{2+}$ ceases to enter, but extrusion through the exchanger continues, so intracellular [$Ca^{2+}$] falls.**


BUT at the same time, you have this efflux, this transport of calcium ions outside of cells by an exchanger, which is the Na+-Ca+2 exchanger, which transports calcium ions outside of the cell in exchange for sodium ions to the inside.
This leads to the decrease in the intracellular calcium even further

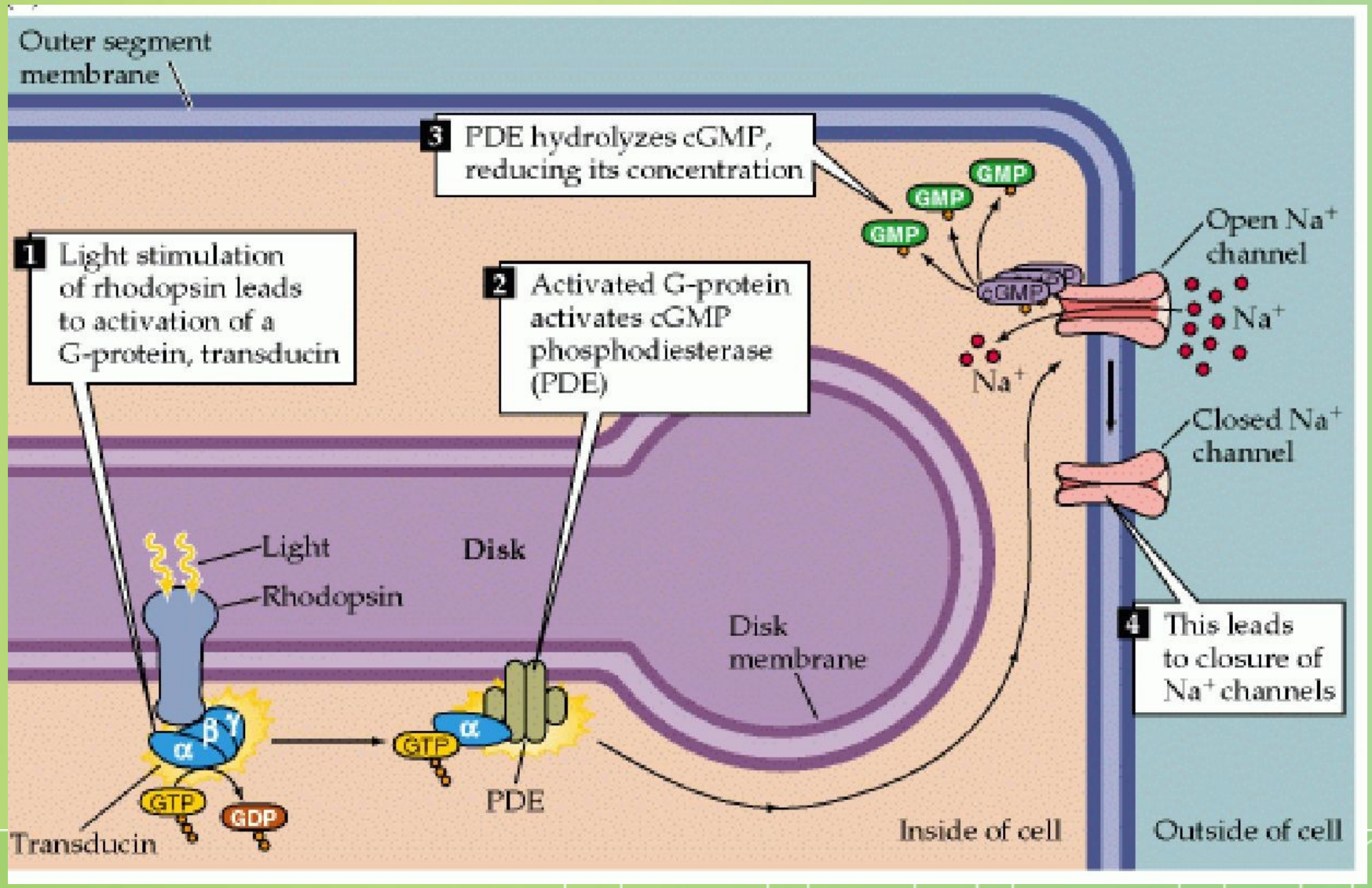
# Creating an image

The picture can't be displayed.

- 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.



The picture can't be displayed.



The picture can't be displayed.

# *Signal amplification*

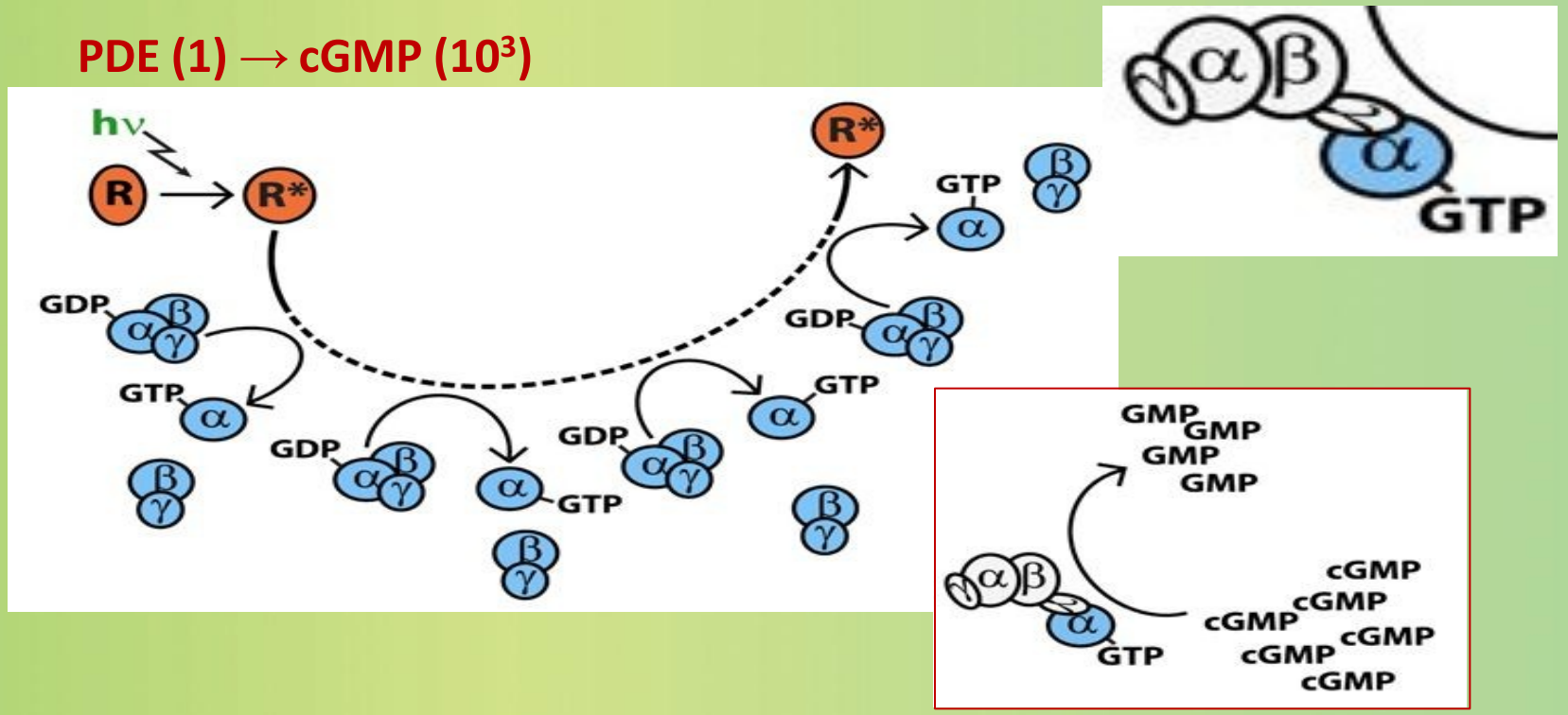
One photon is very little in terms of  rod cells, it doesn't really activate that many rod cells, so the signal must be amplified so that we can see.

The picture can't be displayed.

**Rhodopsin (1) → Transducin (10 to >3000)**

**Transducin (1) → PDE (1)**

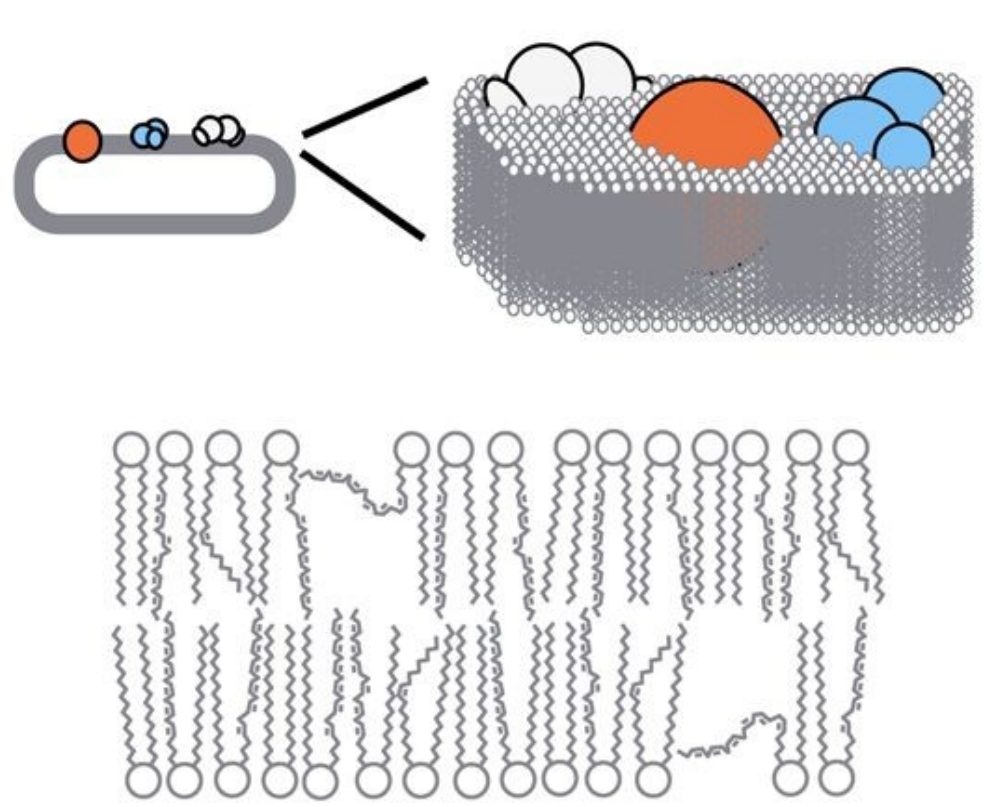
**PDE (1) → cGMP ($10^3$)**




Since there are many experiments done on many systems (mice, human, etc.) there is a wide range of how many transducing molecules one rhodopsin activates but the doctor said that he would say, it is an average of 500 transducing molecules.
There is no signal amplification at the level of PDE, but then one PDE molecule forms one thousand cGMP molecules which would then effect that many ion channels.

# Facilitation of transduction

The picture can't be displayed.



1. 2-dimensional surface
2. Low in cholesterol and high content of unsaturated fatty acids
3. Cooperativity of binding: The binding of one cGMP enhances additional cGMP binding and channel opening (n = ~3)
4. 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 to 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.***

One very important component is the membrane of the cells themselves.
Notice that all of these components are membrane bound.
The membrane of rod cells I very viscous and this means its flexible and it is easy for the proteins to move in a 2D space.

The picture can't be displayed.

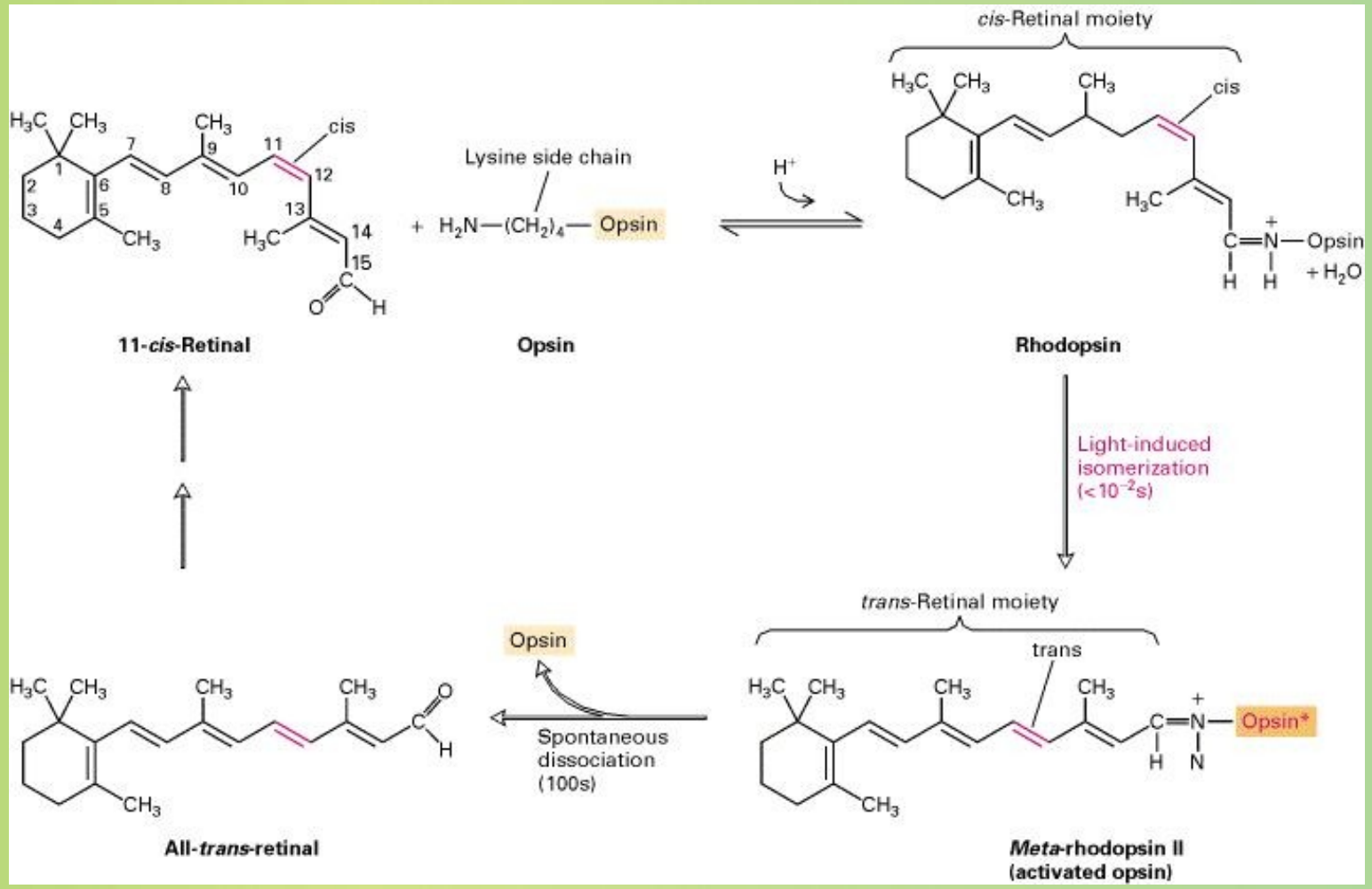
The way we see is not interrupted images, rather we see continuous images, and what facilitates this is that we have signal termination at the same time of activation, so that we have a renewal of images, regeneration of the signal.

# *Signal termination*

# Mechanism I

## Unstable all-trans rhodopsin complex

The picture can't be displayed.



There are different mechanisms of termination at different levels; rhodopsin, transducin, the channels, the PDE, all the players are regulated.
They are not in order.

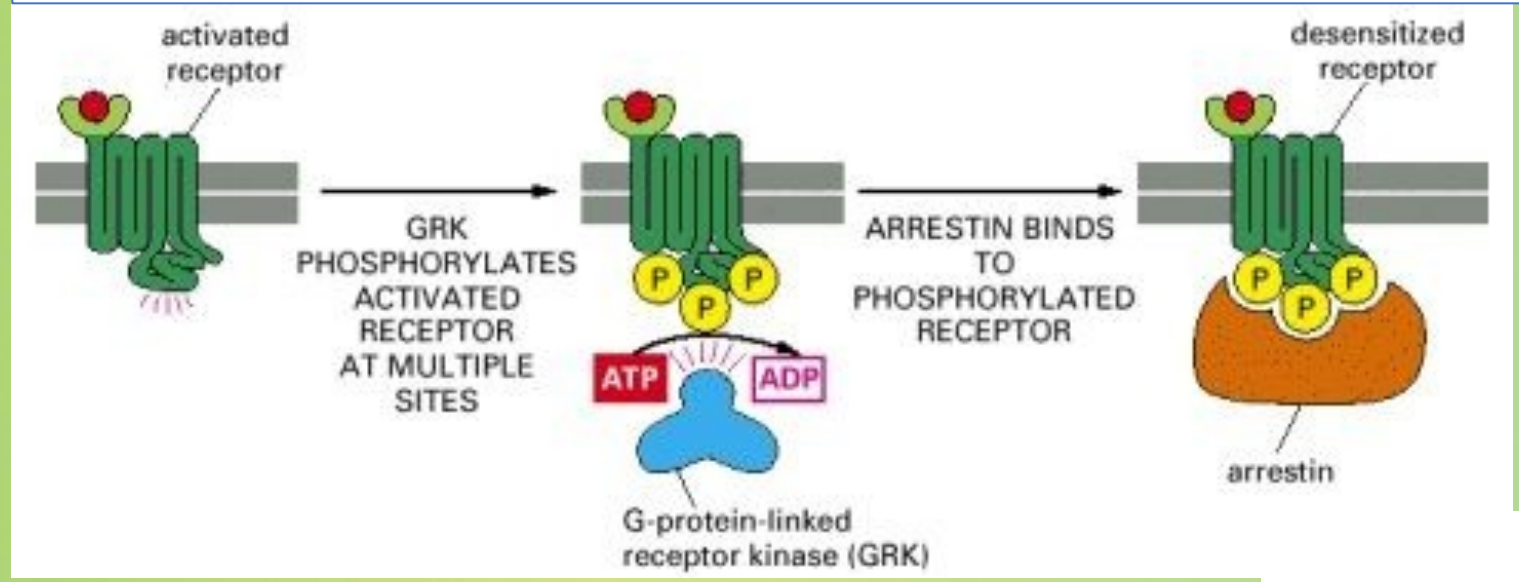
This mechanism is that when we have the 11- cis retinal changing to the all trans molecule, this causes a change in the structure of rhodopsin into meta rhodopsin and the interaction between the trans retinal molecule to opsin becomes unstable.
This causes the release if the all trans molecule and the rhodopsin becomes opsin and it goes back to its inactive conformation and cannot activate transducins anymore.

The all trans retinal molecule can be converted back into 11-cis retinal and can bind to opsin once again to regenerate the signal.

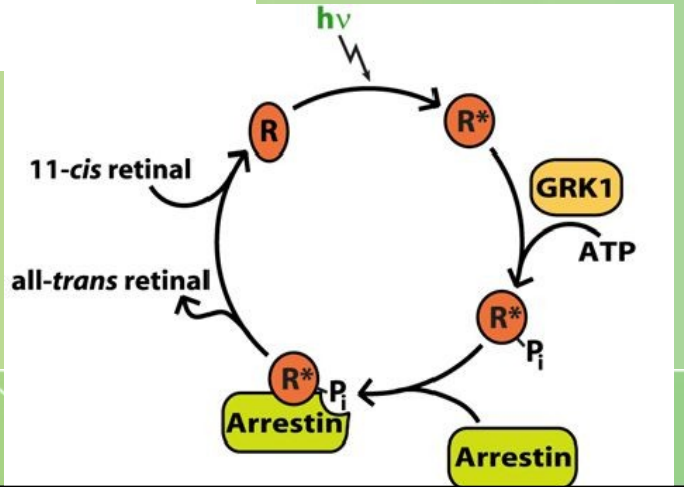
# Mechanism II Arrestin binding

The picture can't be displayed.

rhodopsin activated >> activation of the kinase (phosphorylates rhodopsin) >> reduces the activity of the rhodopsin >> interacting the molecule with arrestin >> preventing the interaction between rhodopsin and transducin >> termination of the signal



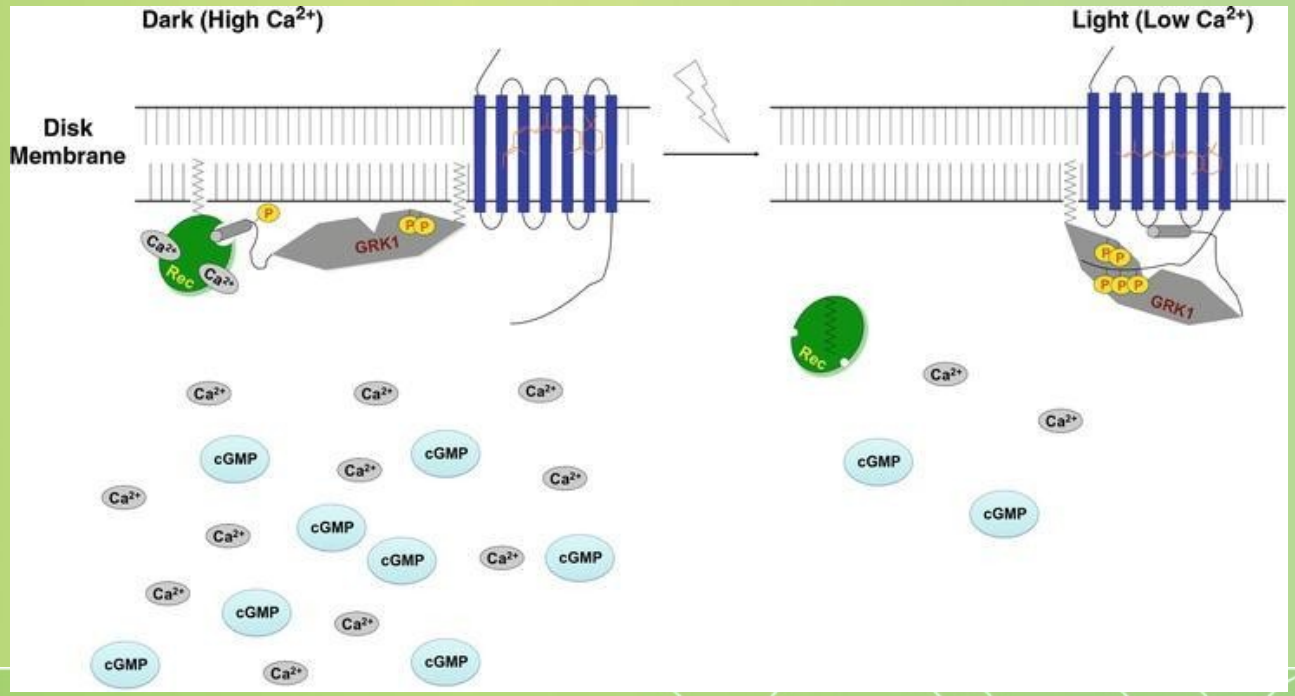

- **Rhodopsin kinase 1 (GRK1) phosphorylates the C-terminus of R*.**
- **Phosphorylation of R* has two effects:**
- **1. It decreases transducin activation**
- **2. It facilitates binding to arrestin, which completely quenches its activity, and releases of the all *trans-retinal* regenerating rhodopsin.**



# Mechanism II (cont.)

## GRK1 and recoverin

- GRK1 is more active at low [$Ca^{2+}$]. Why?
- In the dark, $Ca^{2+}$ ions bind to a protein called recoverin allowing it to anchor to the membrane, bind to GRK1, and inhibit it.
- In contrast to $Ca^{2+}$-free recoverin that does not bind to GRK1, the recoverin-$Ca^{2+}$ complex binds to the N terminus helix of GRK1,



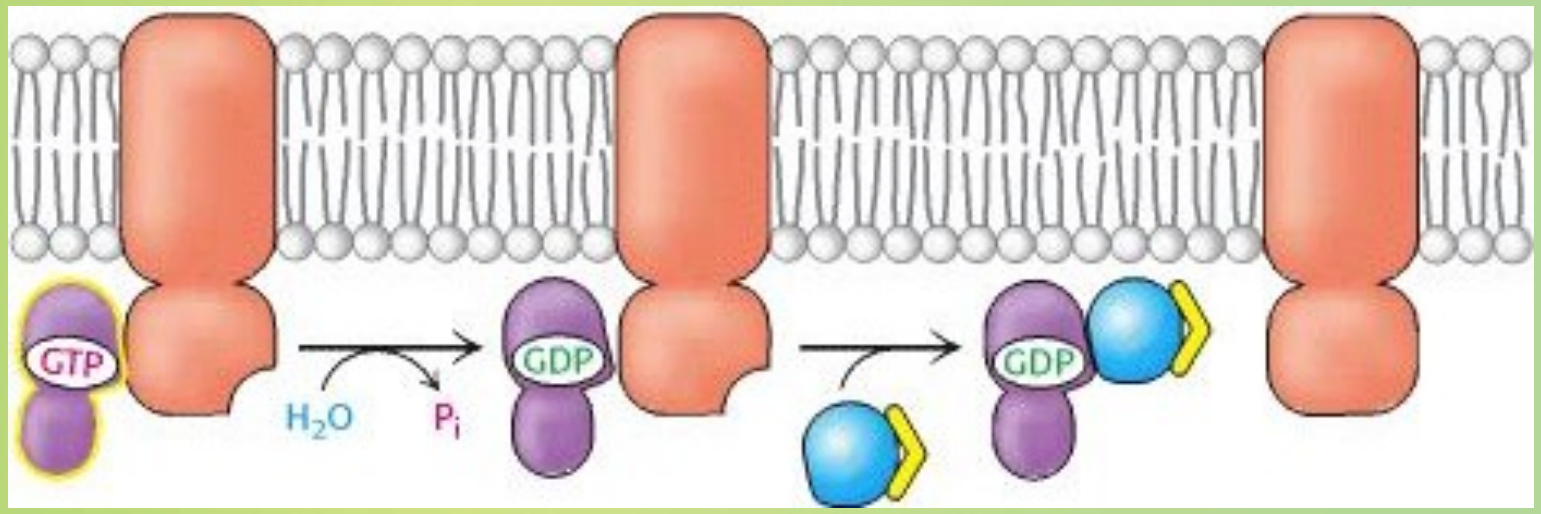

- *$Ca^{2+}$-Calmodulin (CaM) also binds to GRK1 and inhibits it.*

# Mechanism III

## Intrinsic GTPase activity of G protein

The picture can't be displayed.

G protein is active when bound to GTP, when GTP converts to GDP this inactivates tranducin



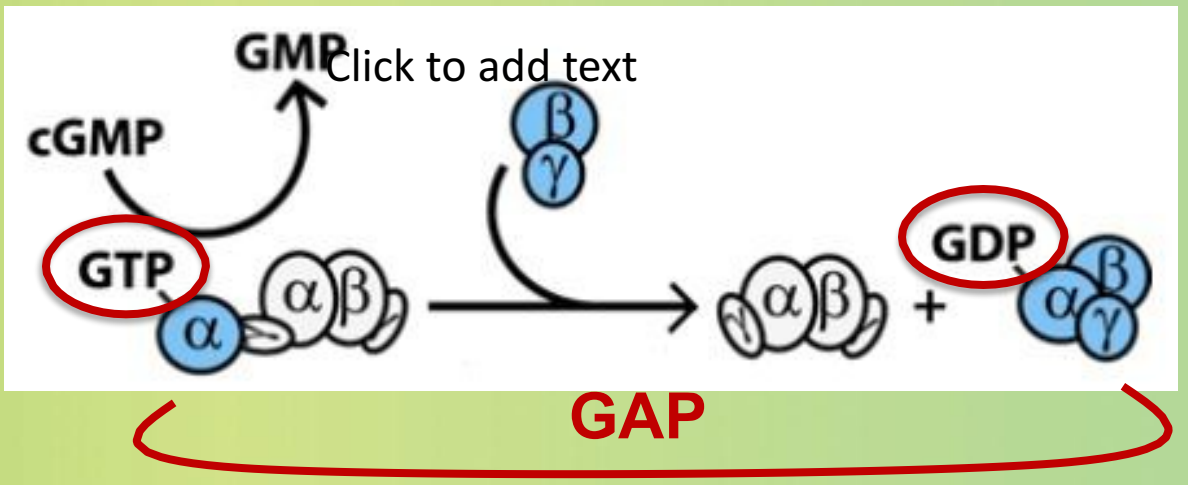

- Transducin has an intrinsic GTPase activity that hydrolyzes GTP to GDP.
- Upon hydrolysis of GTP to GDP, transducin α subunit releases the PDE γ subunit that re-inhibits the catalytic subunit.
- Transducin α-GDP eventually combines with transducin βγ

# Mechanism IV

## Facilitation of GTPase activity of G protein

The picture can't be displayed.

- **GTP hydrolysis is slow intrinsically, but it is accelerated by 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, until transducin α-GTP binds PDEγ.**



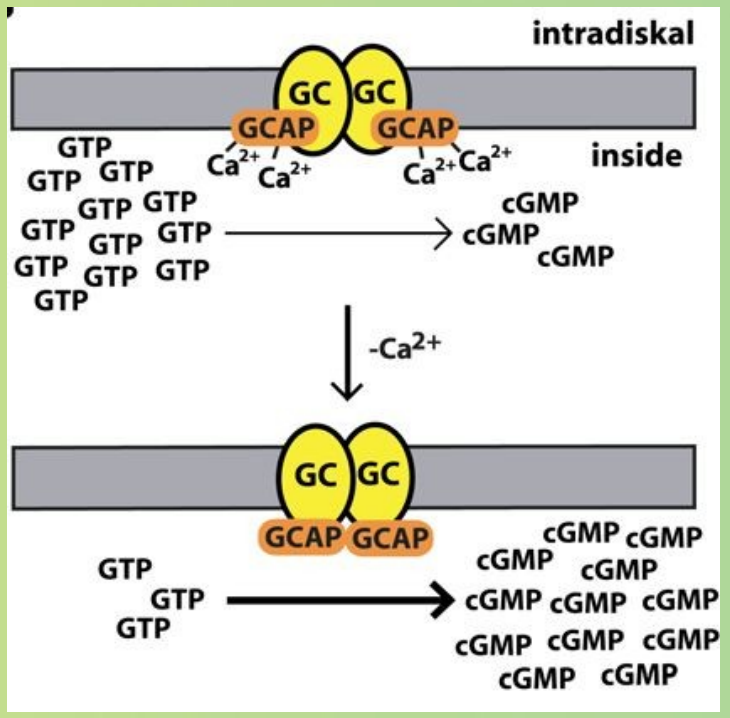

**The inhibition of the Gα subunit by GTP hydrolysis and, hence, dissociation from PDE is the rate limiting step in the recovery of rod response to light.**

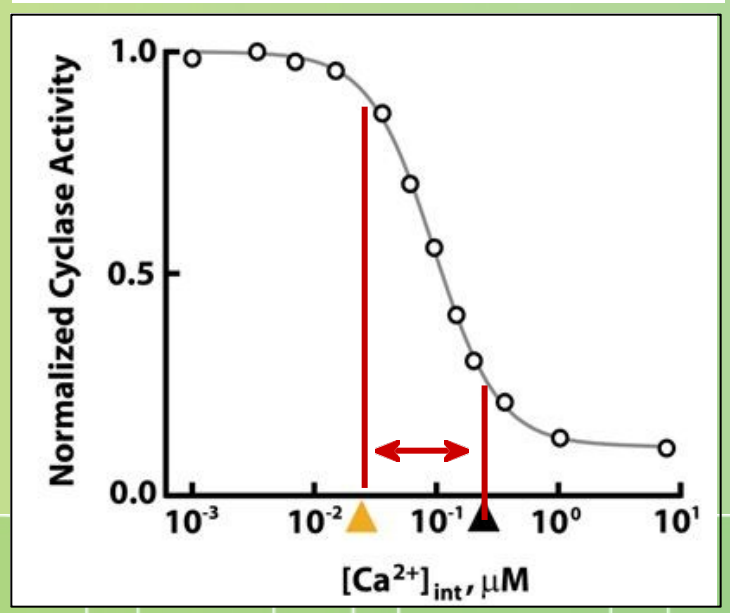
# Mechanism V

## Guanylate cyclase

- In the dark, guanylate cyclase-activating proteins (GCAPs) bind $Ca^{2+}$ blocking their activation of guanylate cyclase.
- A decrease in intracellular [$Ca^{2+}$] causes $Ca^{2+}$ to dissociate from GCAPs leading to full activation of guanylate cyclase subunits, and an increase in the rate of cGMP synthesis.

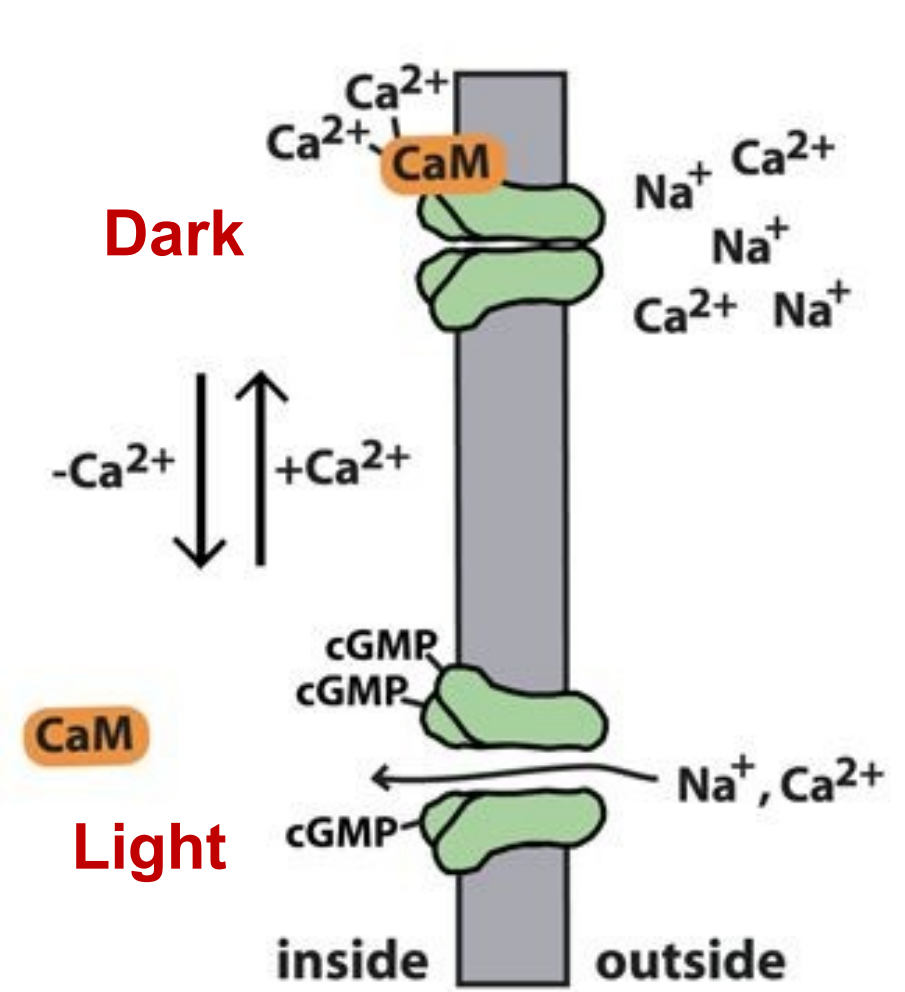
guanylate cyclase forms cGMP from GTP
so, its job is to keep the channels open when Ca++ goes down.



# Mechanism VI

## Ca-calmodulin *and cGMP-gated channels*




- In the dark, $Ca^{2+}$-Calmodulin (CaM) binds the channel and reduces its affinity to cGMP and shuts it down.
- During visual transduction, the decrease in intracellular [$Ca^{2+}$] causes CaM to be released, the affinity towards cGMP increases, and the channel reopens in response to the slightest increase to cGMP.

- In the dark calmodulin is bound to Ca++ ions and closes the channels as a feedback mechanism.
- Only 1% of the channels are open in the dark
- When Ca goes down, calmodulin is released, causes opening of the channels,
and Ca into cells to regenerate the signal

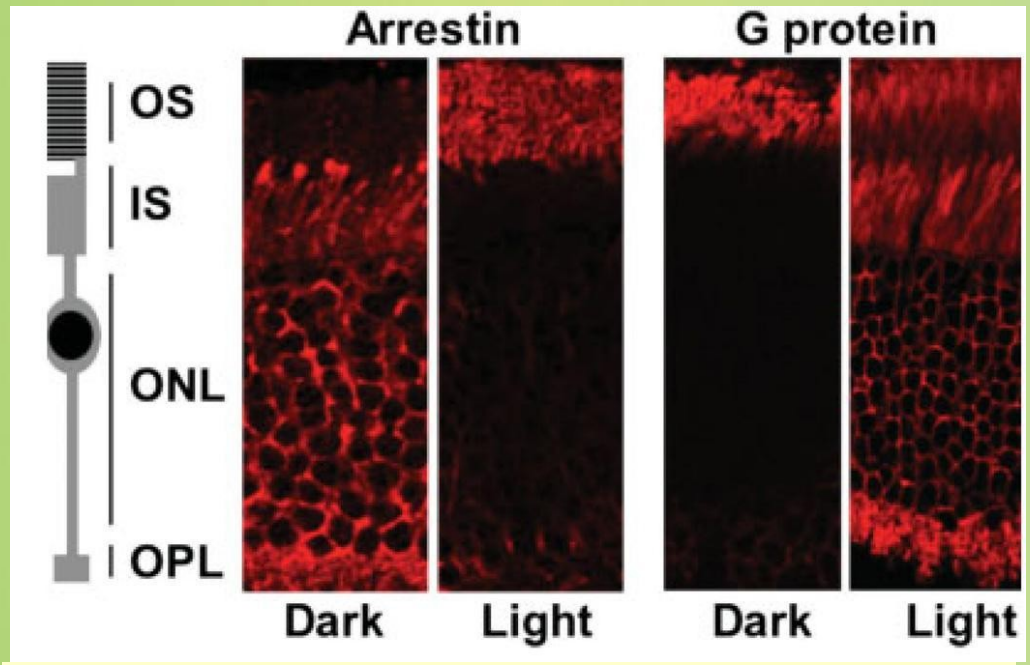
- ***Note: $Ca^{2+}$-Calmodulin (CaM) also binds to GRK1 and inhibits it.***

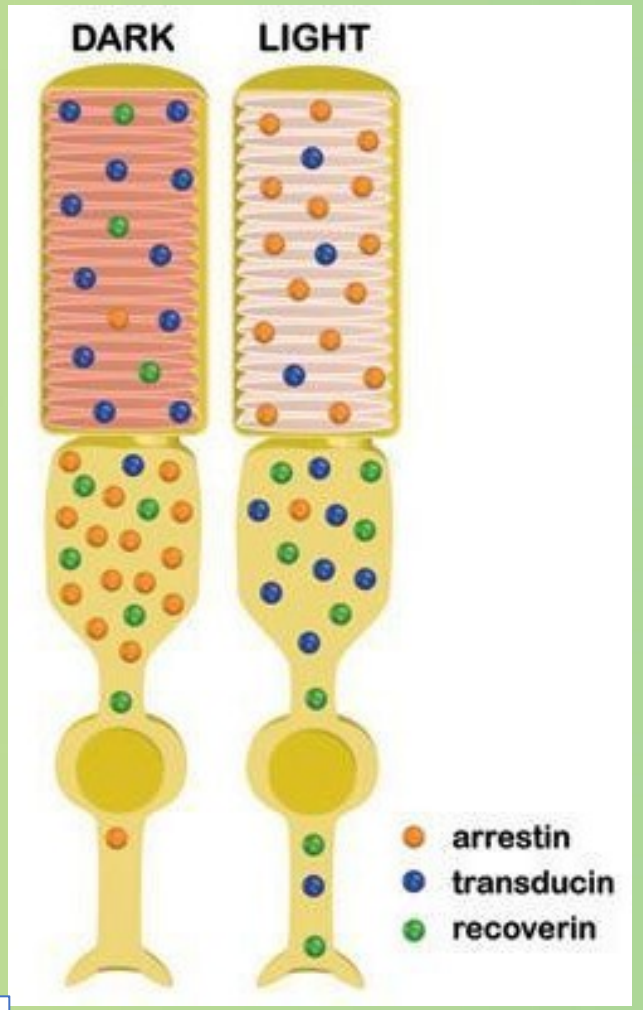
The picture can't be displayed.

# *Adaptation to light/dark conditions*

# Arrestin/recoverin/transducin distribution

The picture can't be displayed.




- In dark, the outer segment contains high levels of transducin and recoverin and low levels of arrestin (low inhibition; ready to be activated).
- In light, it is the opposite (high inhibition; ready to be inactivated).

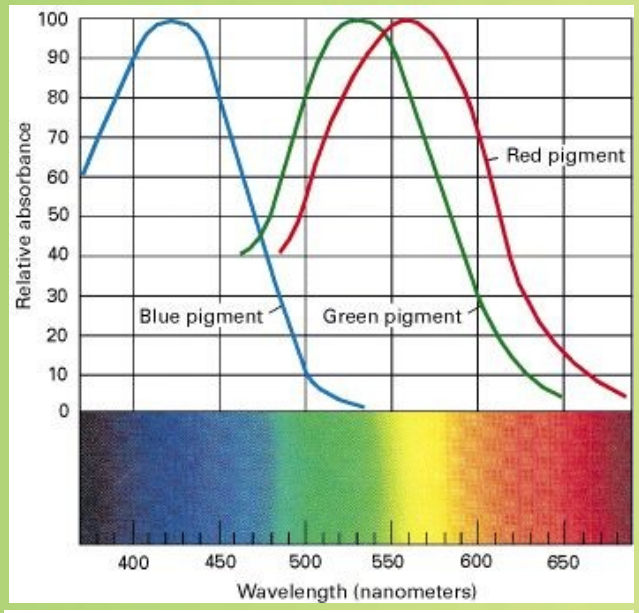
in the light, there's no need to have a very strong signal (high transducin) and vice versa.

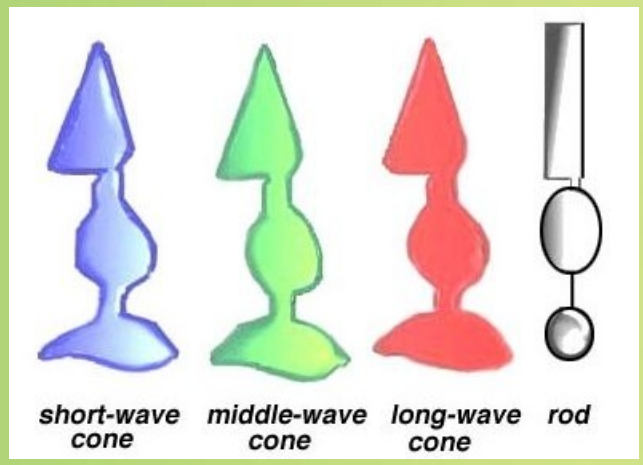
The picture can't be displayed.

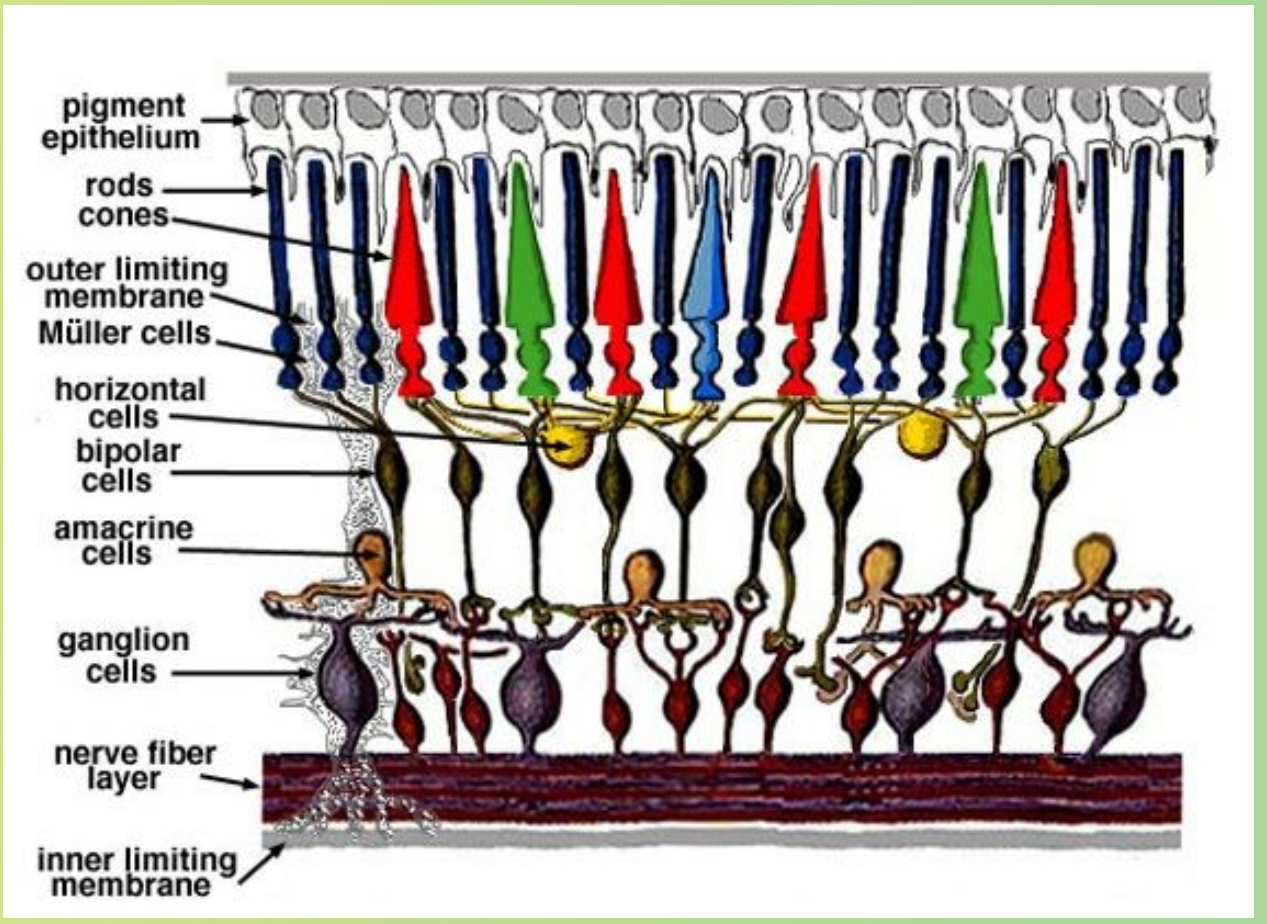
# Color vision

# Cone photoreceptor proteins

The picture can't be displayed.



The picture can't be displayed.

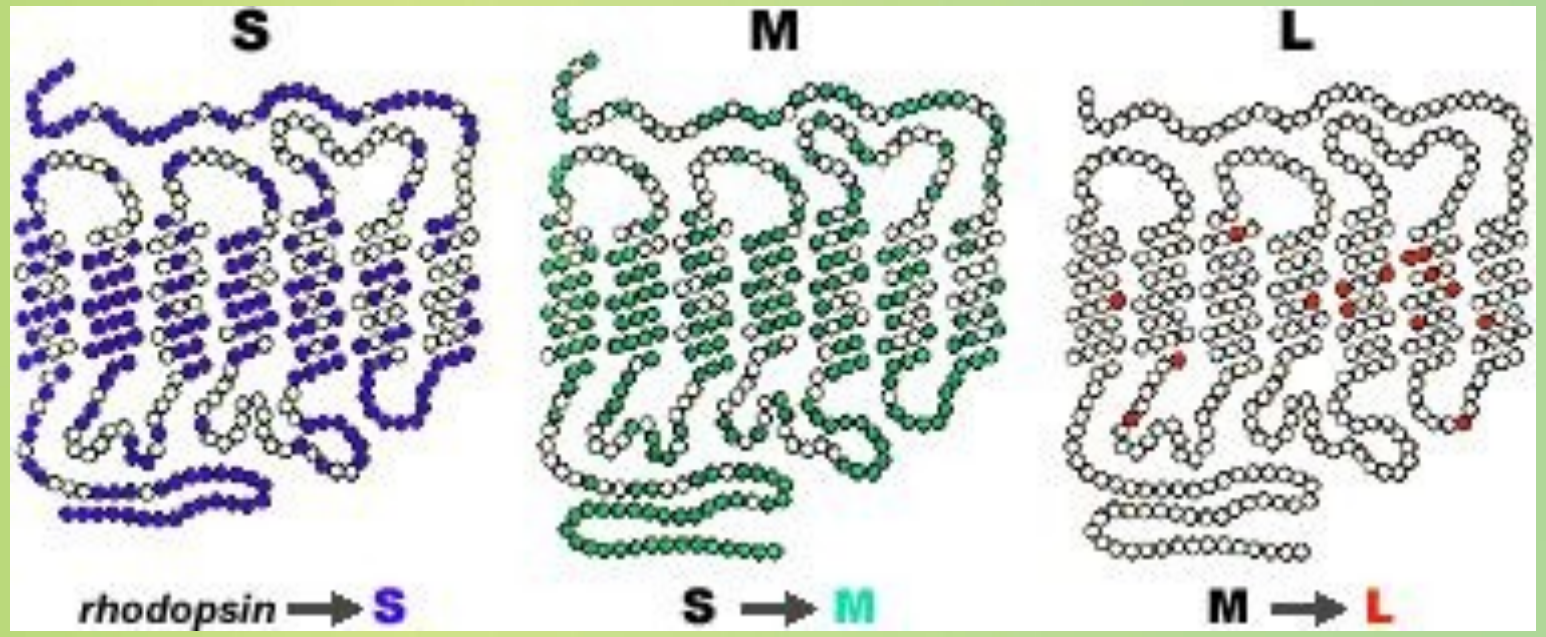
- Cone cells are responsible for visualizing colors, and we have 3 types of cone cells, each one is responsible for visualizing colors at a certain wavelength, (Blue, Green, and Red).
- Notice there are a lot of overlapping in the green/red wavelength.

- Rods are more sensitivity because they are higher in number and because we have multiple rod cells connected to one neuro ( in cones one cell per one neuron), but cones give a sharper image due to the 1:1 cone with ganglion cell (compared to 60:1 in rods) so the brain knows exactly the location of that cone.

# How different are they?

The picture can't be displayed.

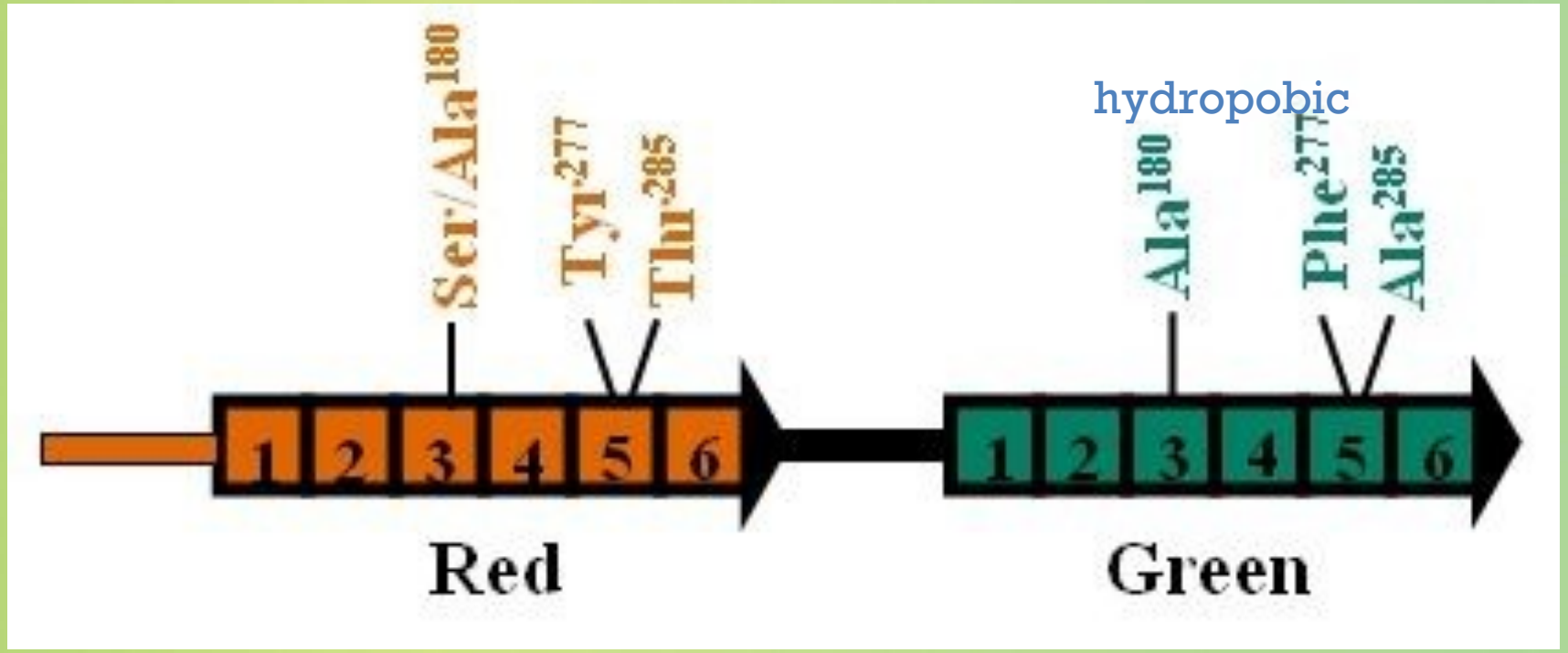
short wavelength | intermediate wavelength | low wavelength




- 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.
- Each of the cone photoreceptors vs. rhodopsin ≈ 40% identical.
- The blue photoreceptor vs. green and red photoreceptors = ≈ 40% identical.
- The green vs. red photoreceptors > 95% identical.

# Three important aa residues

The picture can't be displayed.




**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).**

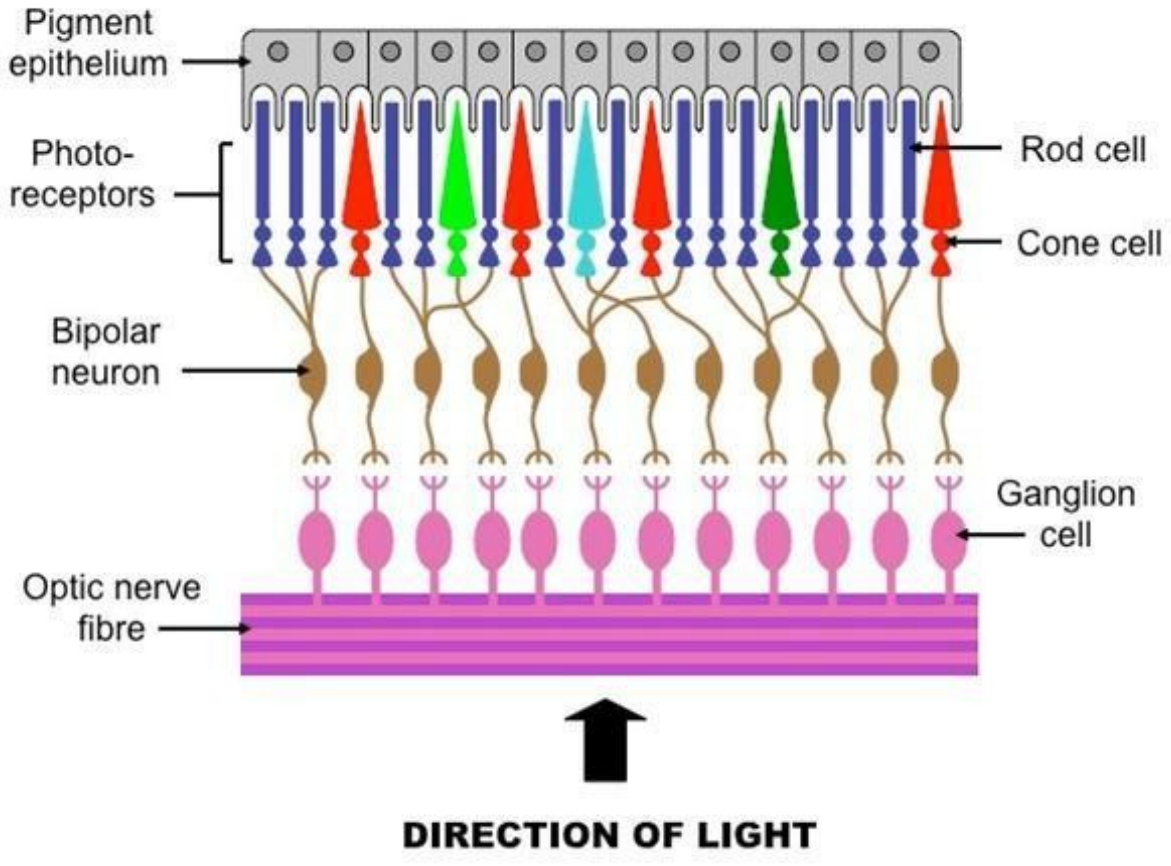
Single nucleotide polymorphism makes every color unique to the person that sees it.

# Rods vs. cones

The picture can't be displayed.

- **Light absorption, number, structure, photoreceptors, chromophores, image sharpness, sensitivity (amplification)**

**The image is sharper in bright light but in dark isn't sharp**
**Because multiple rods connected to one neuron so the brain doesn't know exactly from where it got the image**
**On the other hand each cone is connected to one neuron so the brain an determine exactly from where the image**




We see better in dark light than bright light in case of sensitivity beca have more rods than cones

**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 nerve fiber.**

The picture can't be displayed.

# Color blindness

# Chromosomal locations

The picture can't be displayed.

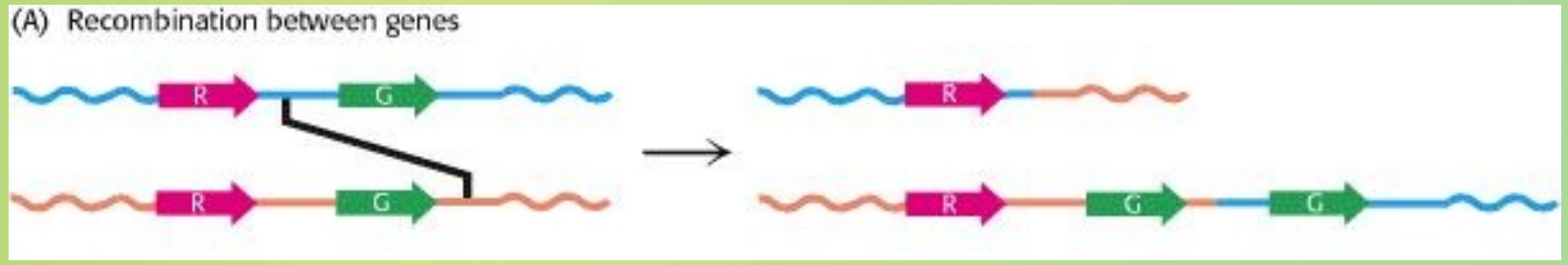
- The "blue" opsin gene: chromosome 7
- The "red" and "green" opsin genes: X chromosome
- The X chromosome normally carries a cluster of from 2 to 9 opsin genes.
- Multiple copies of these genes are fine.

- Almost 6% of males are color blind mainly green and red, because the opsin gene is X linked chromosome (more common in males)

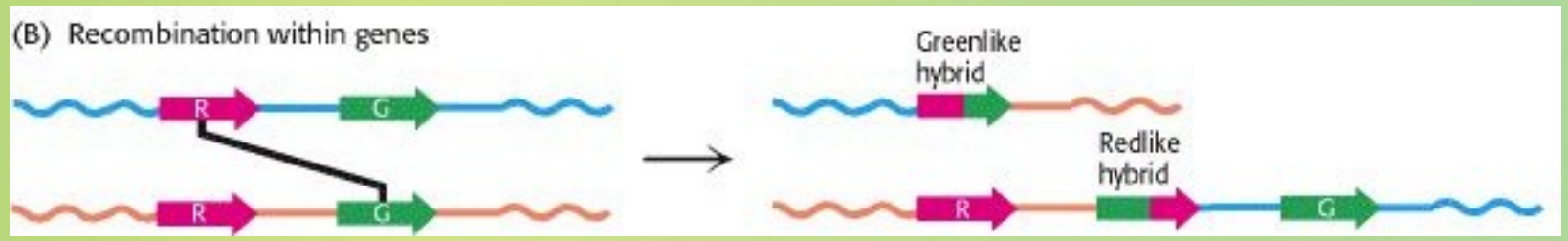
# Red-green homologous recombination

The picture can't be displayed.

- **Between transcribed regions of the gene (inter-genic)**




- **Within transcribed regions of the gene (intra-genic)**



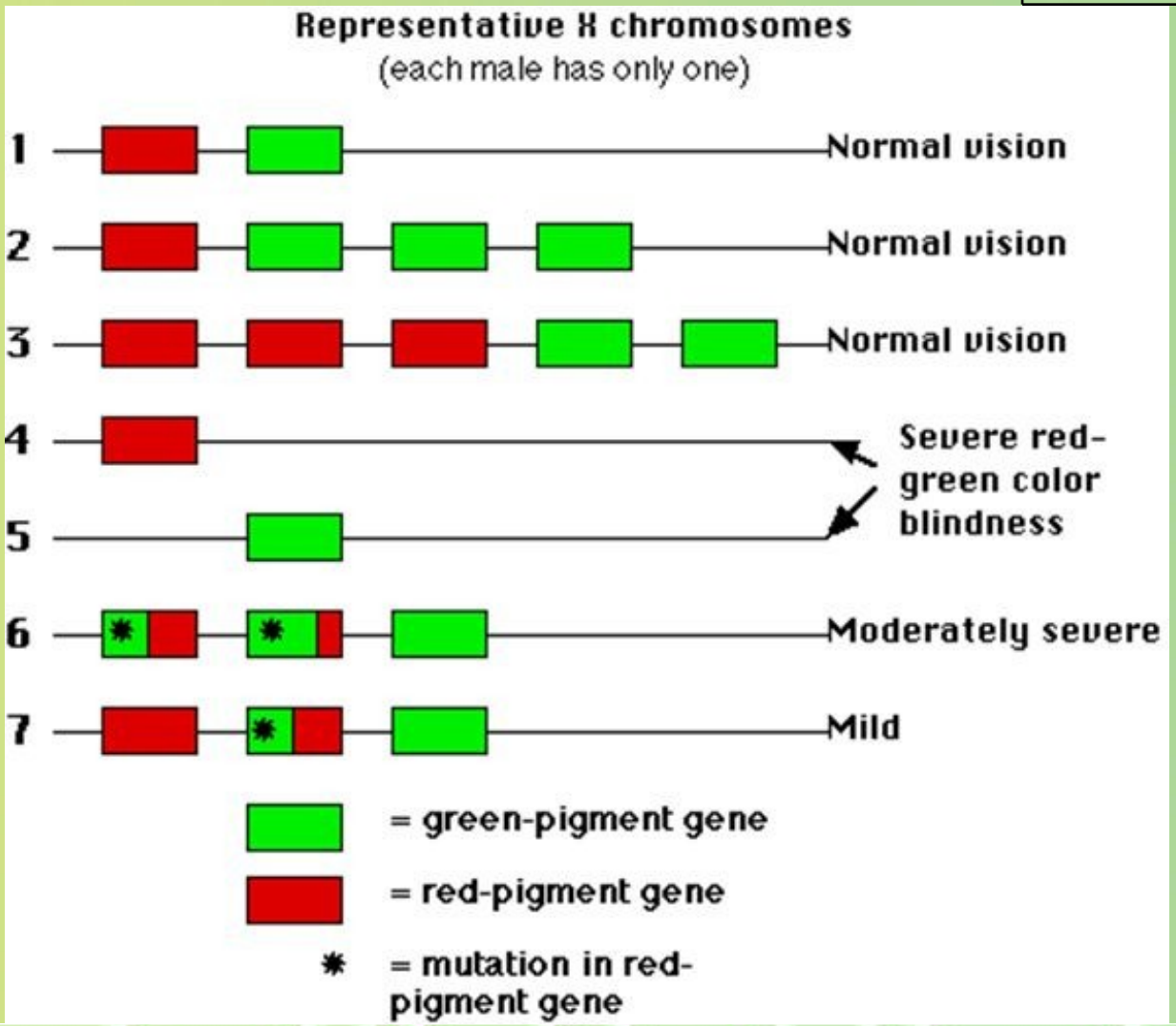

- The amino acid sequence between green opsin and red opsin is 95% identical, so it's more common to have mutations or polymorphisms in green or red.
- In the metaphase when chromosomal material are inlined, we can have exchange of the genetic material between the two X chromosomes, resulting in an X chromosome with a missing/extra red/green opsin (could be intra-genic or inter-genic)
- This what leads to differences among individuals

# Genetic probabilities

The picture can't be displayed.

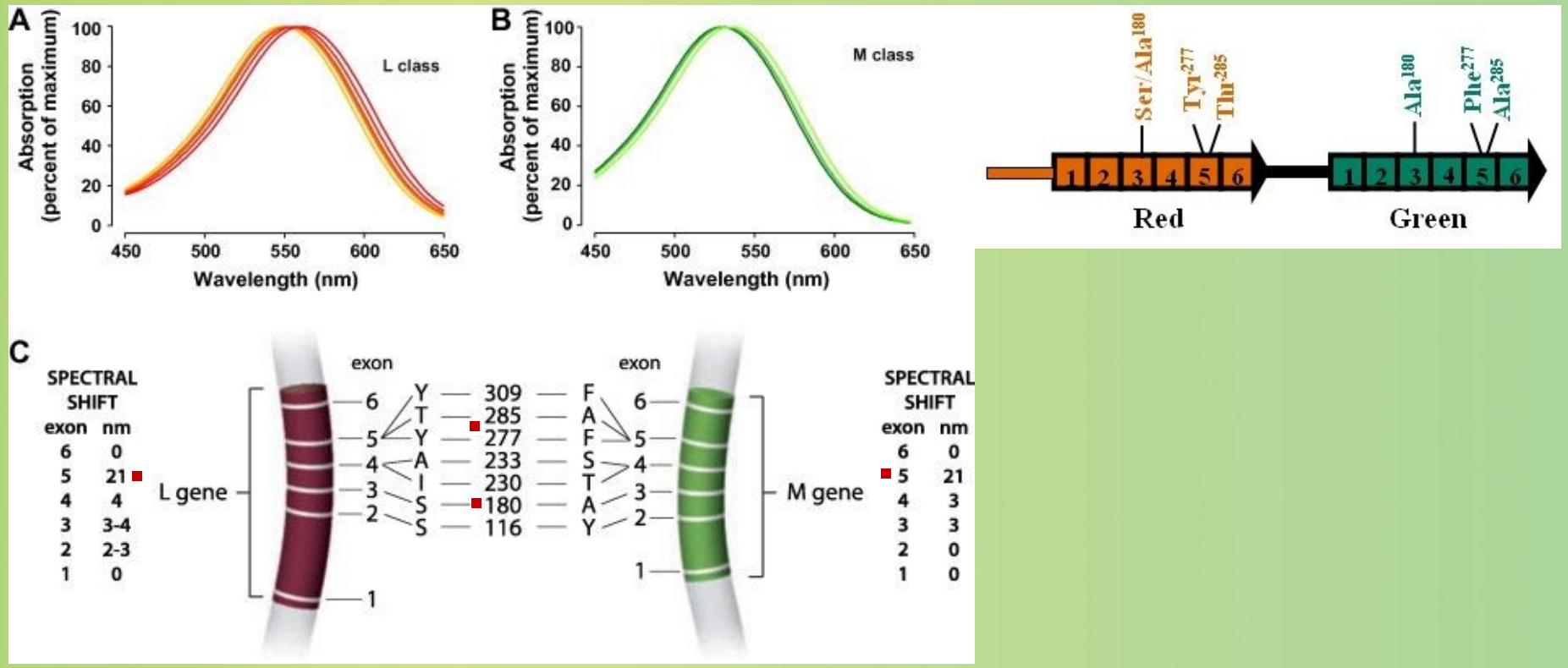
Mutations affect how we see the colors

Color blindness is more common for green and red



# Spectral tuning

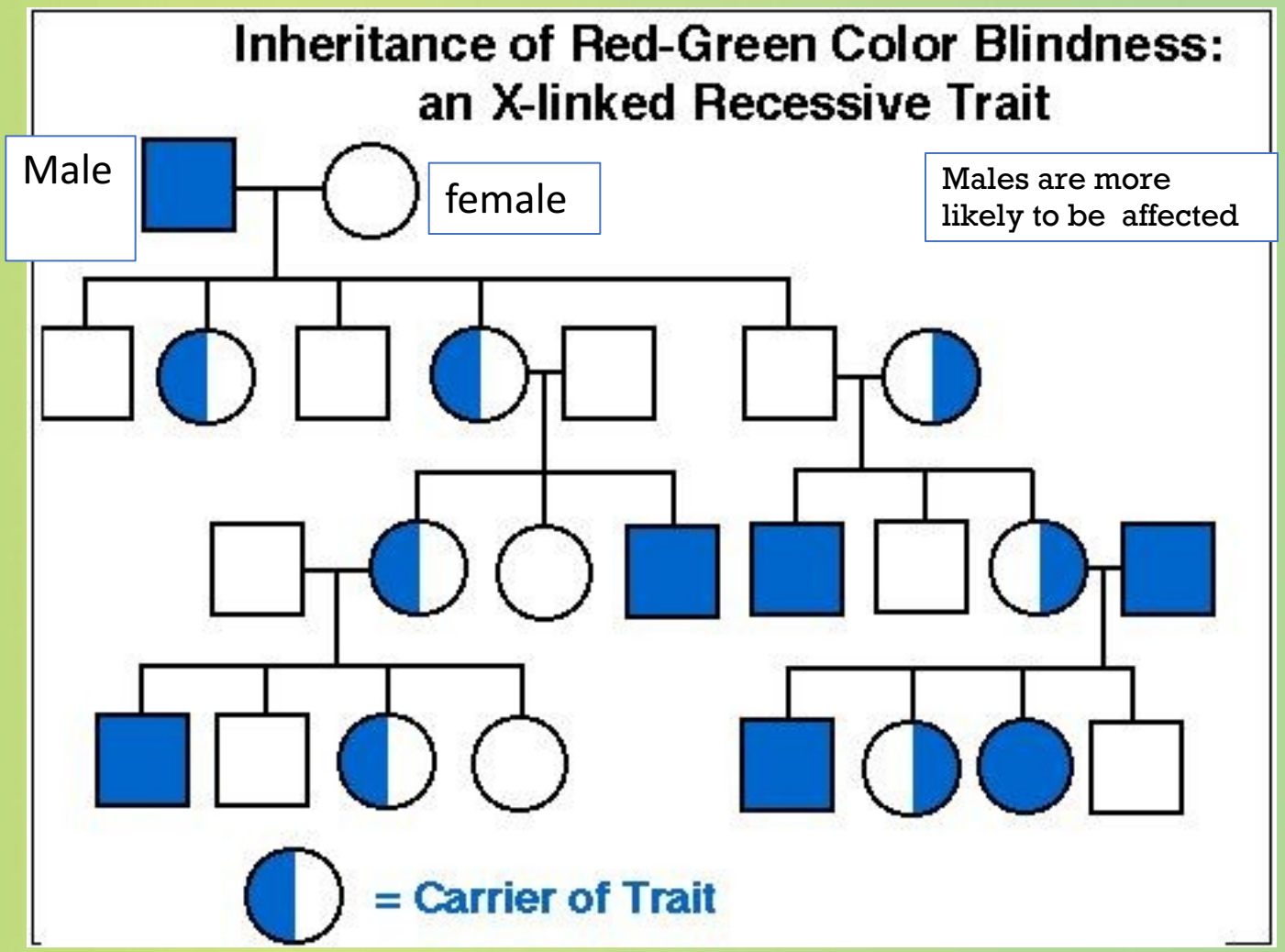
The picture can't be displayed.




- **The substitutions at positions 277 and 285 account for about 20 nm of the difference in peak sensitivity.**
- **Serine (S) vs. alanine (A) at position 180 produces a measurable shift in the spectrum.**

# Pedigree

The picture can't be displayed.



# Examples

The picture can't be displayed.

## Red blindness

## Green blindness

https://www.buzzfeed.com/crystalro/red-color-vision-test

# Only People Who Can See RED Really Well Can Read These Words

The picture can't be displayed.