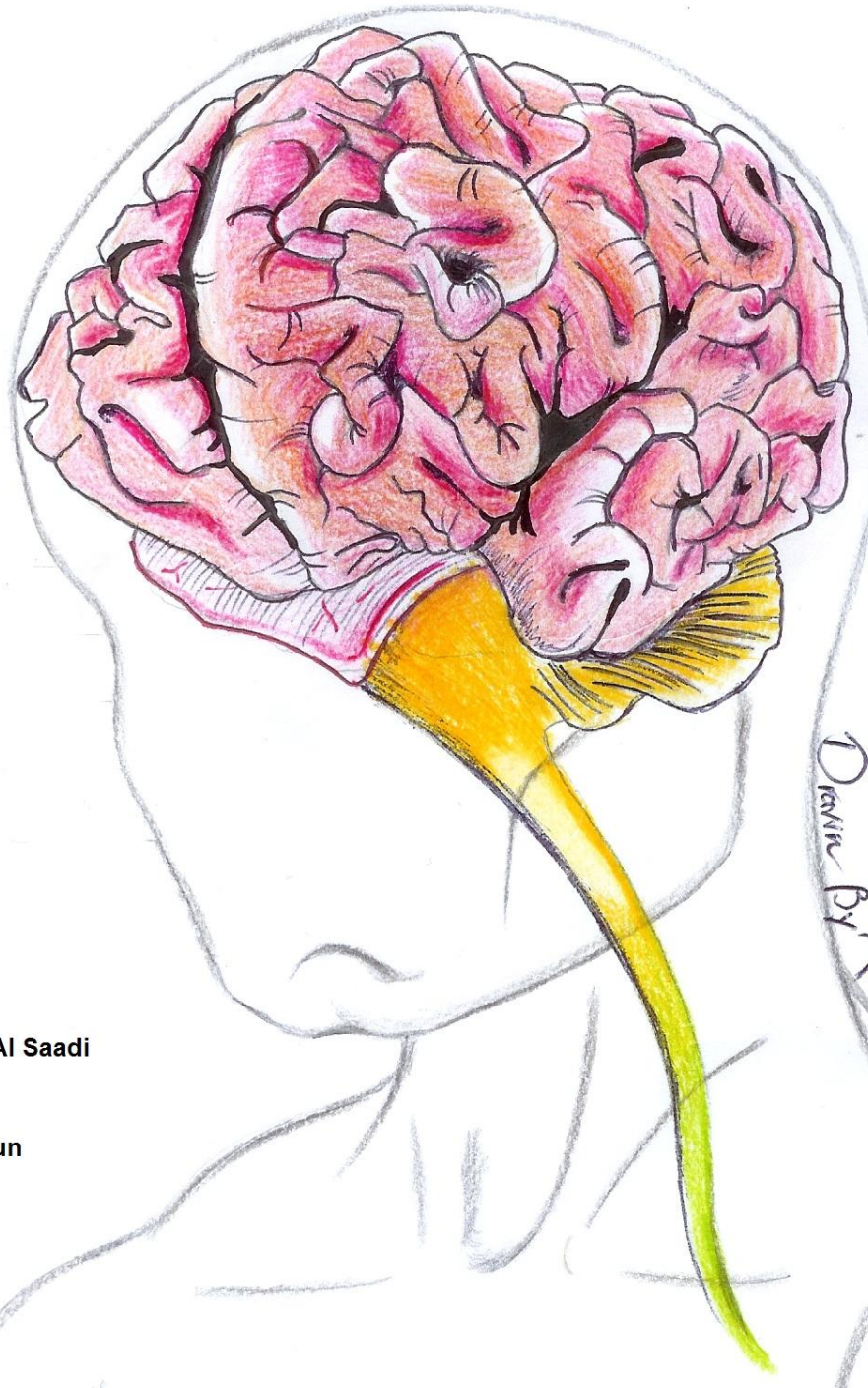
CENTRAL NERVOUS SYSTEM

- ○ Handout
- ★ Sheet
- ○ Slide

- ○ Anatomy
- ○ Physiology
- ○ Pathology
- ★ Biochemistry
- ○ Microbiology
- ○ Pharmacology
- ○ PBL



Drawn By Tariq Bushnaq...

Done By : **Nijmeh Al Saadi**

Dr. Name : **Mamoun**

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There is absolutely no need for you to refer back to the set of slides provided by the professor. Everything present in the slides was incorporated here, including the figures.

The differences between rods and cones are of great importance. Also of importance is the activation and the amplification of signal transduction. In addition, the different mechanisms by which signals are terminated are important. Pedigrees are common in the exam.

According to the professor, these are the references that you should use:

- Photoreceptors and Visual Pigments:
  - Webvision: The Organization of the Retina and Visual System (http://www.ncbi.nlm.nih.gov/books/NBK11522/#A127)
  - The Molecular Design of Visual Transduction (https://www.biophysics.org/portals/1/pdfs/education/Phototransduction.pdf)
  - Biochemistry (http://www.ncbi.nlm.nih.gov/books/NBK22541/#A4618)
- Vitamin A and Carotenoids:
  - Lippincott Williams & Wilkins, p.381-383

Lecture Outline:

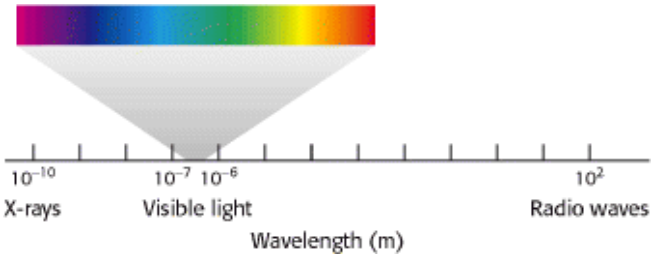
- Visual transduction (dim vs. bright light)
  - Components (cells and molecules)
  - Mechanisms of activation, amplification, and termination
- Color blindness
- Metabolism of vitamin A

Animation Movie:

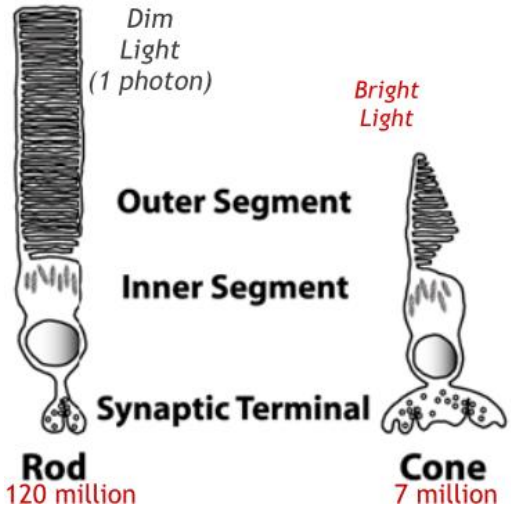
http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/webvision/photomv3-movie1.mov

Visual Pigments

Basics of Human Vision:

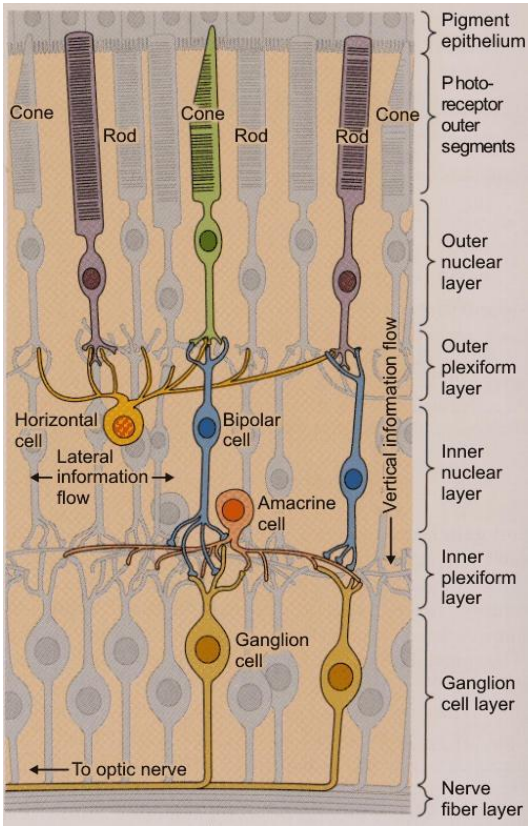


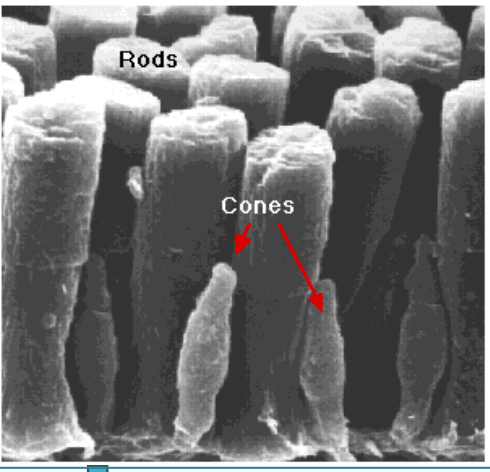
Humans have the ability to see only a narrow range of light. Humans do not possess the ability to see ultraviolet (UV) light.



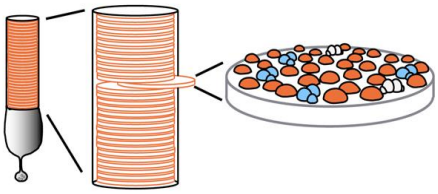
Rods and Cones:

There are two types of cells responsible for vision. These are the rod cells and the cone cells, or simply the rods and cones. These differ in their shape, number, levels of sensitivity and sharpness, and function. However, they share the same regulatory mechanisms. For example, humans have 120 million rod cells, which are responsible for vision in the dark or dim light. On the other hand, humans have 7 million cone cells, which are responsible for vision in bright light (back then sitting in the lecture, we see each other due to cones activation). Looking at their structures, each type was named according to its shape. They are composed of an inner and an outer segment. The outer segment is responsible for visual transduction. 4:00



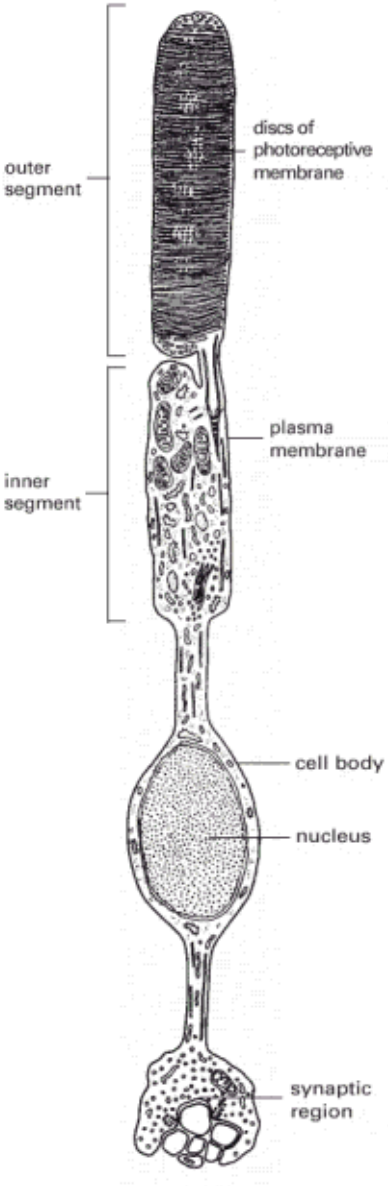


How they really look like…
This is how these cells are seen under a scanning electron microscope (SEM).



More on rod cells:
Rod and cone cells share the same mechanisms. However, more is known about rod cells than cone cells.

The outer segment of Rod cells is composed of hundreds of discs. Looking at an individual disc, a plasma membrane composed of the signal transaction machinery (the core things responsible for vision) can be seen.

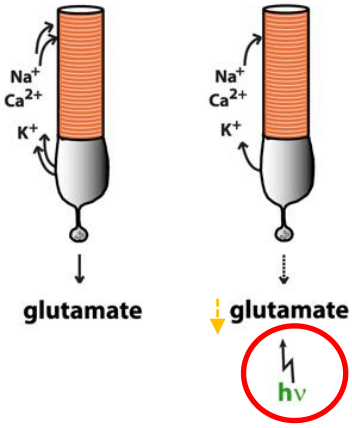


- Na+ and a lesser amount of Ca2+ enter through cyclic nucleotide-gated channels in the outer segment membrane
- K+ is released through voltage-gated channels in the inner segment.
- Rod cells depolarize.
- The neurotransmitter glutamate is released continuously.

The dark current:

Usually, in the dark, the rod cells are depolarized as a result of the opening of the channels, which allows for the entry of sodium and calcium ions.

When light hits these cells, the channels will close. This decreases the entry of ions into the cells



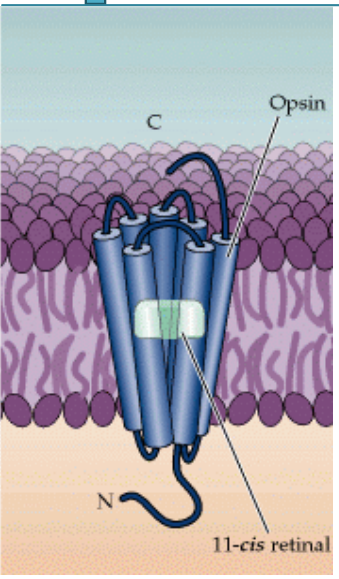
1. Channels in the outer segment membrane close, **the rod hyperpolarizes**
2. Glutamate release **decreases**.

The reduction in the release of glutamate is the signal. Meaning that the inhibitory signal of glutamate will end. So, when light hits these cells, the cells will be hyperpolarized, allowing for signal to be transduced.

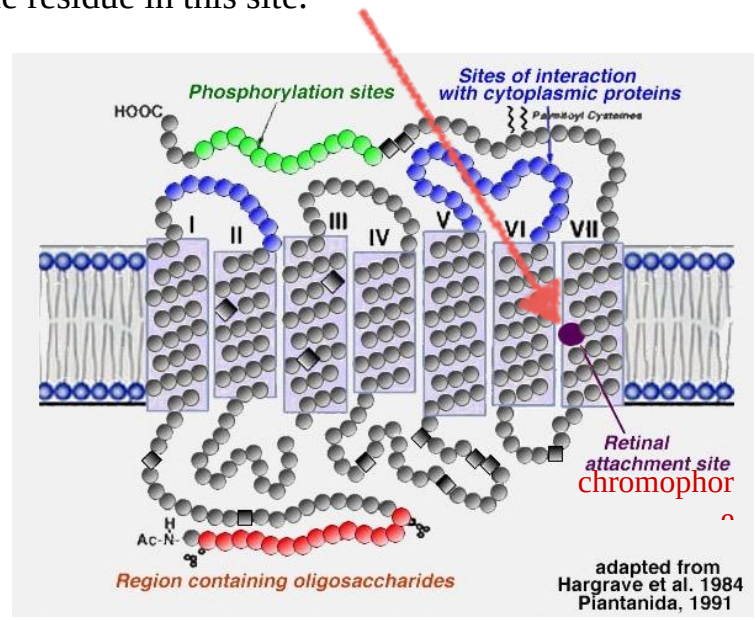
Generation of Vision Signals:

The players (i.e. the proteins responsible for visual transduction):

- Rhodopsin
- Transducin
- Phosphodiesterase
- Na^+-gated channels
- Regulatory proteins

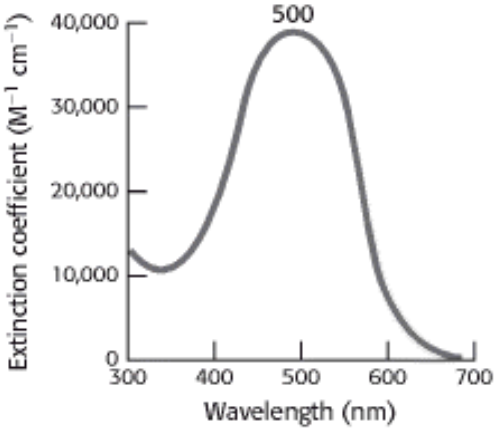


Rhodopsin:
It is a 7-transmembrane domain protein.
Chromophore, the molecule responsible for signal induction, binds to a lysine residue in this site.

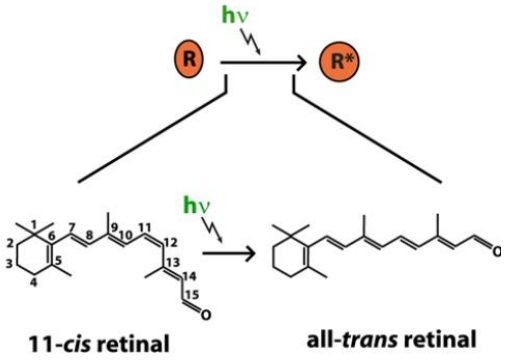


chromophor
e

Light absorption by rhodopsin:
Rhodopsin can absorb a wide range of light wavelengths, allowing us to see all colors. The peak is about 500-510 nm.



11-cis-retinal:



The chromophore is derived from vitamin A, and is known **as 11-cis-retinal**. The name comes from the fact that the molecule has a double-bond on carbon number 11. When it is hit by light, the electrons will be activated, resulting in electron rearrangement. This rearrangement changes the double-bond from cis to trans, forming **all-trans** retinal. This small change in the structure of this molecule results in a change in the structure of rhodopsin as a whole. The speed by which this molecule changes structure is 10^{-13} sec. It's amazing how in Femtoseconds it can change structure of the protein.

10^{-13} sec

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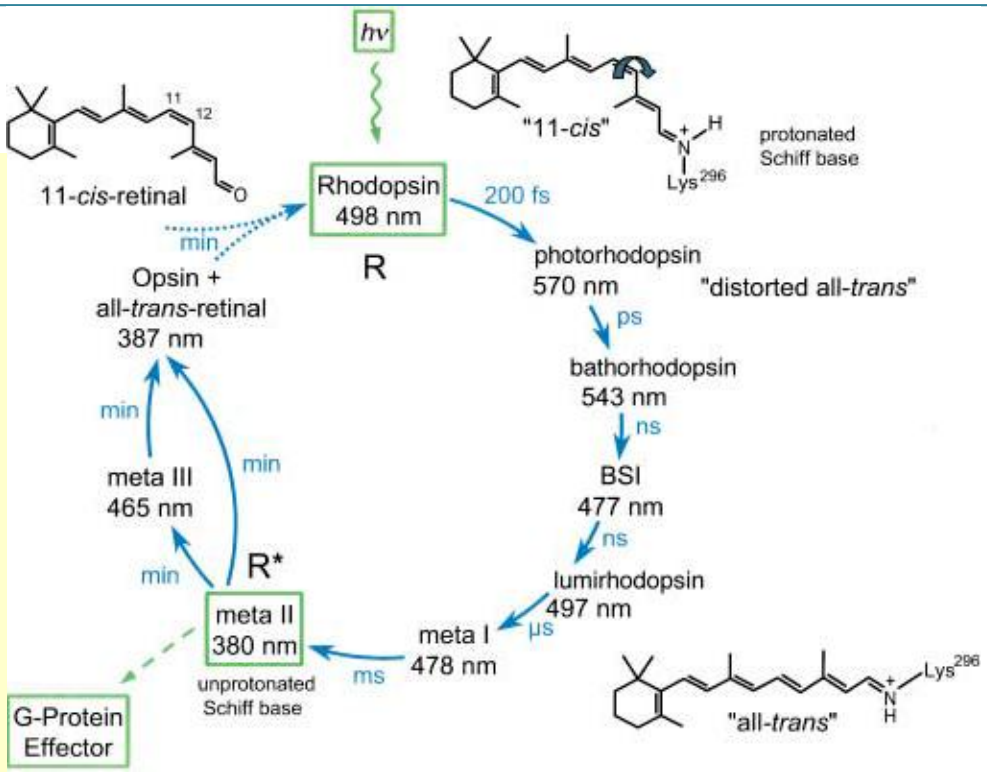
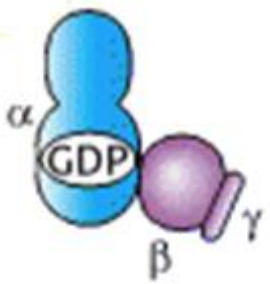
Rhodopsin Intermediates

- By itself, 11-cis retinal absorbs near UV light. But opsin perturbs the distribution of the electrons exiting 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.



Opsin bound to 11-cis-retinal is known as rhodopsin. Activated rhodopsin is in the form of **meta-rhodopsin II**.
When 11-cis-retinal changes to all-trans retinal, it disturbs the structure of the whole rhodopsin protein, resulting in a lot of repulsion and electron rearrangement. The rhodopsin molecule becomes activated and it undergoes changes in its structure. Each resulting structure is given a certain name and can absorb light at a different specific wavelength (the numbers with nm in the figure). In other words, as rhodopsin changes structure, it absorbs light at different wave lengths. This explains why rhodopsin has the ability to absorb light at a wide range of wavelengths.
The most important structure is known as meta-rhodopsin II. This structure is capable of activating signal transduction. If the signal stopped before reaching this structure, it won't be transduced.
What actually happens is that the protein keeps changing its structure until the **all-trans retinal** is **released** and **converted back to 11-cis-retinal**, which then can bind to the same or another Opsin protein.

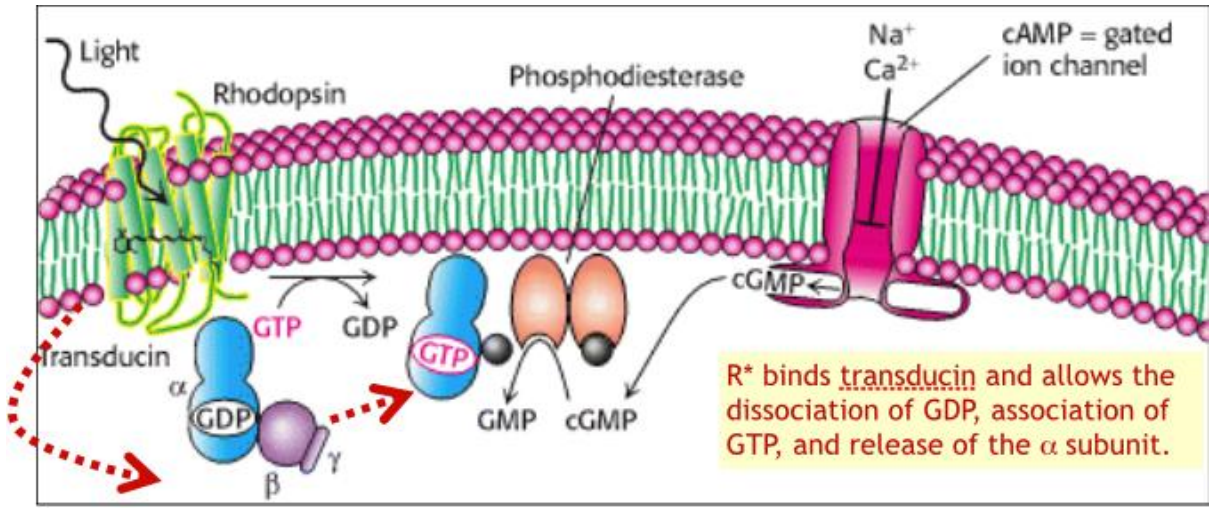


Rhodopsin does not stop at a certain structure, eventually the cycle is completed and the all-trans retinal is released, leaving inactivated opsin. Inactivated opsin binds to 11-cis-retinal and the cycle starts again (i.e. light hits 11-cis-retinal…).

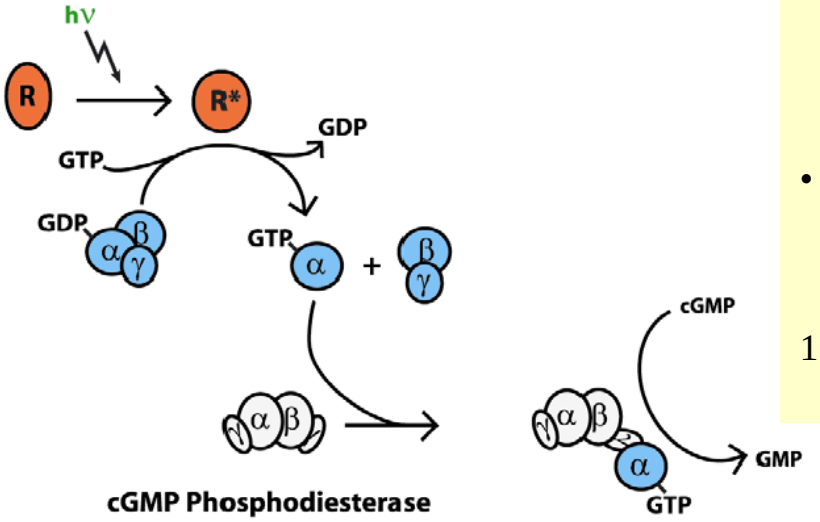
Transducin → Phosphodiesterase (PDE):
The activated rhodopsin activates a G-protein known as transducin (in rod cells).

Transducin is a large heterotrimeric G protein, consisting of α, β, and γ subunits. β and γ are inhibitory subunits. α is the active subunit that binds to GTP when activated. In its inactive state, transducin's α subunit has a GDP bound to it.

Meta-rhodopsin II interacts with transducin, resulting in a GDP-GTP exchange.



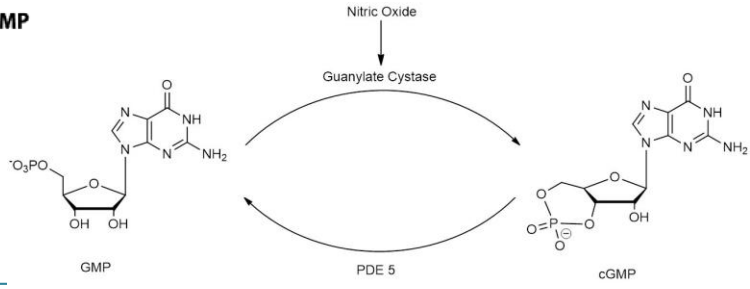
The α subunit is released and it travels along the plasma membrane to activate the enzyme phosphodiesterase. Phosphodiesterase (PDE) converts cGMP to GMP. cGMP has the ability to bind to ion channels (e.g. sodium-gated channels) activating them and allowing for the entry of ions into the cells. When cGMP is converted to GMP, the channels close, preventing the entry of ions (Na and Ca ions) into the cells. This



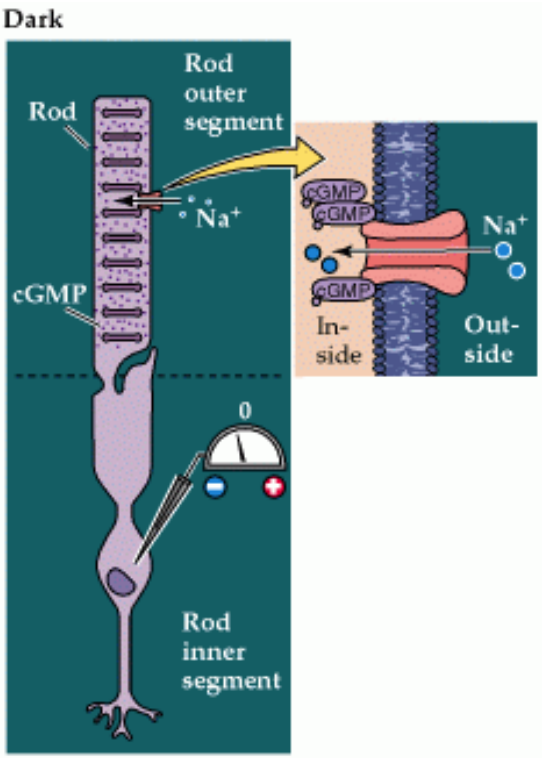
- PDE consists of four subunits, α, β, and two γ subunits. The γ subunits are inhibitory, while the α and β are the two catalytic subunits that convert cGMP to GMP.
- PDE is a heterotetramer that consists of a dimer of two catalytic subunits, α and β subunits, each with an active site inhibited by a PDE γ subunit.

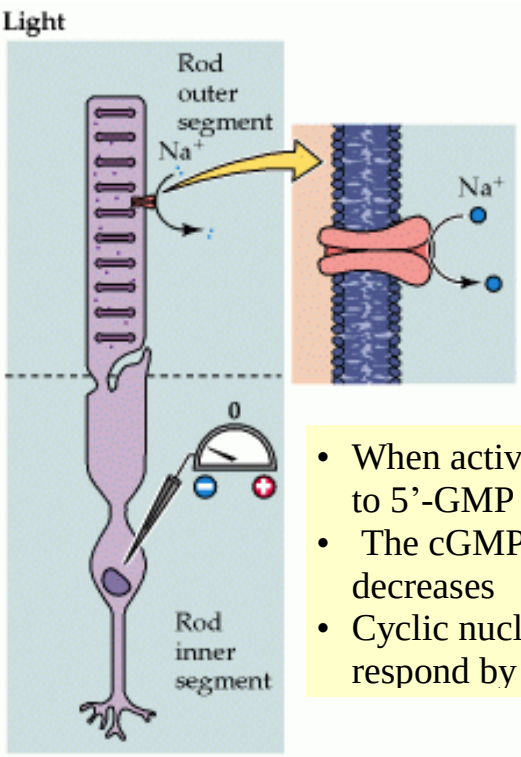
1. The activated transducin α subunit-GTP binds to PDE γ and relieves the inhibition

results in hyperpolrization of the cells.



Activation of phosphodiesterase:



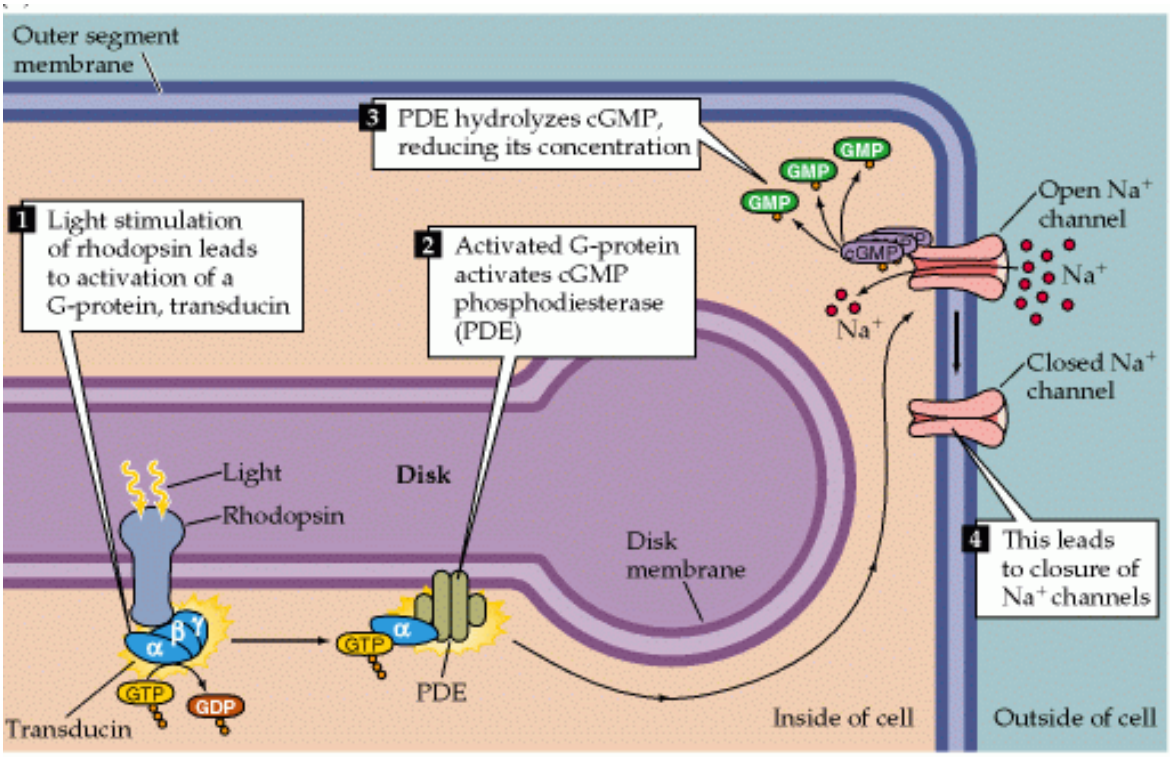


cGMP-gated channels:

The whole picture

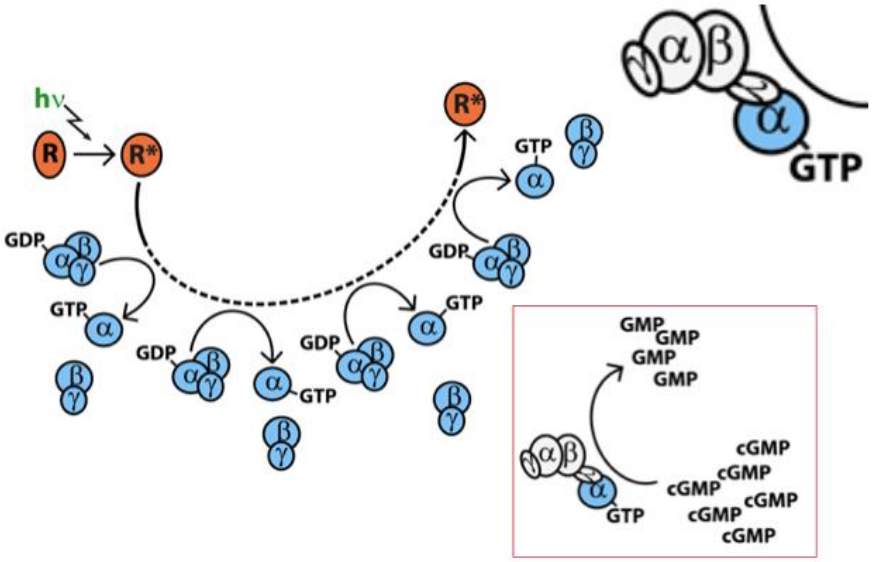
- When activated, PDE hydrolyzes cGMP to 5'-GMP
- The cGMP concentration inside the rod decreases
- Cyclic nucleotide-gated ion channels respond by closing

Hyperpolarization is important in the prevention of the release of glutamate, thus relieving its inhibitory effect. This results in activation of signals in neighboring cells. These signals then reach the brain indicating the presence of light.



Overall, a single photon closes about 200 channels and thereby prevents the entry of about a million Na+ ions into the rod.

Signal Amplification:

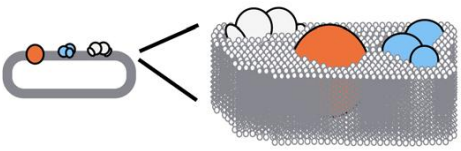


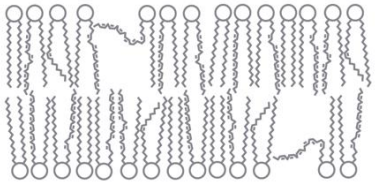
How is that?

Rhodopsin (1) → Transducin (500)

Transducin (1) → PDE

PDE (1) → cGMP (10^3) molecules are converted to GMP
The conversion of 1000 cGMP can result in the closure of 200 channels.
In total, 100000 (200x500) channels are closed.?





Facilitation of transduction:

- A specific characteristic of the rod cells is their plasma membrane.

• For signaling to be activated, signaling molecules must **interact in the proper orientation.** The fact that all signaling molecules are embedded in the plasma membrane means that they are fixed in a certain position and that they can only travel in 2 dimensions. This increases the probability of their collision in general and their interaction in the proper orientation as well. Collision is a random process, which makes any interaction between these molecules hard to achieve if they were free in the cytoplasm, let alone their interaction in the proper orientation.

- The plasma membrane of rod and cone cells is **highly viscous** (very fluidic) due to its **low cholesterol and high unsaturated fatty acids** content. This facilitates the movement of protein molecules in the plasma membrane.

- **Cooperativity of cGMP**. The release of one cGMP makes it easier for the next cGMP to be released, resulting in the closure of the channel. The binding of one cGMP also makes it easier for the next one to bind, resulting in the opening of the channel.

2. 2-dimensional surface
3. low in cholesterol and high content of unsaturated fatty acids
4. Cooperativity of binding: The binding of one cGMP enhances additional binding and channel opening (n = ~3)
5. 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.

In short:

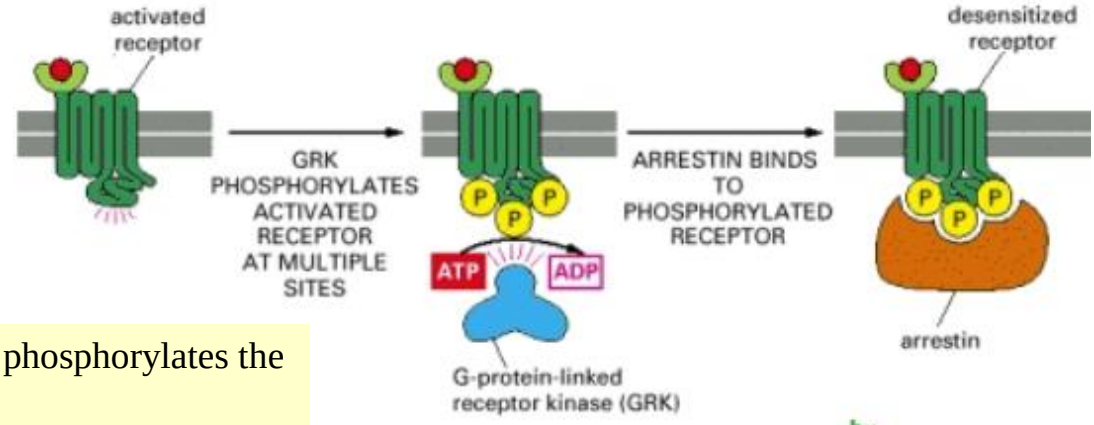
Signal Termination:

The termination of the signal is of extreme importance in uninterrupted visioning of movement.

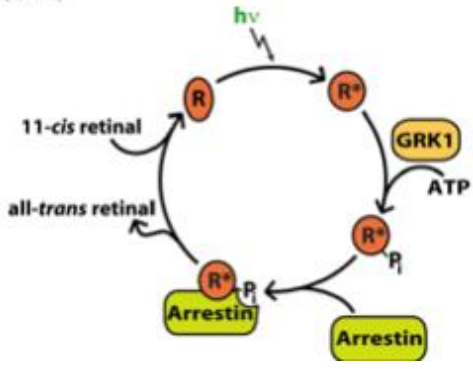
Mechanism I
Arrestin binding

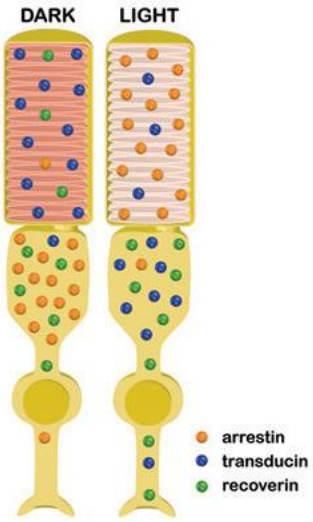
Arrestin has a high affinity for the activated form of rhodopsin. However, it binds only to the phosphorylated ones.
Rhodopsin → activation → phosphorylation by a kinase → binding of arrestin
The purpose of phosphorylation is: 1- Delay the process of inhibition of rhodopsin and thus allow for the activation of signal transduction

2- Terminate signal transduction



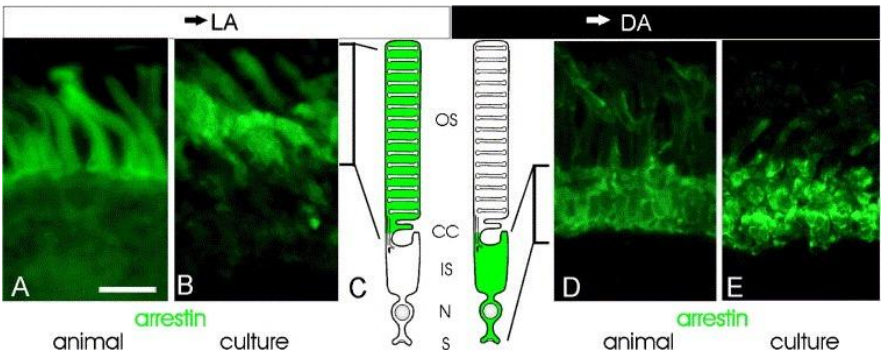
- Rhodopsin kinase (GRK1) phosphorylates the C-terminus of R*.
- Phosphorylation of R* decreases transducin activation and facilitates binding to arrestin, which completely quenches its activity, and releases of the all *trans-retinal* regenerating rhodopsin.





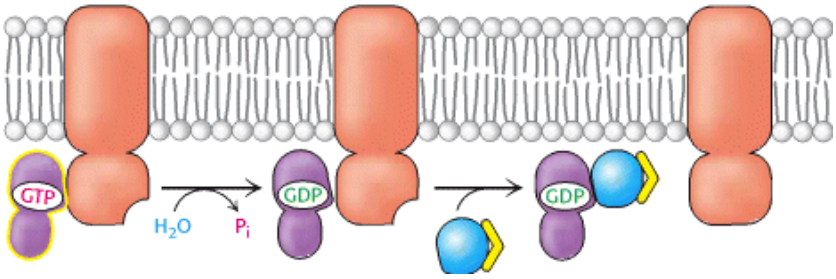
Mechanism II
Arrestin/transducin distribution

The localization of arrestin and transducin differs. In the dark, **arrestin** is in the inner segment of the rod cells (kept away) to prepare rhodopsin to be activated. In the light, arrestin is translocated from the inner segment to the outer segment in order to terminate the signal transduction. As for transducin, in the dark it is located in the outer segment. However, when light hits rod cells transducin activates signaling and goes all the way down to the inner segment in order to terminate the signaling pathway.



- In dark, the outer segment contains high levels of transducin and low levels of arrestin.
- In light, it is the opposite.

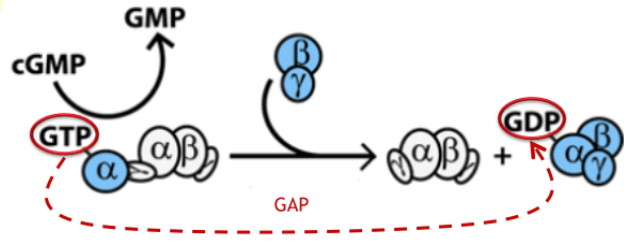
Mechanism III



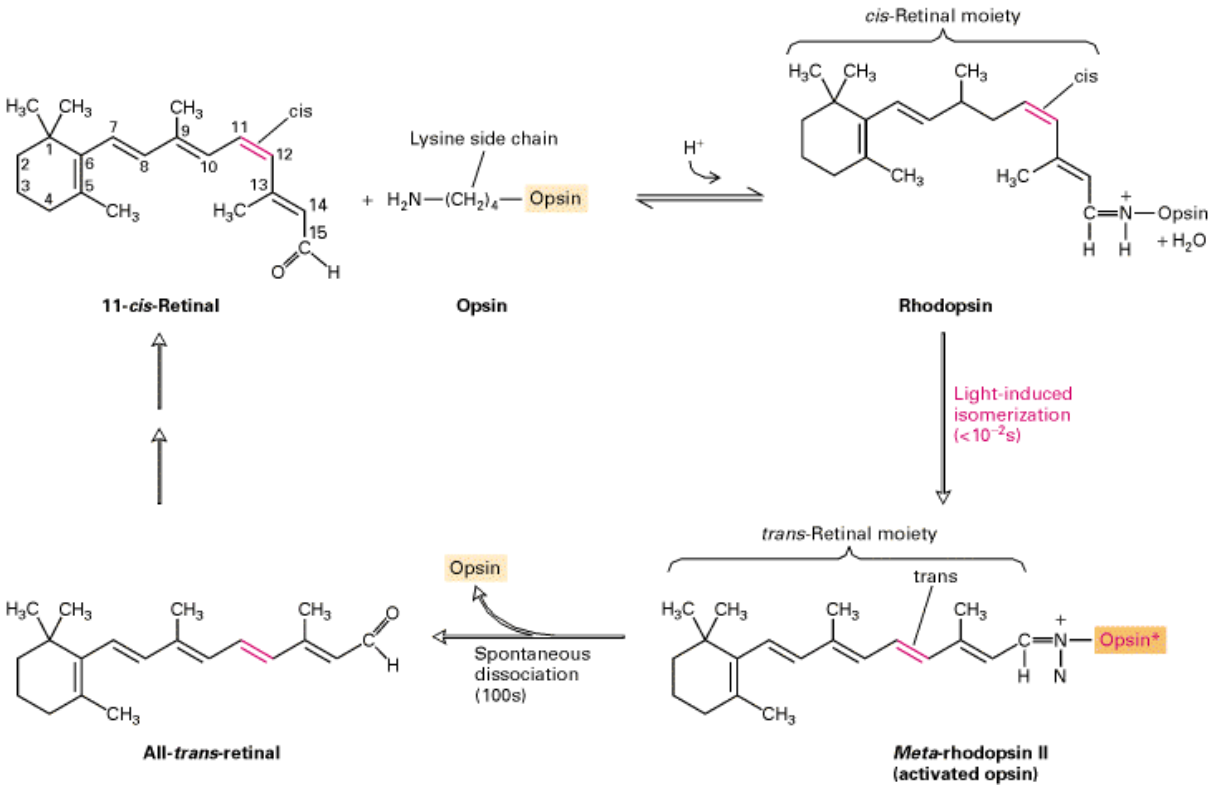
- α subunit of transducin has an intrinsic GTPase activity that hydrolyzes GTP to GDP.
- Upon hydrolysis of GTP to GDP, transducin α subunit releases the PDE γ subunit that re-inhibits the catalytic subunit.
- Transducin α-GDP eventually combines with transducin βγ
- When the α subunit is activated, GTP is hydrolyzed, and transducin is inhibited. This is because when α is bound to GDP, it then can bind to the inhibitory subunits, β and γ.

Intrinsic GTPase activity of G protein
Mechanism IV
Facilitation of GTPase activity of G protein

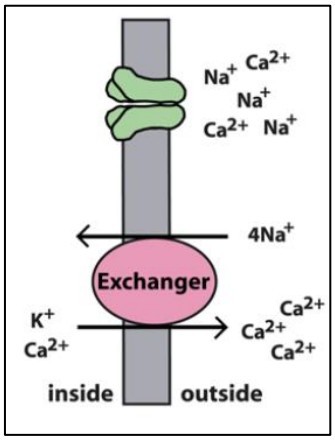
- GTP hydrolysis is slow intrinsically, but it is accelerated by the GAP (GTPase Activating Protein) complex.
- To ensure that transducin does not shut off before activating PDE, transducin and the GAP complex have a low affinity for each other, until transducin α-GTP binds PDEγ.



Mechanism V
Unstable all-trans rhodopsin complex
As previously mentioned, rhodopsin changes structures in different



conformations. The reason behind that is that the rhodopsin molecule containing all-trans retinal is not stable due to the instability of their interaction, resulting in the release of all-trans retinal molecule.



Feedback regulation by calcium ions (Other mechanisms dependent on Calcium ions):

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

The channels allow for the entry of not only Na ions but also Ca ions into the cells. An exchanger that results in the extrusion of Ca ions is present. So when the channel closes, Ca+2 ions do not enter the cell but they still **leave** the cell. So the amount of calcium inside the cell is reduced.

Activation

[cGMP]↓ → Ion channels closed → [Ca^{2+}]↓ → Guanylate cyclase activity increased → [cGMP]↑

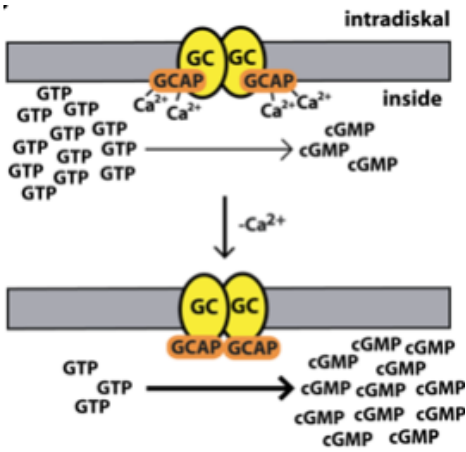
Recovery

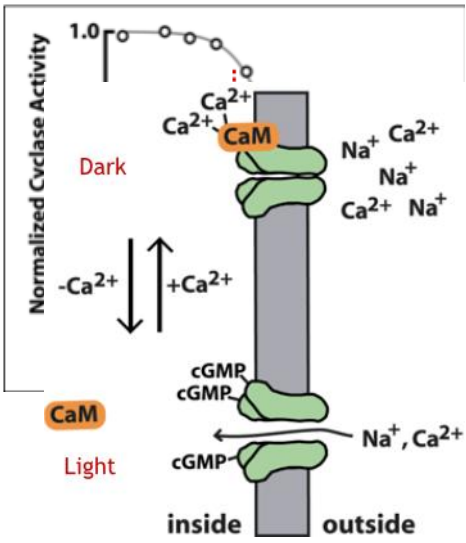
500 nM

50 nM

Feedback regulation/inhibition:
cGMP decreases as a result of the activation of signal transduction by light. The ion channels close and the amount of Ca ions inside the cell decreases dramatically. This activates **guanylate cyclase**, which converts GTP into cGMP, resulting in an increase in the concentration of cGMP inside the cell. cGMP binds to the channels, opening them and allowing for the entry of Na and Ca ions. This results in depolarizing of the plasma membrane.

Mechanism VI
Guanylate cyclase
Guanylate cyclase is bound to a regulatory protein which binds Ca ions when it is active. When the concentration of Ca ions is high, the regulatory protein inhibits the activity of the enzyme. When the concentration of Ca ions decreases, the regulatory protein can no longer inhibit the enzyme GC, resulting in its activation. cGMP levels thus increase.
This is rather odd. However, this takes place to ensure that not all ion channels open and close all at once, thus maintaining a state of balance.
A little change in the concentration of calcium ions inside the cell can result in a dramatic change in the activity of gauanylate cyclase.

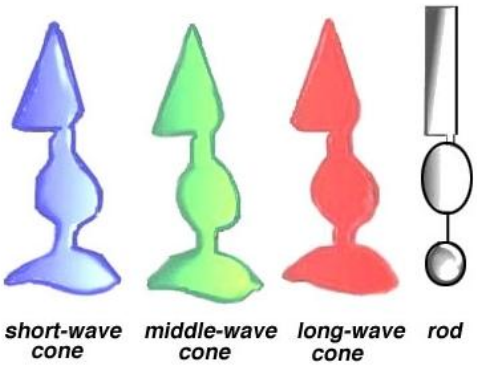




- In the dark, guanylate cyclase-activating proteins (GCAPs) bind Ca^{2+} blocking their activation of guanylate cyclase.
- A decrease in intracellular [Ca^{2+}] causes Ca^{2+} to dissociate from GCAPs leading to full activation of guanylate cyclase subunits, and an increase in the rate of cGMP synthesis.

Mechanism VII

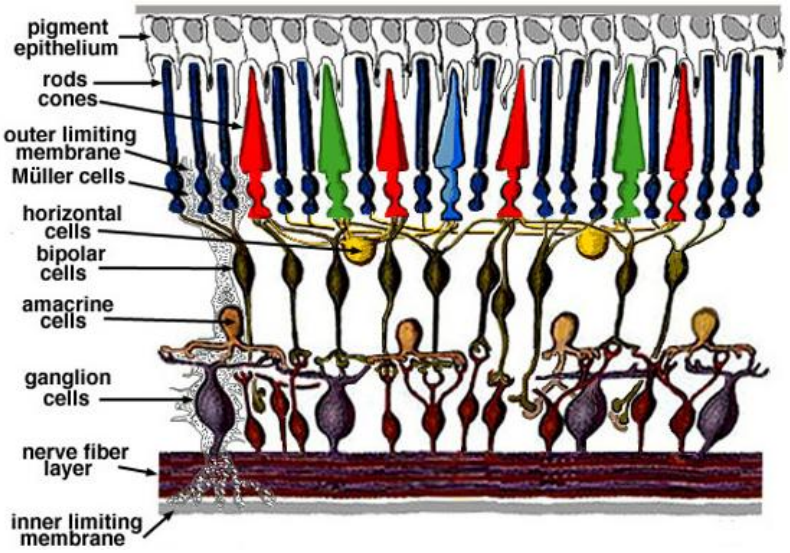
- In the dark, Ca^{2+}-Calmodulin (CaM) binds the channel and shuts it down.
- During visual transduction, the decrease in intracellular [Ca^{2+}] causes CaM to be released, and some channels reopens at lower levels of cGMP as a result of the presence of light. This balances out the amount of Na and Ca ions inside the cells.

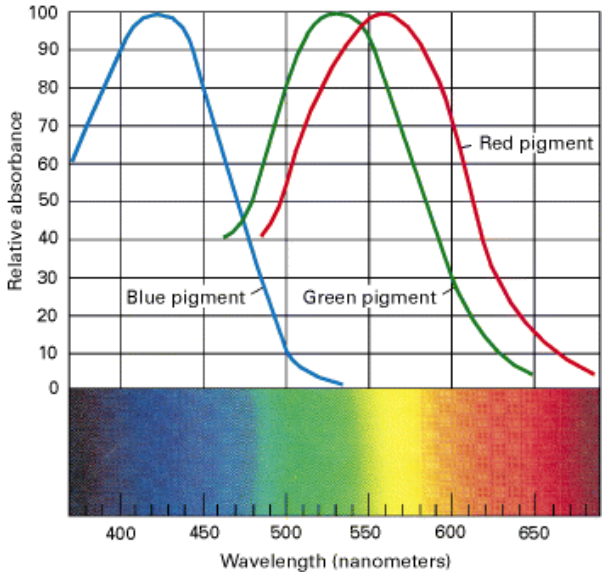


Ca-calmodulin
Even though most of the channels are open in the dark, some of them are closed because of Ca ions binding to Calmodulin protein.

Colour Vision:

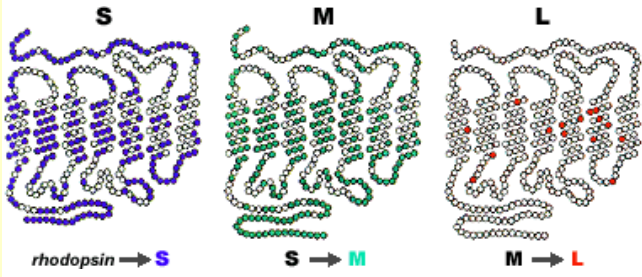
Cone photoreceptor proteins:
The same mechanism of signal transduction exists in cone cells. There are three types of cone cells, each of which absorbs light at a different wavelength. They are divided according to the colour of the wavelength at which they absorb light into: 1- Red 2- Blue 3-Green

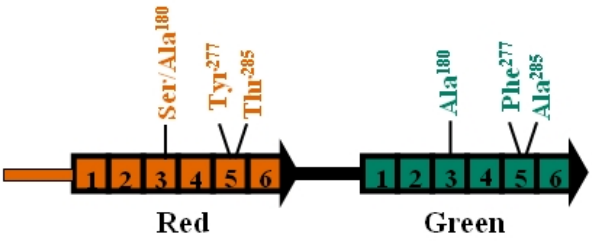




- Cone opsins have similar structures as rhodopsin, but with different amino acid residues surrounding the bound 11-cis retinal; thus they cause the chromophore's absorption to different wavelengths. Overall, they show a lot of homology. For comparison purposes, the white circles indicate homology (same amino acids), while the colored ones are specific for the type of the cone cell.
- 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. (important genetically)

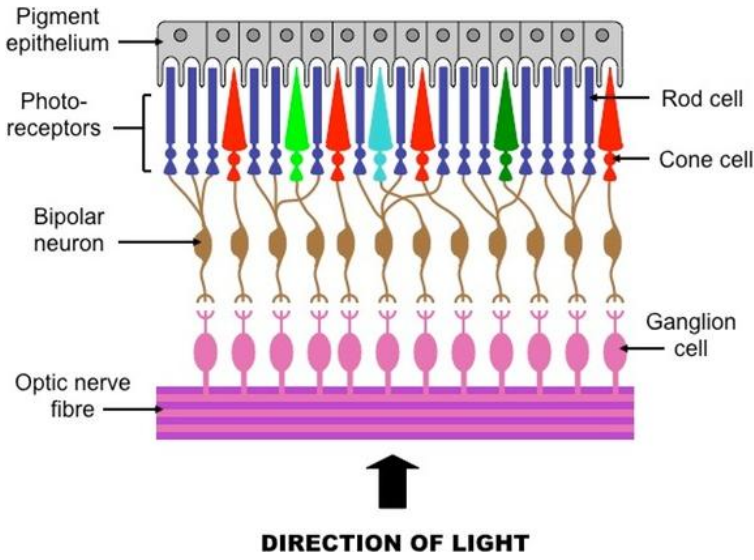
There is an overlap between their peaks. For example, to see orange, the red and green cone cells are activated at different degrees, and the brain would combine their signaling, resulting in the vision of the orange colour.





A hydroxyl group has been added to each amino acid in the red pigment causing a λ_{max} shift of about 10 nm to longer wavelengths (lower energy) [i.e changing any of the amino acids by hydroxylation or dehydroxlation will result in a 10 nm-shift] . This has important individual variations.

How different are they?



Three important aa residues:
The three important residues on red cone cells contain a hydroxyl group, while those on the green cone cells are hydrophobic non-polar amino acid residues.

Rods vs. cones

- Differences in: Light absorption, number, structure, photoreceptors, chromophores, image sharpness, sensitivity
- **Rode cells are more sensitive**.
- Each 1000 rod cells are connected to the brain via one nerve fiber. The brain cannot distinguish the rod cell that sent the signal, resulting in a "fuzzy" image. However, due to their larger number 120 million, a stronger signal is sent to the brain, thus increasing the sensitivity.
- **Cone cells have higher sharpness**.

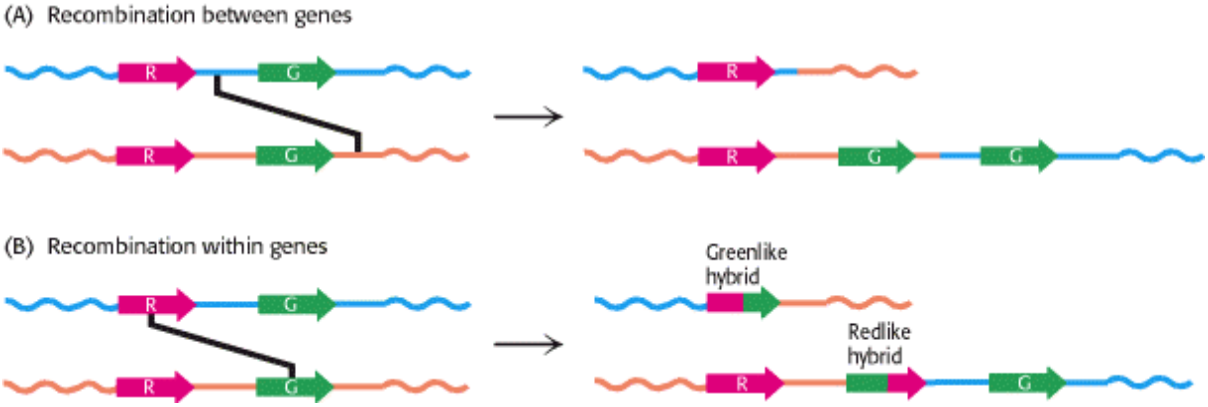
- Each cone cell is connected to the brain via one nerve fiber. The brain can distinguish the exact cone cell that sent the signal, resulting in a sharper image.

Rod cells cannot contribute to light vision. The proof is the presence of a transition period of a few seconds when a person moves from a room with bright light to a room with dim light. It is the time needed for the cone cells to be inactivated and the rod cells to be activated.

Colour Blindness:

Chromosomal locations:

- The "blue" opsin gene: chromosome 7
- The "red" and "green" opsin genes: close to each other on the X chromosome
- The high homology between the red and the green opsin genes has important implications. Due to the similarity in the DNA sequence, when the X chromosomes line out, recombination can take place between these two genes

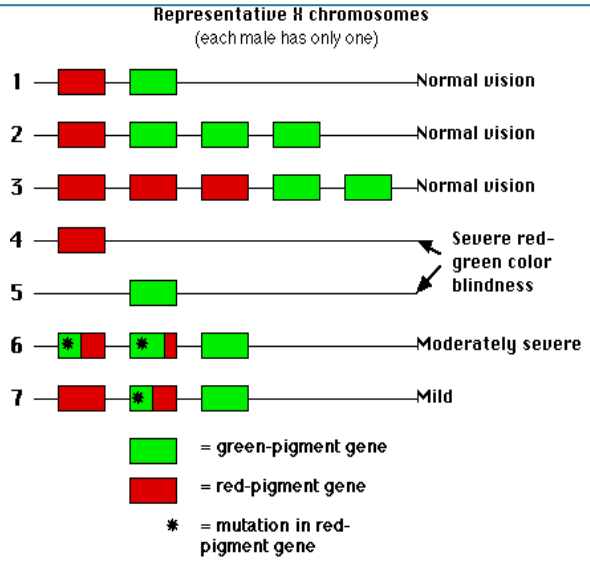


on the different chromosomes. This results in an X chromosome that is missing one gene (cannot see the colour), **or** an X chromosome that has a hybrid gene. A person with a hybrid gene can see combinations of these two colours.

- The X chromosome normally carries a cluster of from 2 to 9 opsin genes
- Multiple copies of these genes are fine. This results in no alteration of vision (normal). This also does not mean that the person is going to have increased ability for the vision of that specific color as all we need is one gene.

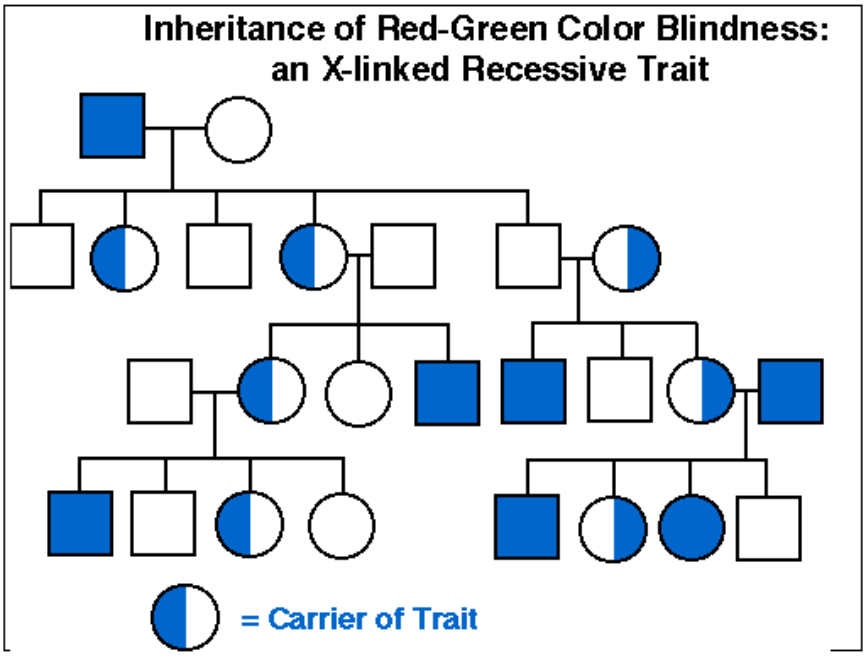
Red-green homologous recombination:

- Between transcribed regions of the gene (inter-genic)
- Within transcribed regions of the gene (intra-genic)



Genetic probabilities

The severity in the case of hybrid genes depends on how much of the other gene is missing.



A lot: moderate-sever

A little: mild

Pedigree

Since these genes are carried on the X chromosomes, males are more affected than females.

If a male is affected, then half of his daughters will be affected? (Correction: yes the doctor said so, not sure if this is true) All of his daughters are carries and none of his sons are affected.

If the mother is a carrier, then there is a 50% chance of her sons being affected and a 50% of her daughters being carriers.

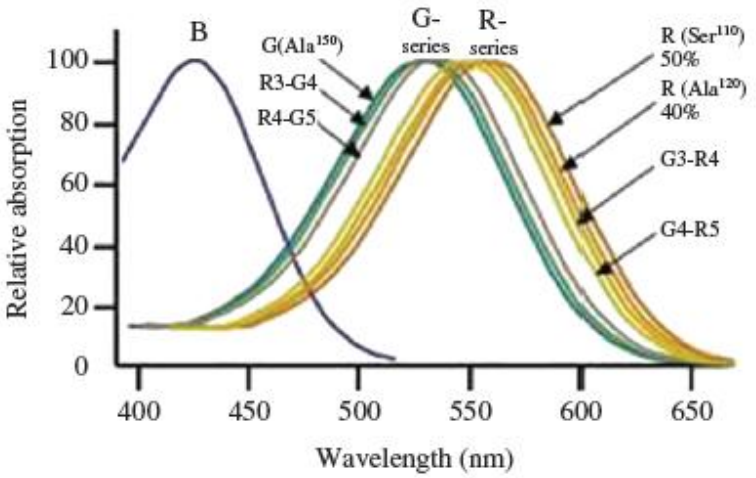
Examples:

Red Blindness

The person is not able to see red. The person sees shades of green rather than red.

Green Blindness

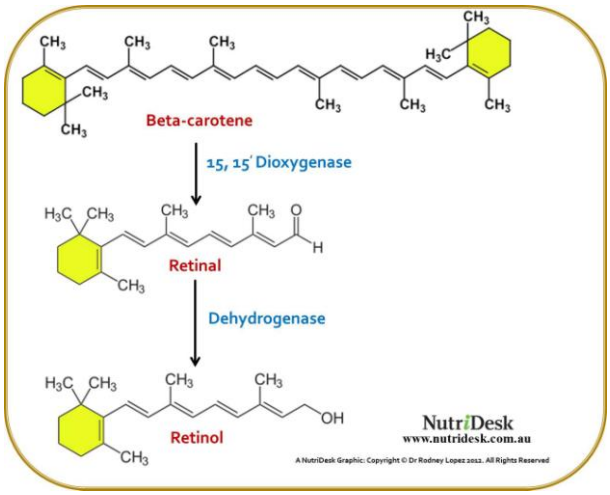
The person is not able to see green. The person sees shades of red rather than green.



Location	180
AA change	Serine Alanine
Wavelength	560 nm 530 nm

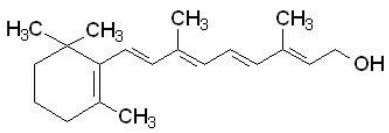
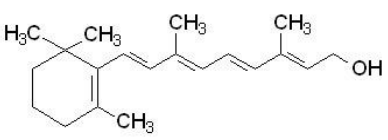
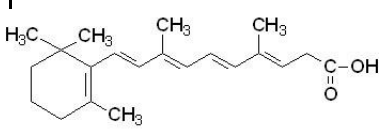
Single Nucleotide polymorphism

The individual differences result from single nucleotide polymorphisms. Recall that changing any of the previously mentioned amino acids by hydroxylation or dehydroxlation will result in a 10 nm-shift. This is exactly the case at hand. There are variations among individuals in how colors are seen if these single amino acids are changed.

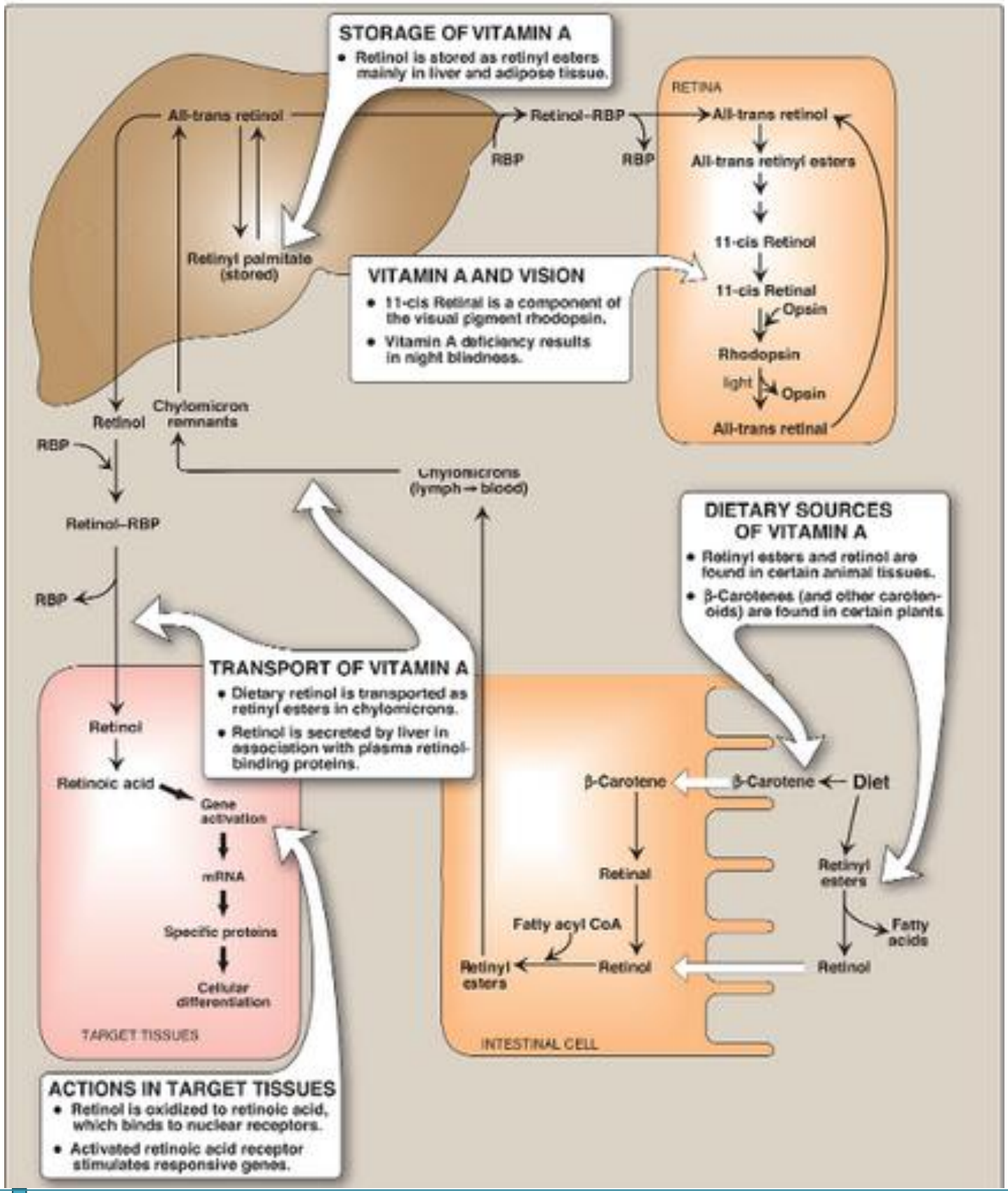


Metabolism of Vitamin A:

Source of vitamin A:
Vitamin A cannot be synthesized, and is thus obtained by diet. Its main source is β-carotene, which is then cleaved into retinal or retinol. There are three forms of vitamin A in the body: retinal, retinol, and retinoic acid depending on the functional group.

		
11-*cis*-retinal	Retinol	Retinoic Acid

Absorption, metabolism, storage, action of vitamin A



Dietary VA is digested in the intestinal lumen and absorbed by enterocytes, which convert β-carotene into retinal then retinol, to which fatty acids are attached. This results in the formation of retinyl esters. REs are packaged into chylomicrons and secreted into the lymphatic circulation. As they travel in the blood stream, chylomicrons are converted by the lipoprotein lipase into chylomicron remnants, which are taken up by hepatocytes, where the REs are hydrolyzed into retinol. Retinol can either be re-esterified into retinyl esters for storage in stellate liver cells in case of excess retinol, or released and transported by retinol-binding proteins to target cells where it is catabolized into retinoid, retinal, retinoic acid (RA), or other metabolites. Retionoic acid is a transcription factor by itself which can heterodimerize with other proteins and translocate into the nucleus, inducing or inhibiting gene expression. Retinoic acid can also result in cancer (due to its role in cell proliferation).

Deficiency of vitamin A:

- Night blindness, follicular hyperkeratinosis, increased susceptibility to infection and cancer and anemia equivalent to iron deficient anemia.

- Prolonged deficiency: deterioration of the eye tissue through progressive keratinization of the cornea (xerophthalmia)