Visual transduction

Prof. Mamoun Ahram
Neuroscience, Biochemistry
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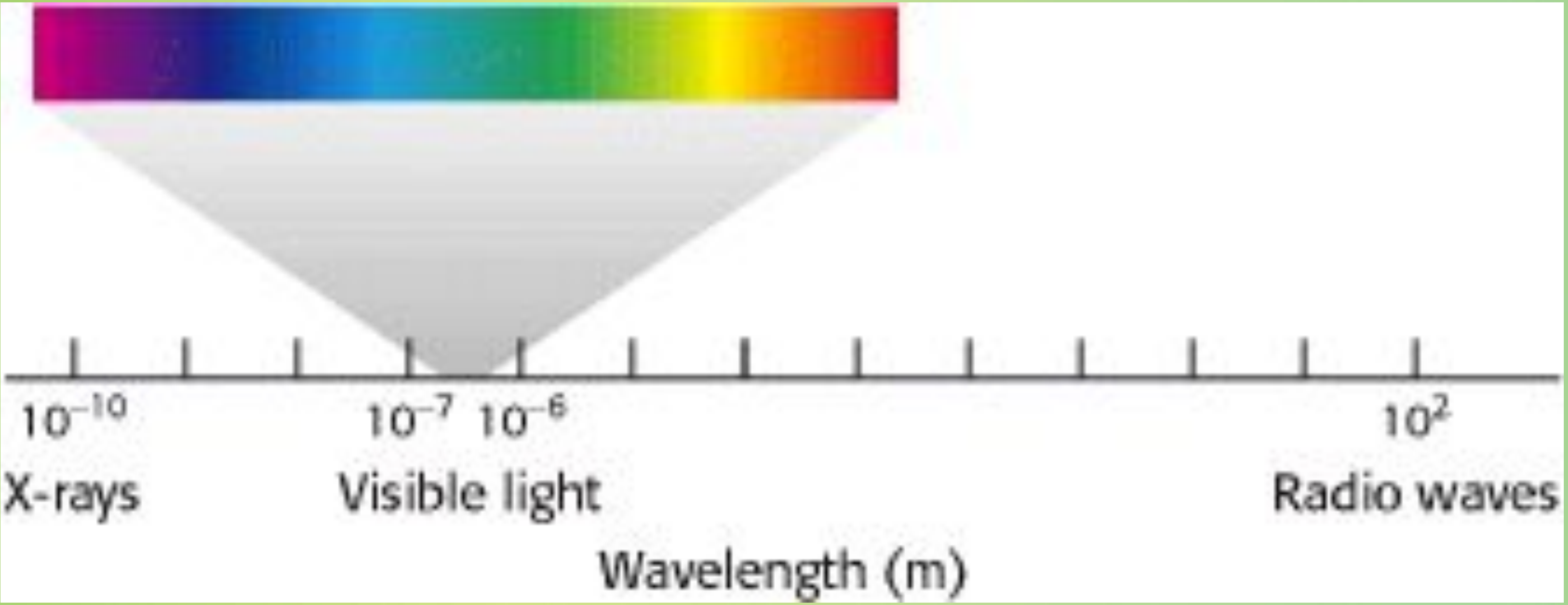
References

- **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)**

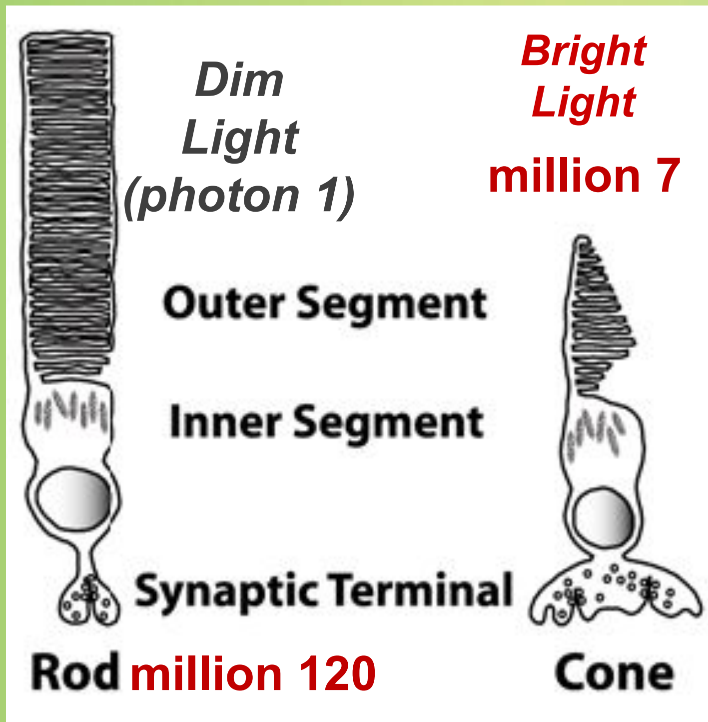
Lecture outline

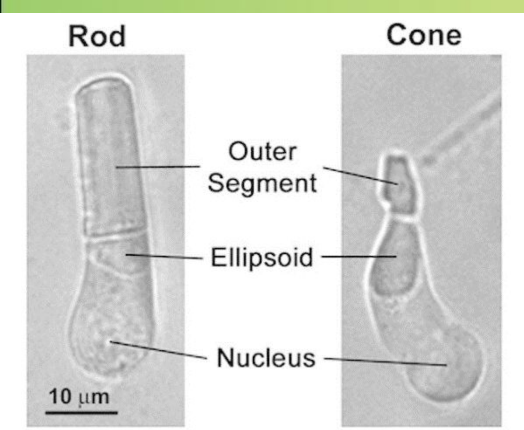
- **Visual transduction (dim vs. bright light)**
  - Components (cells and molecules)
  - Mechanisms of activation, amplification, and termination
- **Color blindness**

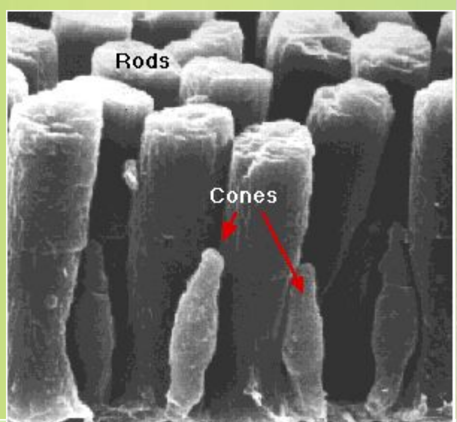
Basics of human vision

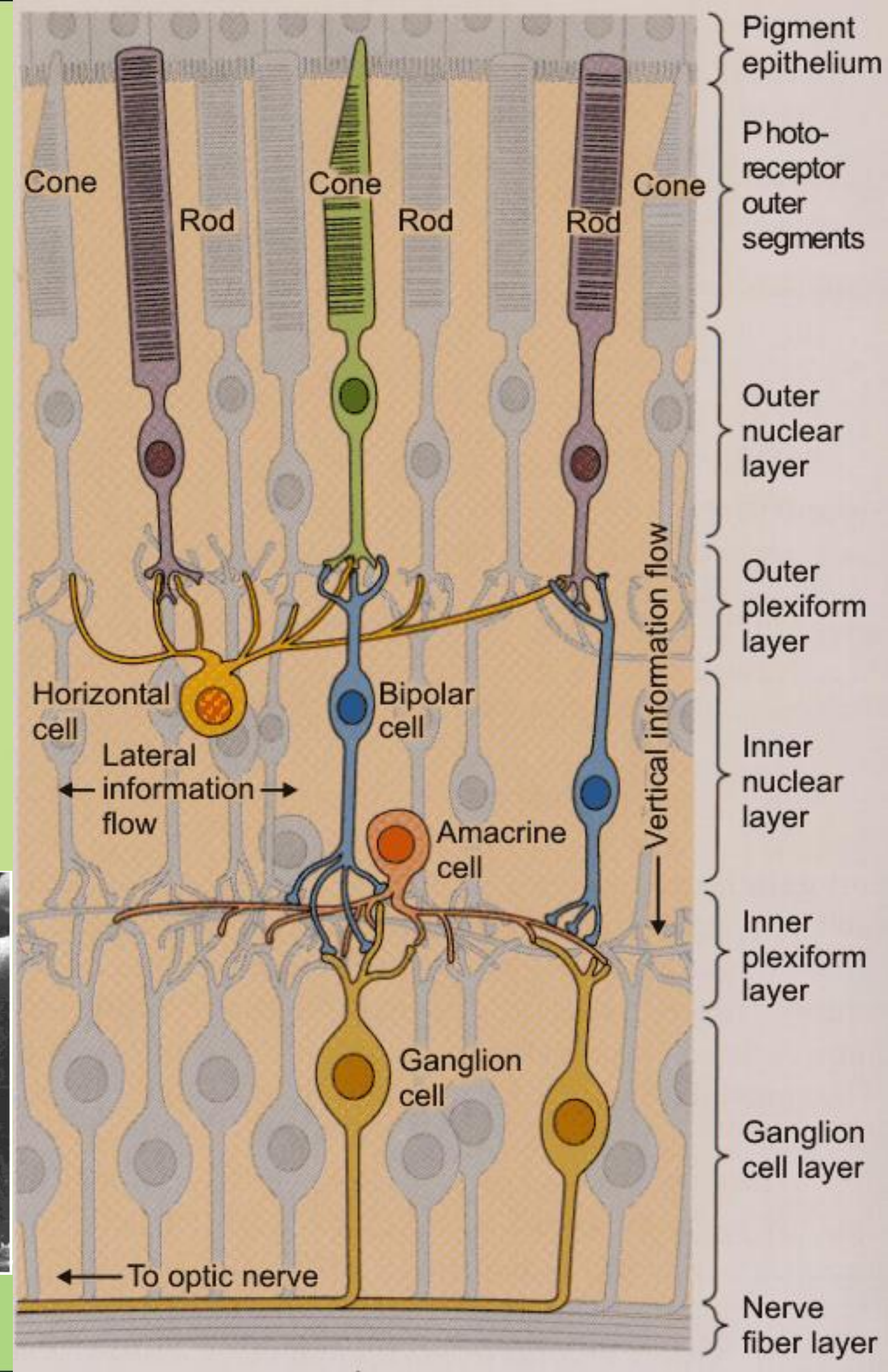


Rods and cones



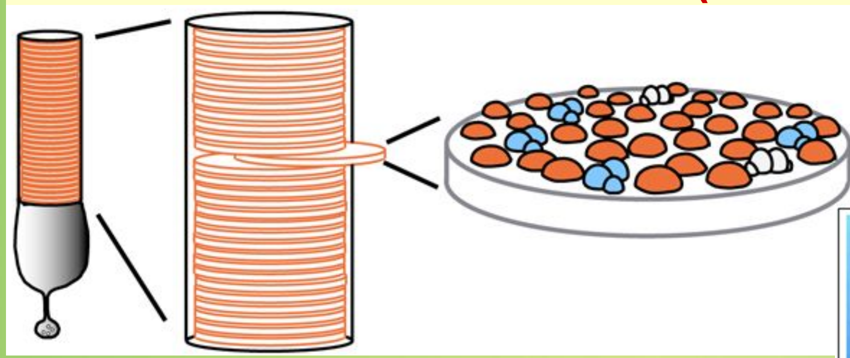




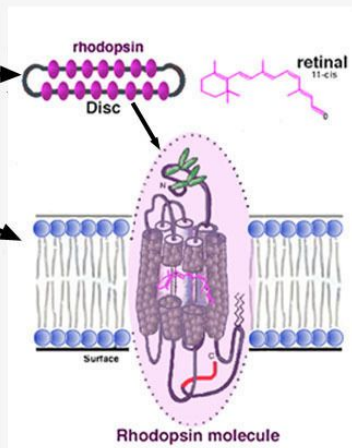


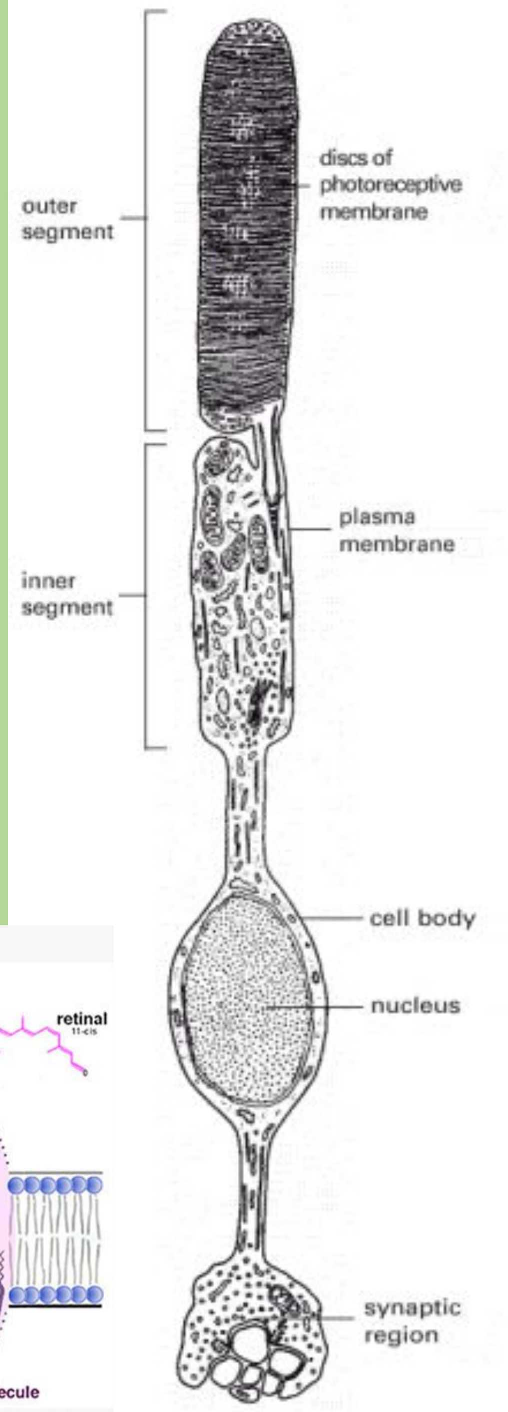
More on rod cells

1. **Rod cells consist of four regions: The inner segment, the cell body (contains the cellular organelles), <u>and the synaptic region</u>.**
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").



outer segment

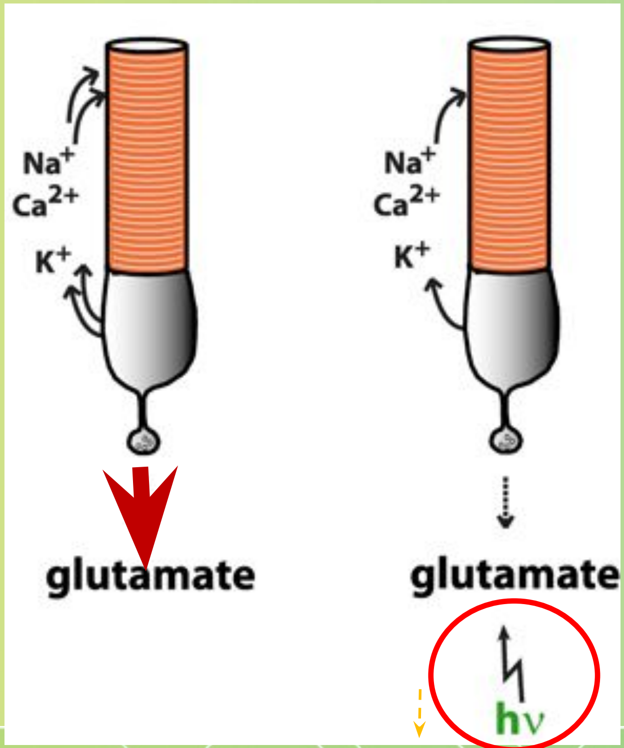




The dark current

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.**

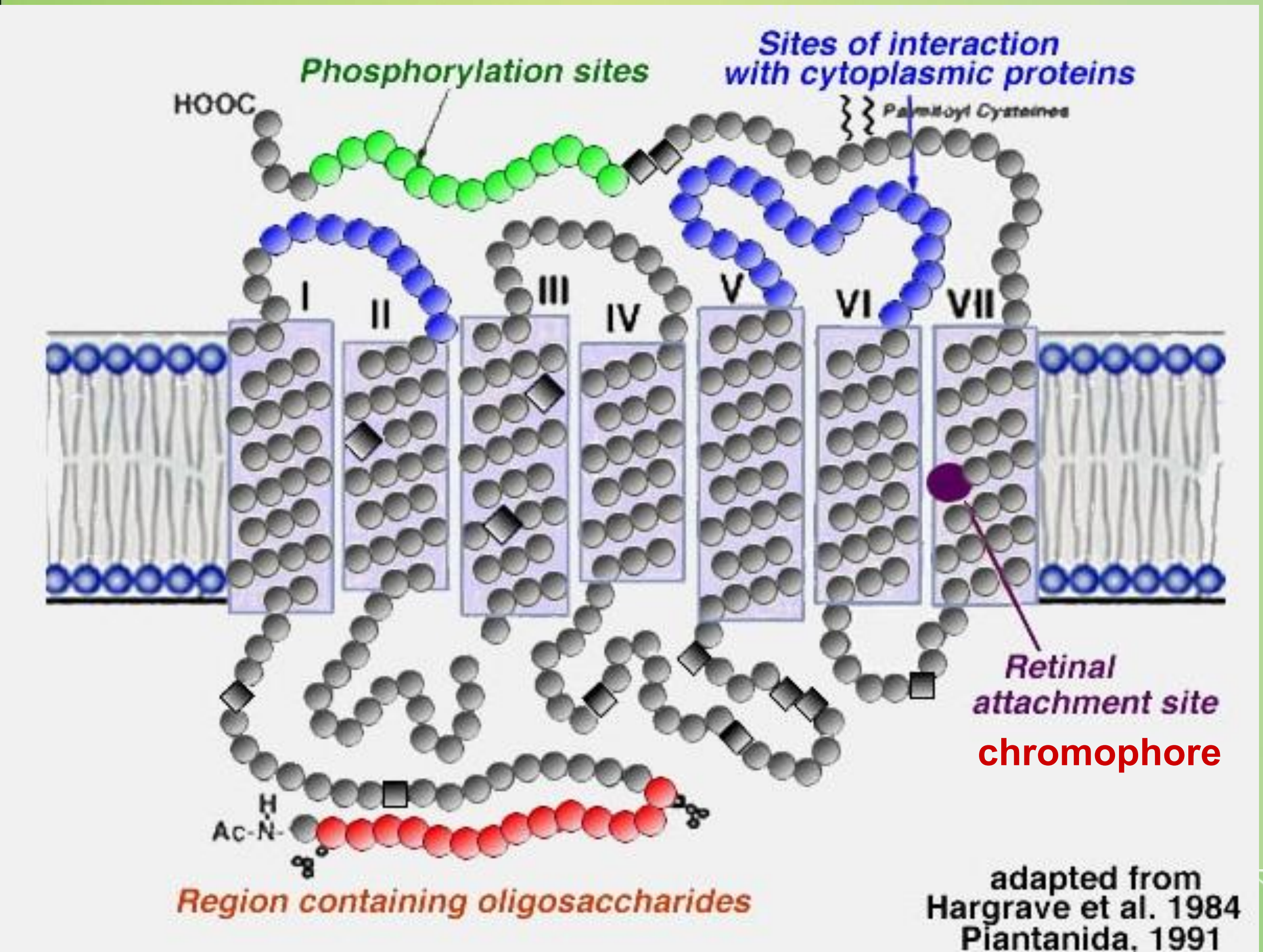
Generation of vision signals

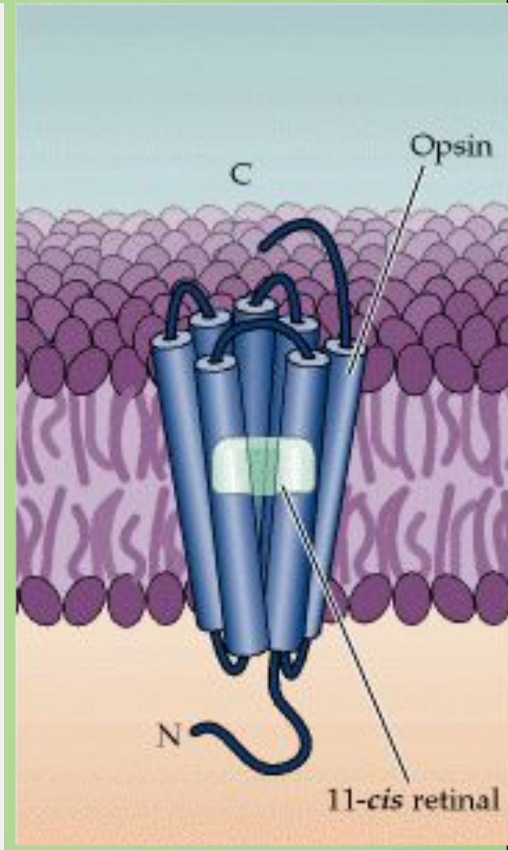
The players

- Rhodopsin (opsin + pigment molecule)
- Transducin
- Phosphodiesterase
- Na^+-gated channels
- Regulatory proteins

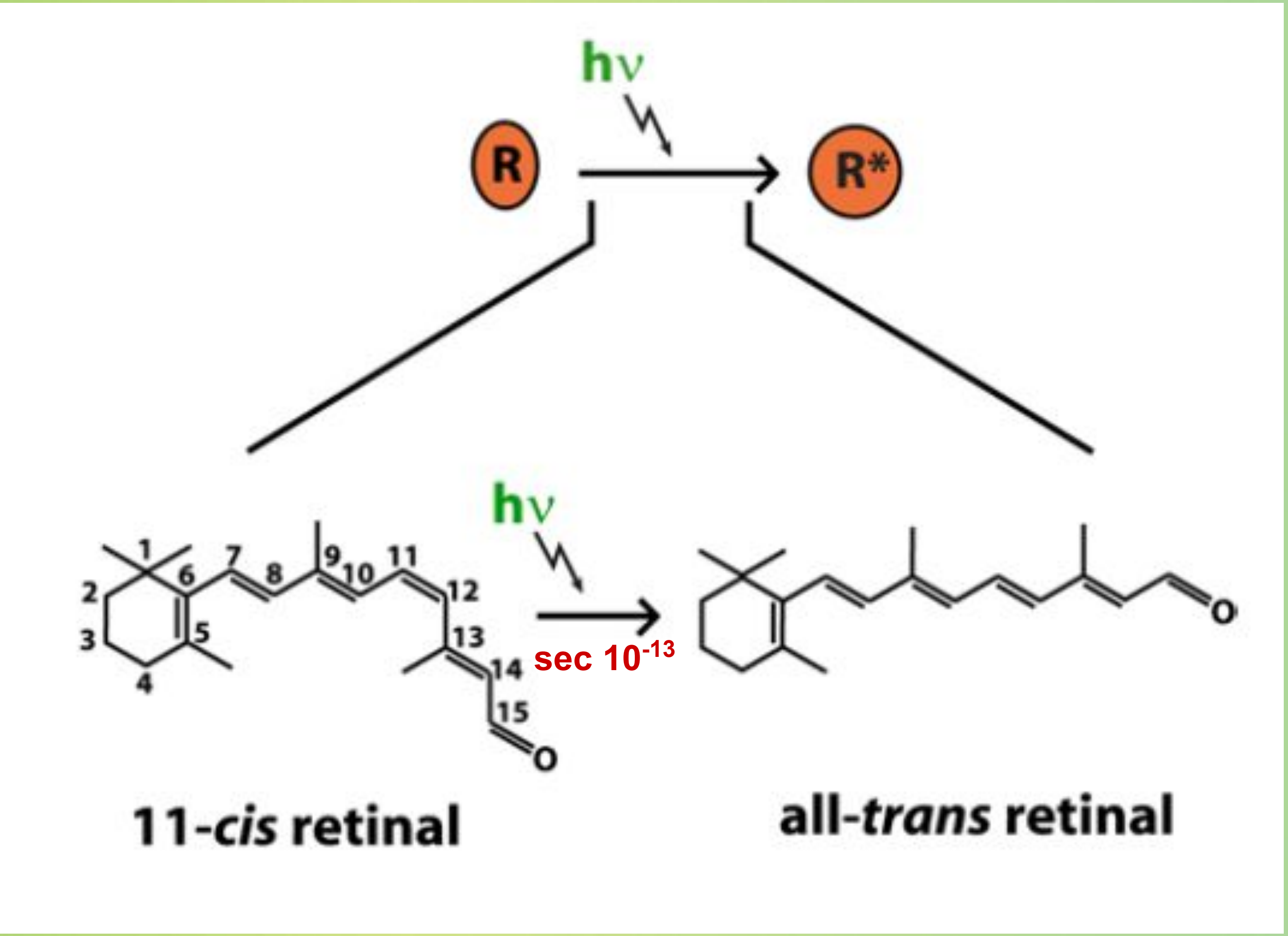
Rhodopsin

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

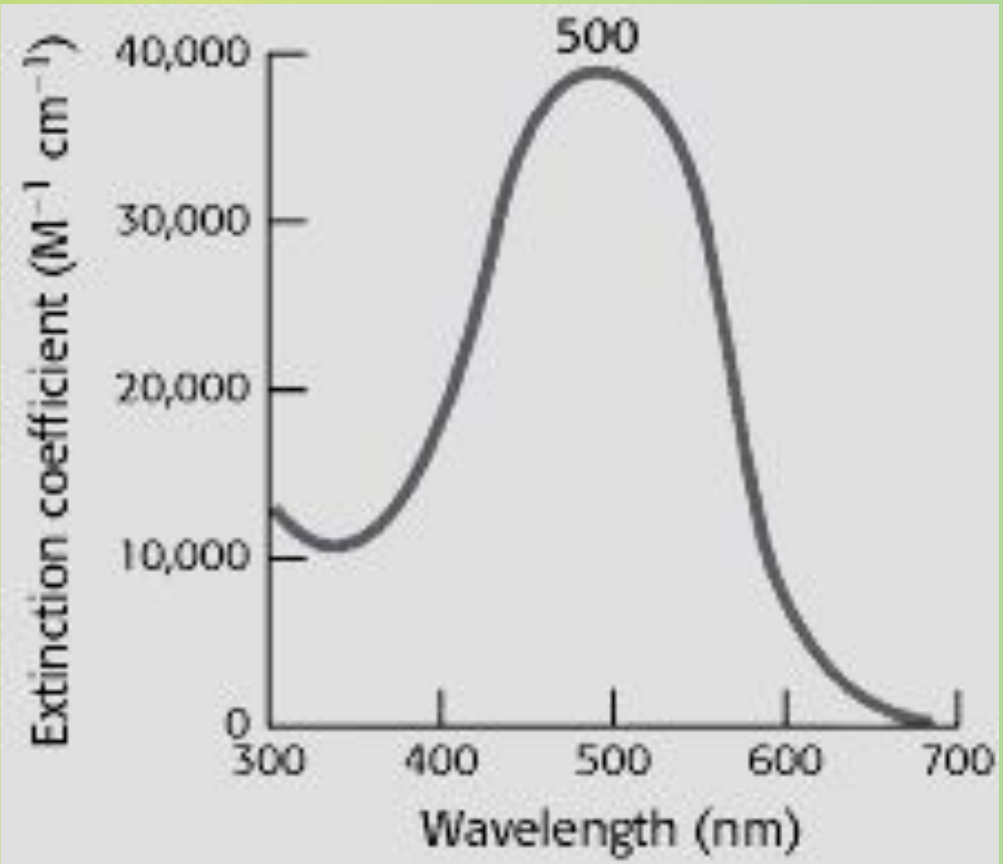




The chromophore (11-cis-retinal)

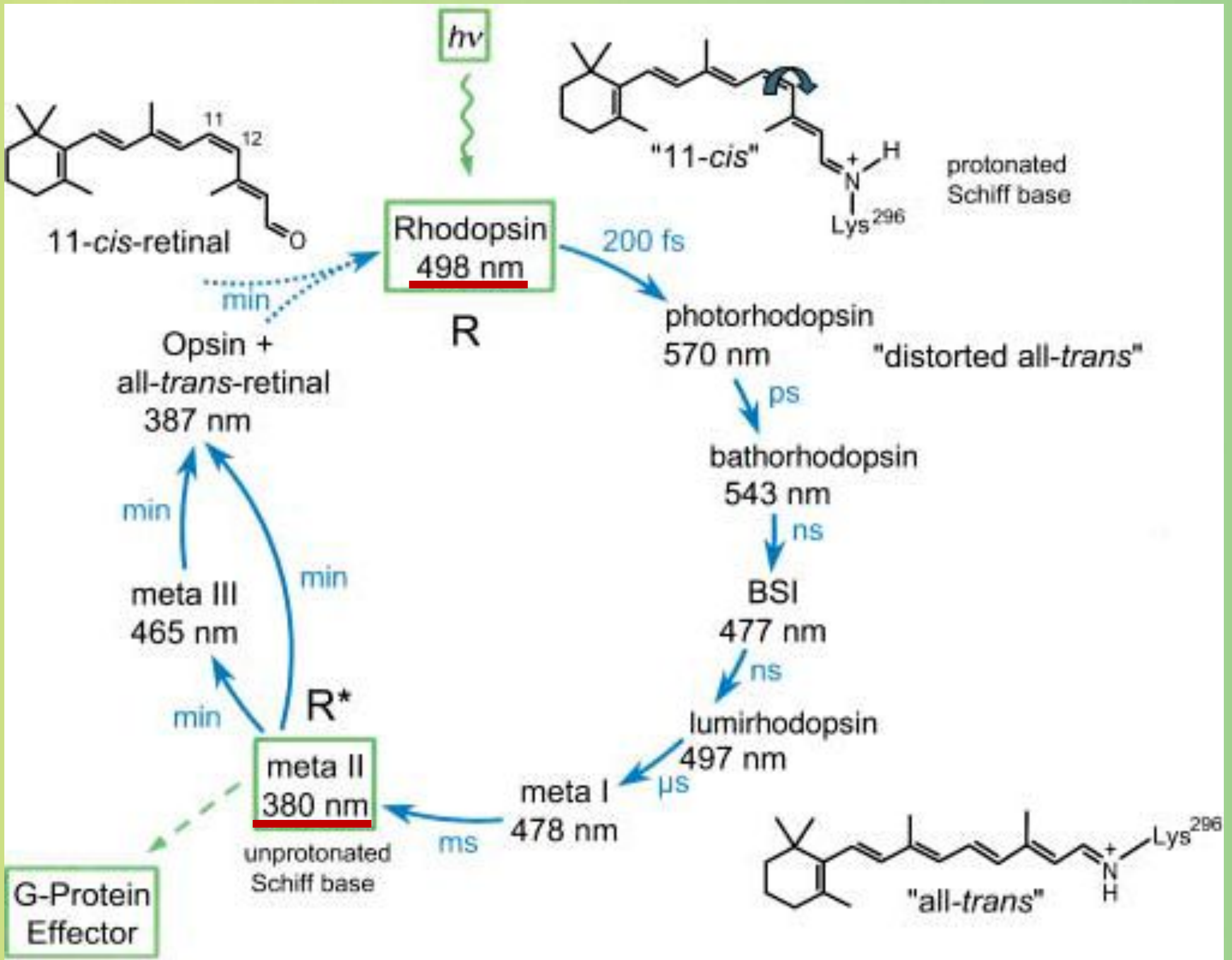


Light absorption by rhodopsin

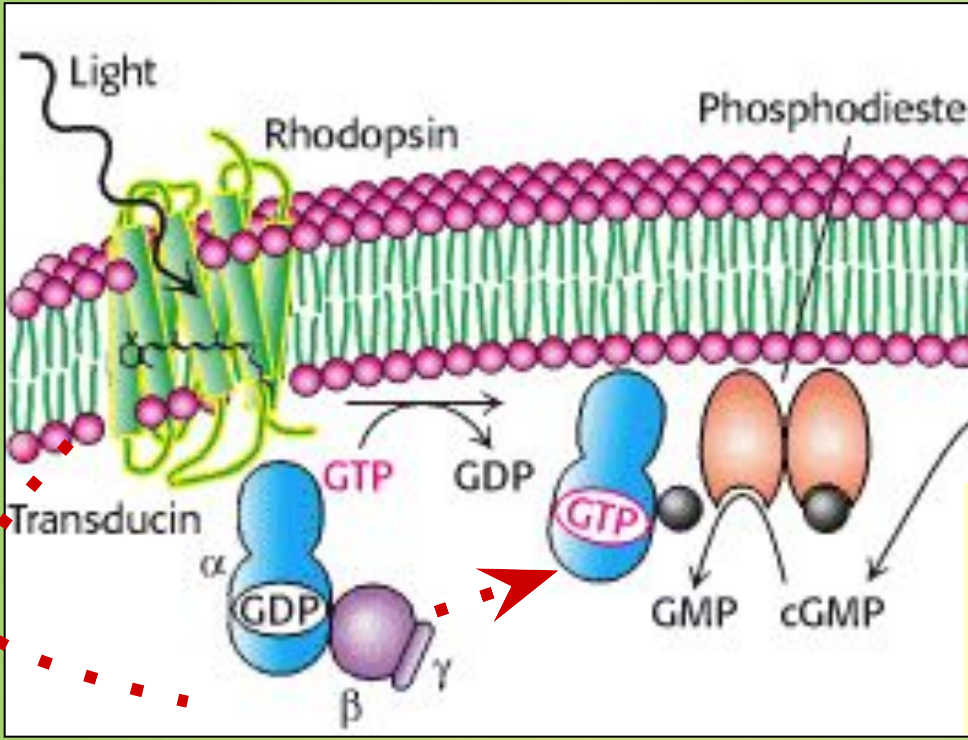


Rhodopsin intermediates

- 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.



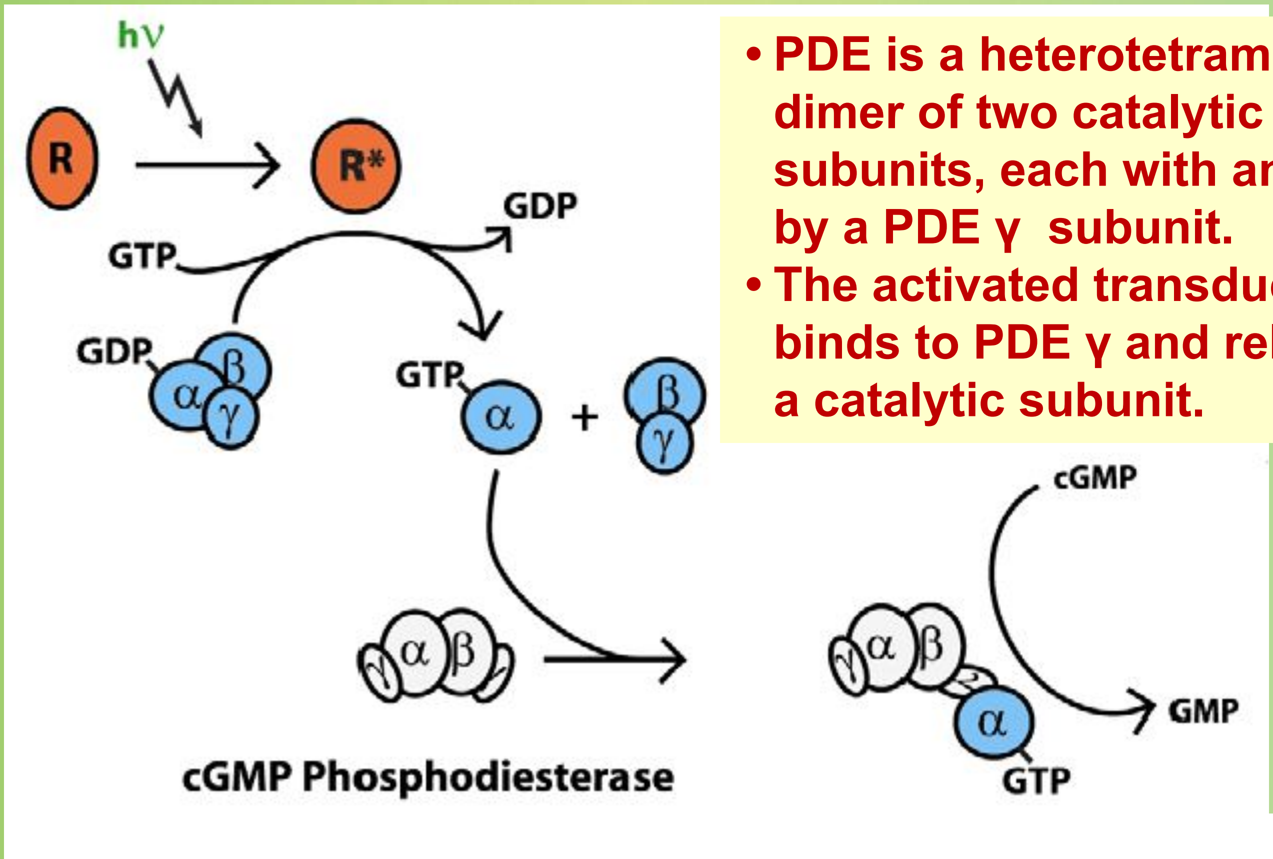
Transducin → Phosphodiesterase (PDE)



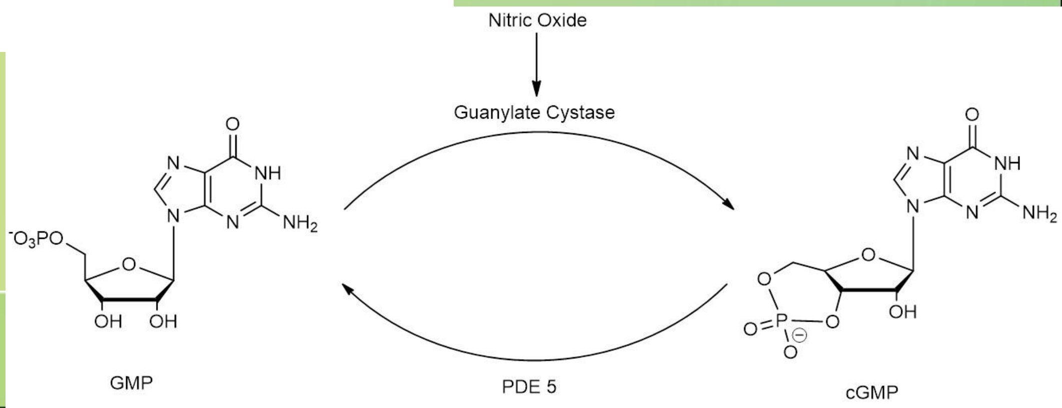
R* binds transducin and allows the dissociation of GDP, association of GTP, and release of the α subunit.

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.

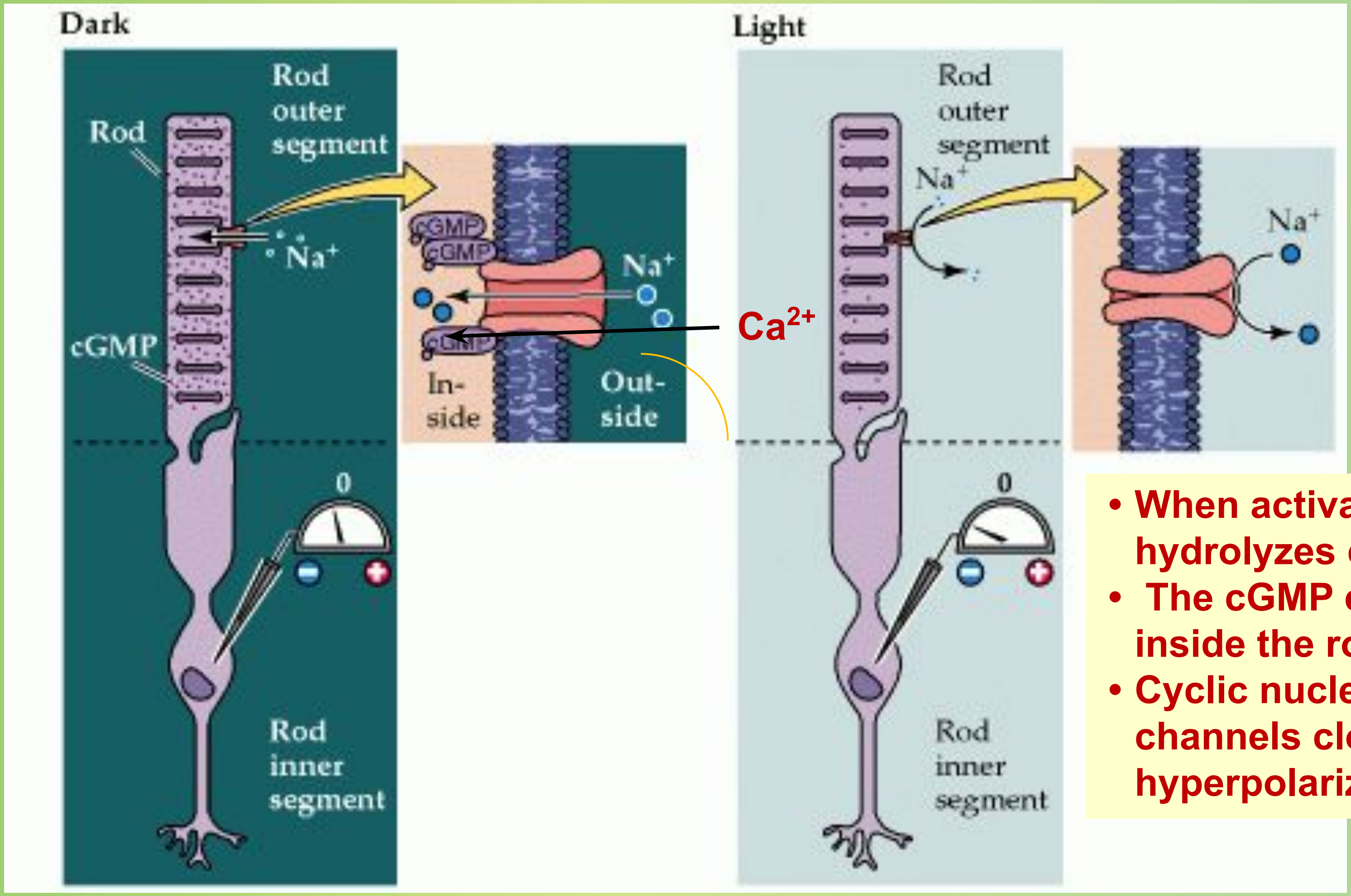
Activation of phosphodiesterase



- 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.

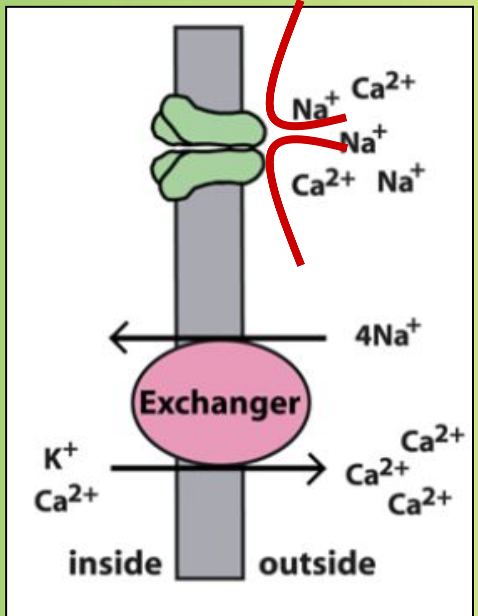


cGMP-gated channels



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

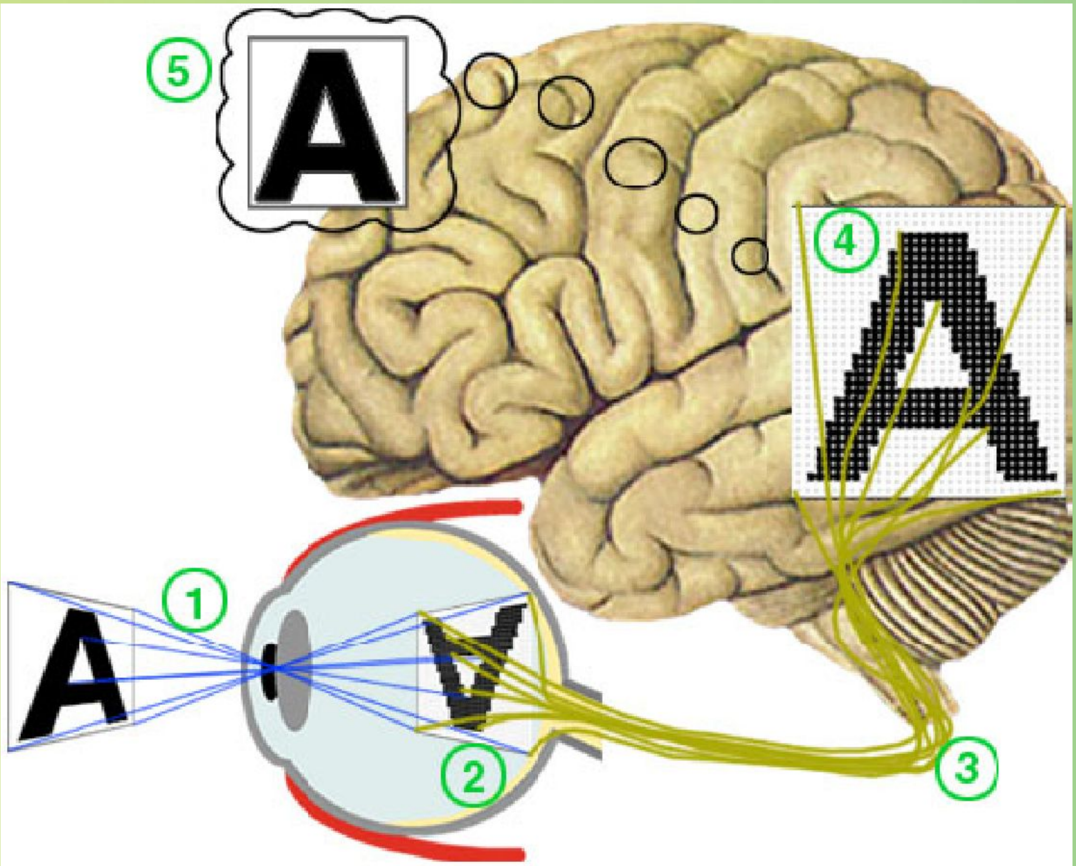
Levels of calcium ions are reduced, too.

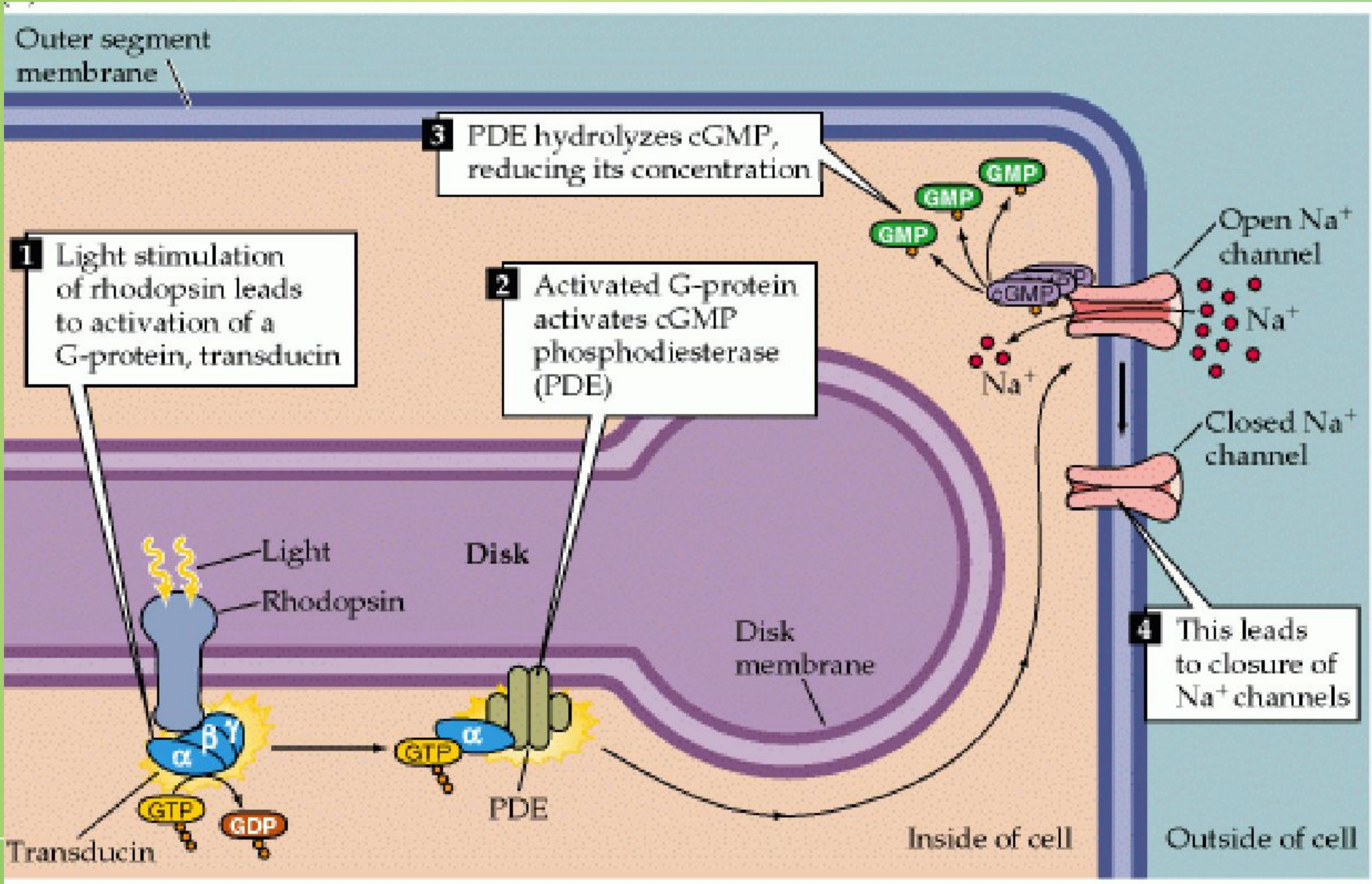


When the channels close, Ca^{2+} ceases to enter, but extrusion through the exchanger continues, so intracellular $[Ca^{2+}]$ falls.

Creating an image

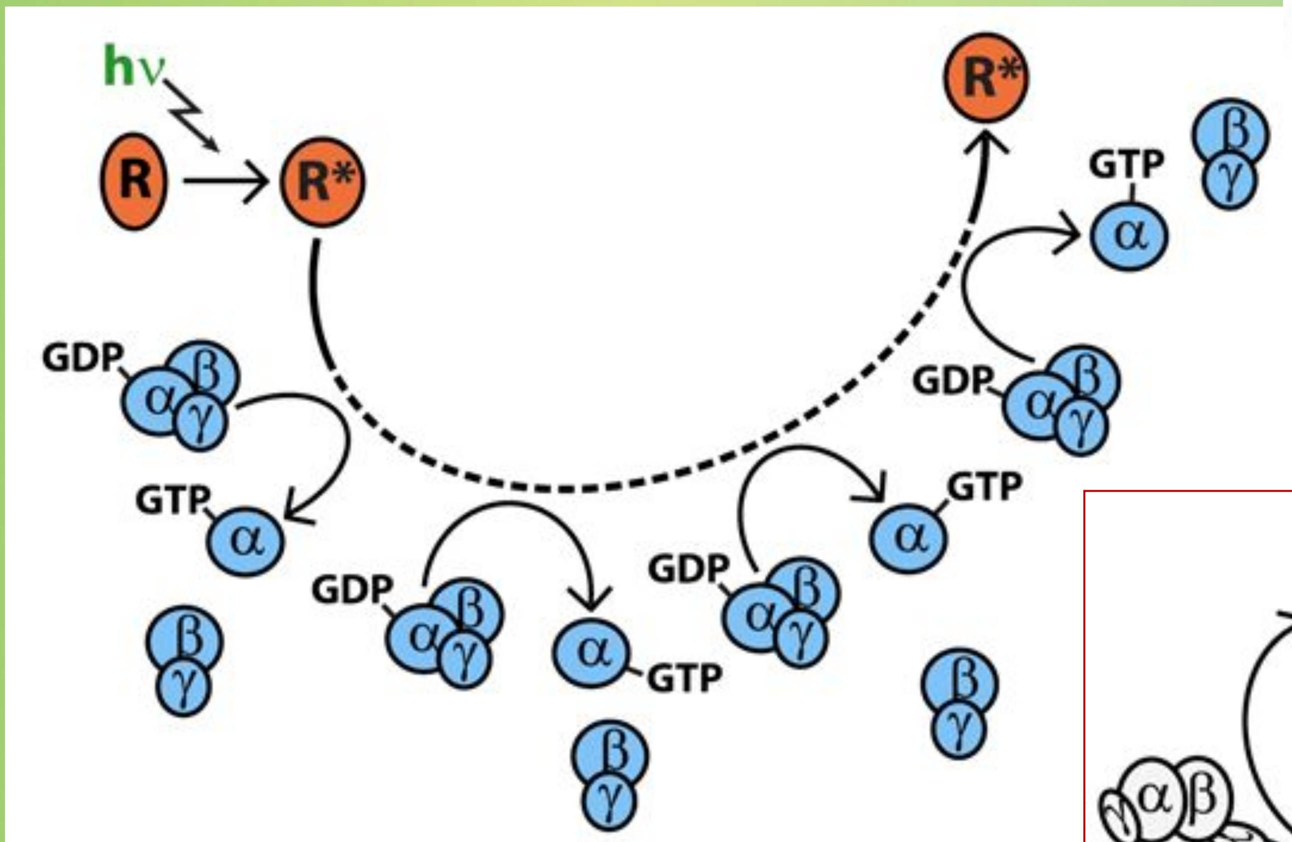
- The large potential difference travels as an electrical impulse down the rod cell to the synaptic terminal and is then transferred to an adjoining nerve cell.
- The nerve cell carries this impulse all the way to the brain.
- The brain then determines where the nerve impulse originated and interprets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

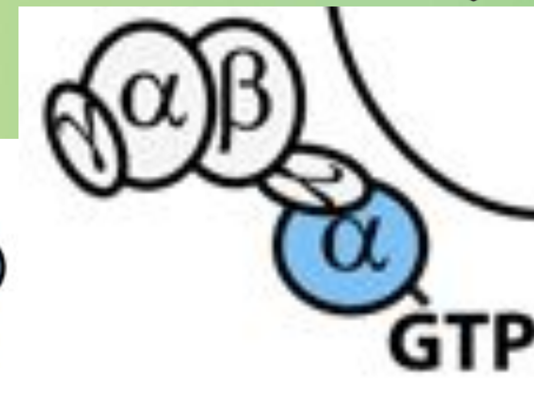


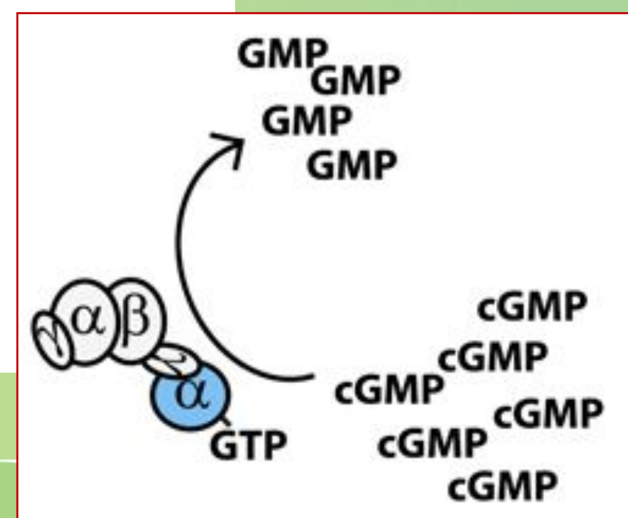

Outer segment membrane
3 PDE hydrolyzes cGMP, reducing its concentration
GMP
GMP
GMP
cGMP
Open Na+ channel
Na+
Na+
1 Light stimulation of rhodopsin leads to activation of a G-protein, transducin
2 Activated G-protein activates cGMP phosphodiesterase (PDE)
Closed Na+ channel
Light
Rhodopsin
Disk
Disk membrane
4 This leads to closure of Na+ channels
α
β
γ
α
GTP
GTP
GDP
PDE
Transducin
Inside of cell
Outside of cell

Signal amplification

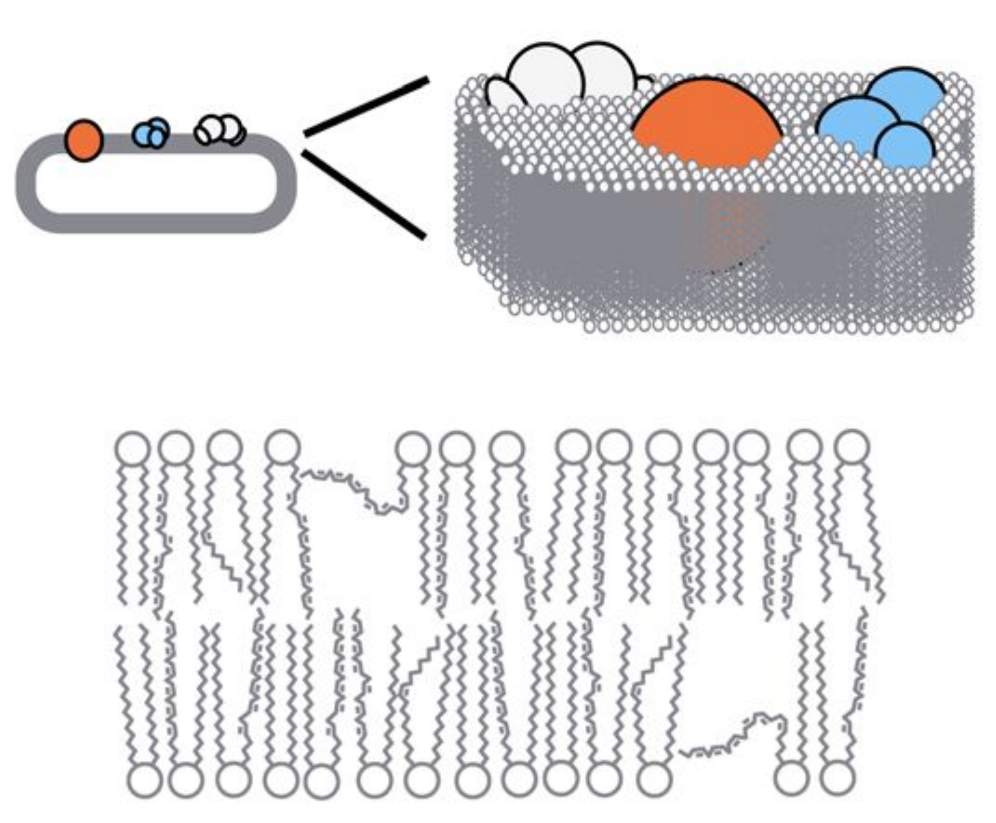
Rhodopsin (1) → Transducin (10 to >3000)
Transducin (1) → PDE (1)
PDE (1) → cGMP (10^3)







Facilitation of transduction



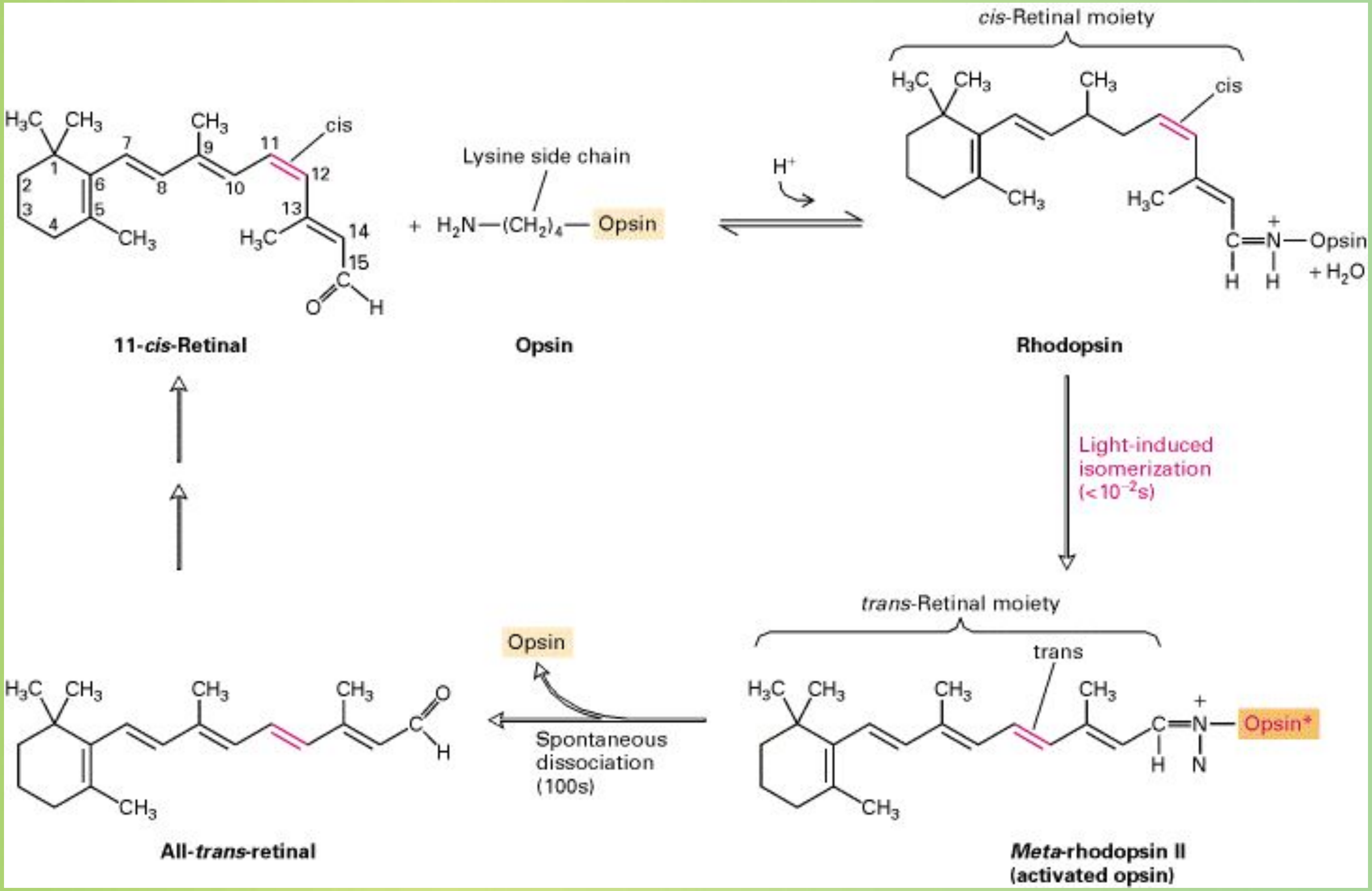
1. **2-dimensional surface**
2. **Low in cholesterol and high content of unsaturated fatty acids**
3. **<u>Cooperativity of binding</u>: The binding of one cGMP enhances additional cGMP binding and channel opening (n = ~3)**
4. **Since multiple cGMP molecules are required to open the channel, it will 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.

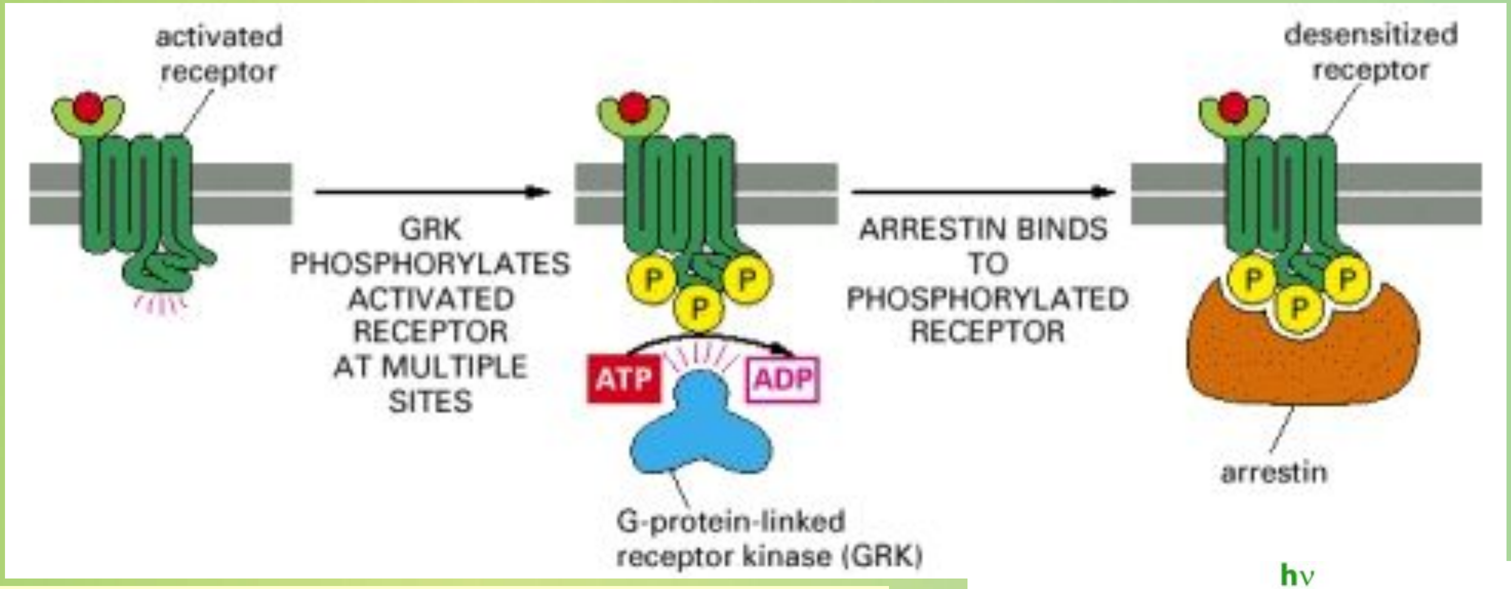
Signal termination

Mechanism I

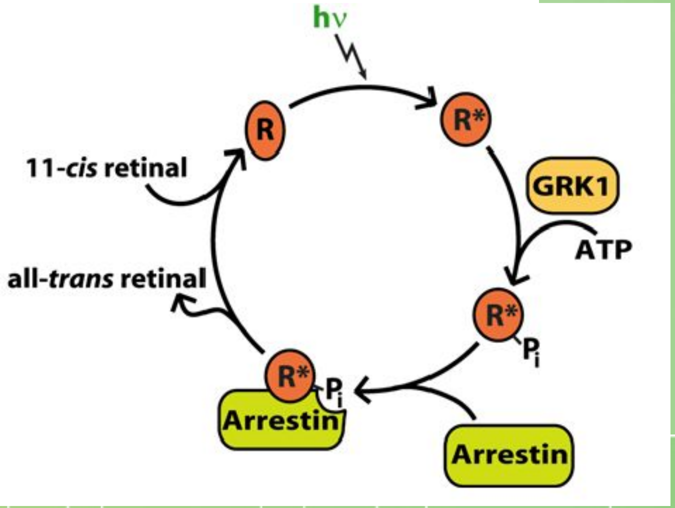
Unstable all-trans rhodopsin complex



Mechanism II
Arrestin binding

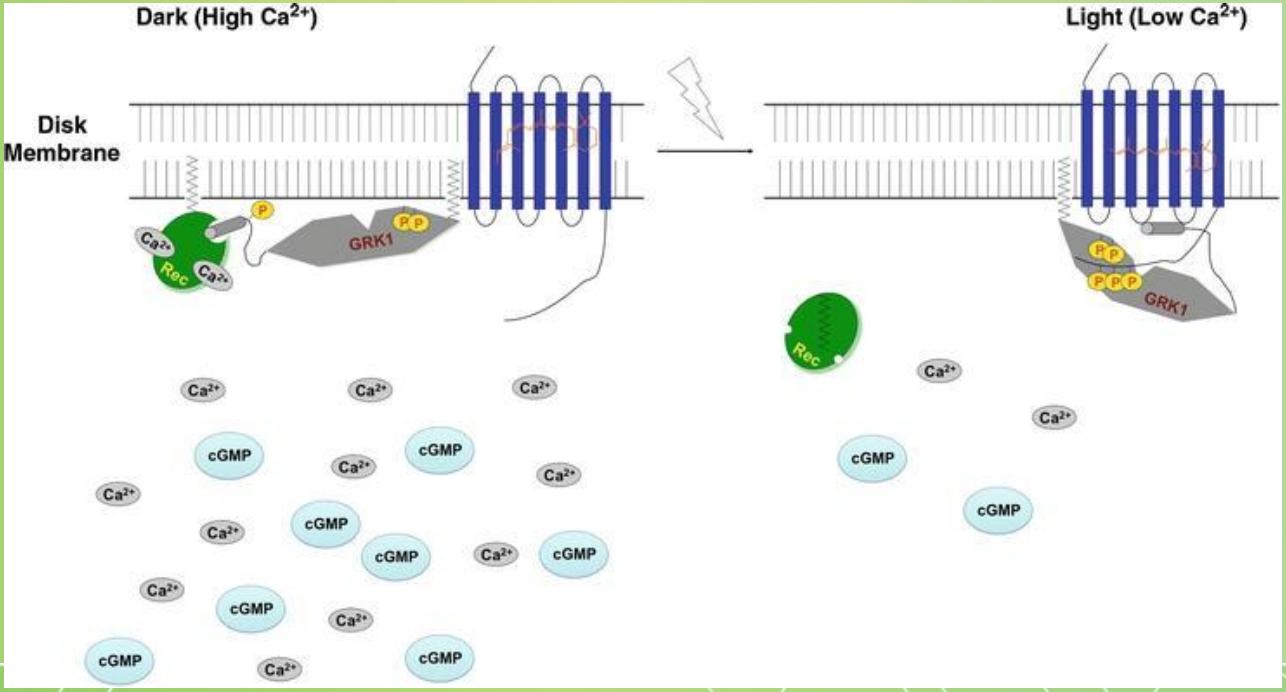


- Rhodopsin kinase 1 (GRK1) phosphorylates the C-terminus of R*.
- Phosphorylation of R* has two effects:
- 1. It decreases transducin activation
- 2. It facilitates binding to arrestin, which completely quenches its activity, and releases of the all *trans-retinal* regenerating rhodopsin.



Mechanism II (cont.)
GRK1 and recoverin

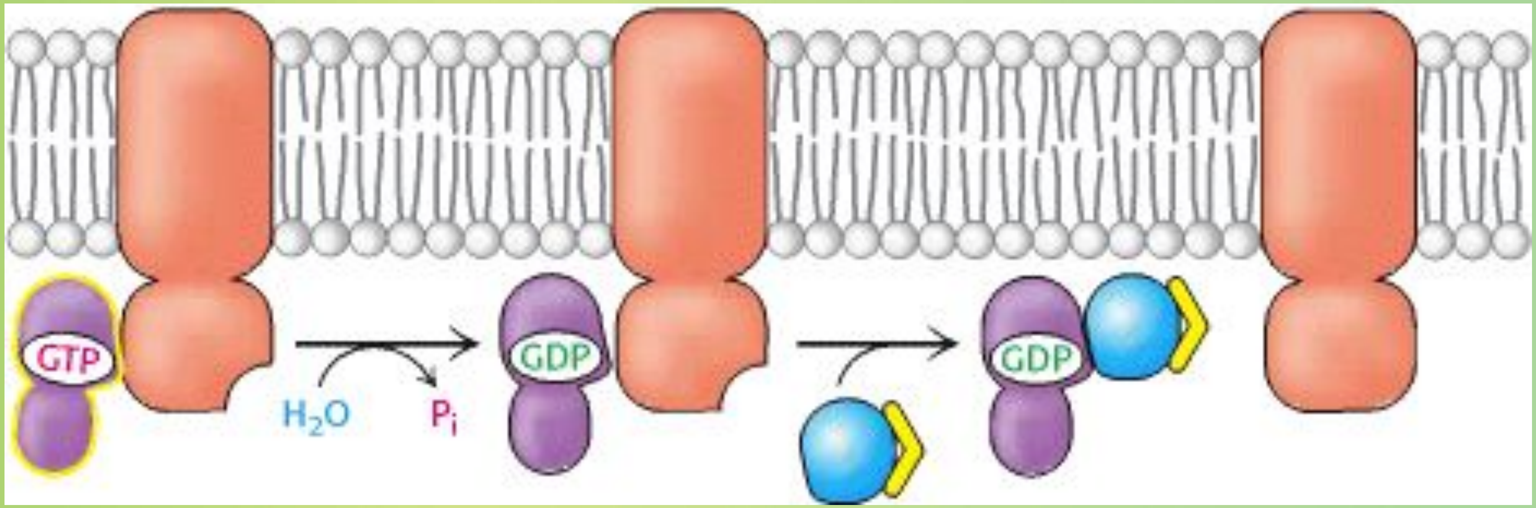
- GRK1 is more active at low [Ca^{2+}]. Why?
- In the dark, Ca^{2+} ions bind to a protein called recoverin allowing it to anchor to the membrane, bind to GRK1, and inhibit it.
- In contrast to Ca^{2+}-free recoverin that does not bind to GRK1, the recoverin-Ca^{2+} complex binds to the N terminus helix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



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

Mechanism III

Intrinsic GTPase activity of G protein

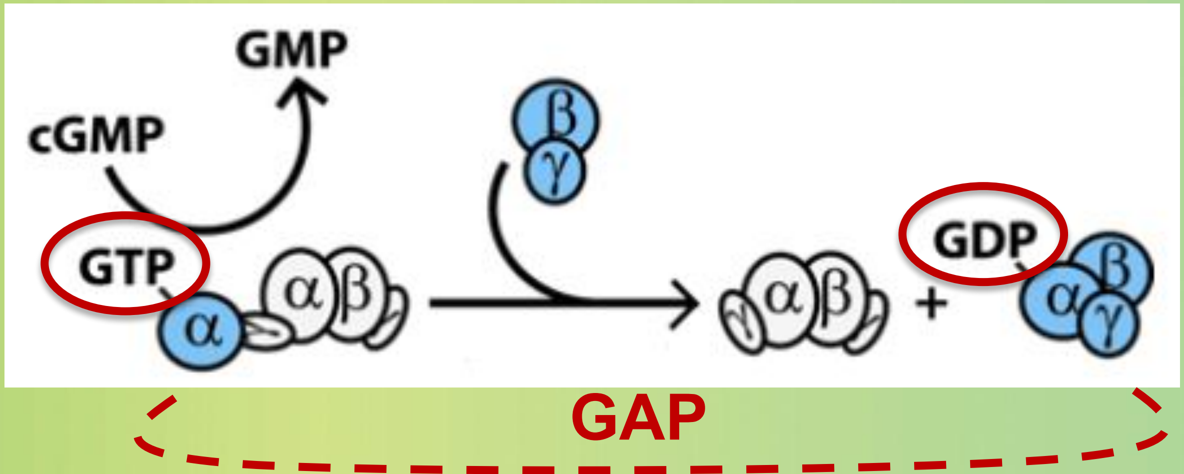


- Transducin has an intrinsic GTPase activity that hydrolyzes GTP to GDP.
- Upon hydrolysis of GTP to GDP, transducin α subunit releases the PDE γ subunit that re-inhibits the catalytic subunit.
- Transducin α-GDP eventually combines with transducin βγ

Mechanism IV

Facilitation of GTPase activity of G protein

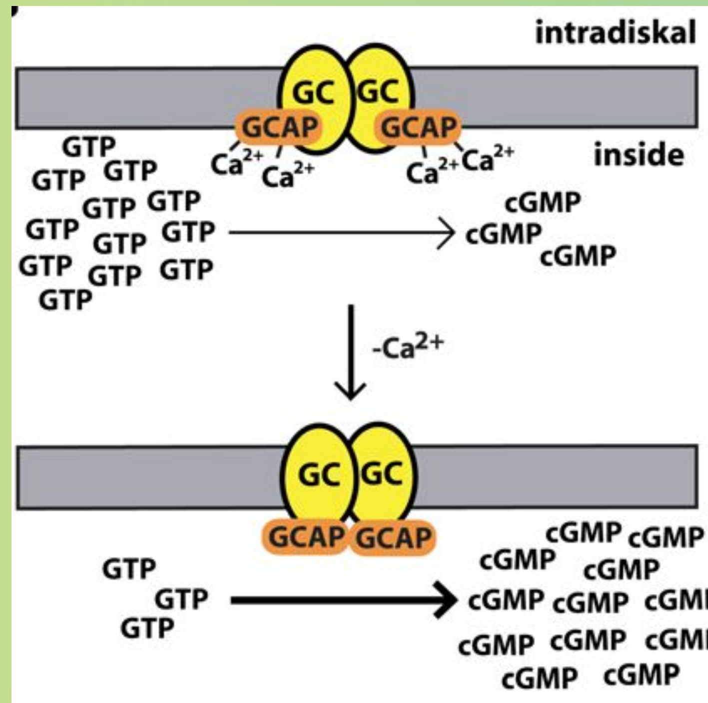
- **GTP hydrolysis is slow intrinsically, but it is accelerated by the GAP (GTPase Activating Protein) complex.**
  - **To ensure that transducin does not shut off before activating PDE, transducin and the GAP complex have a low affinity for each other, until transducin α-GTP binds PDEγ.**

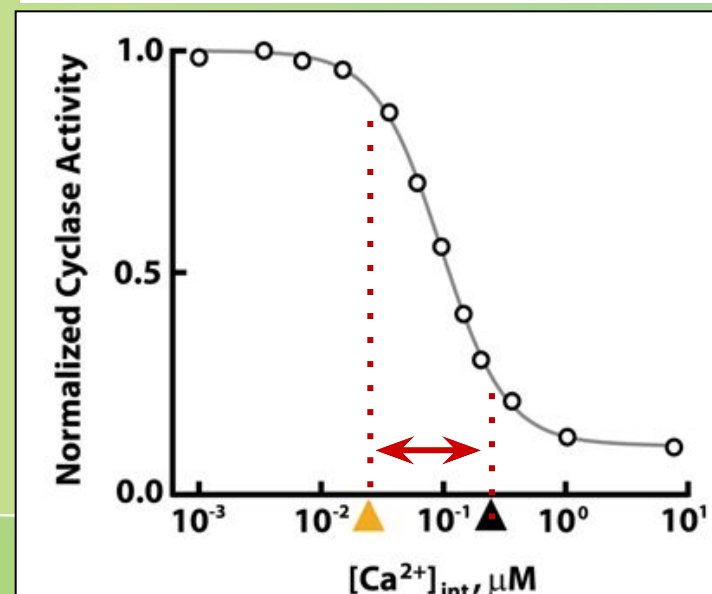


The inhibition of the Gα subunit by GTP hydrolysis and, hence, dissociation from PDE is the rate limiting step in the recovery of rod response to light.

Mechanism V
Guanylate cyclase

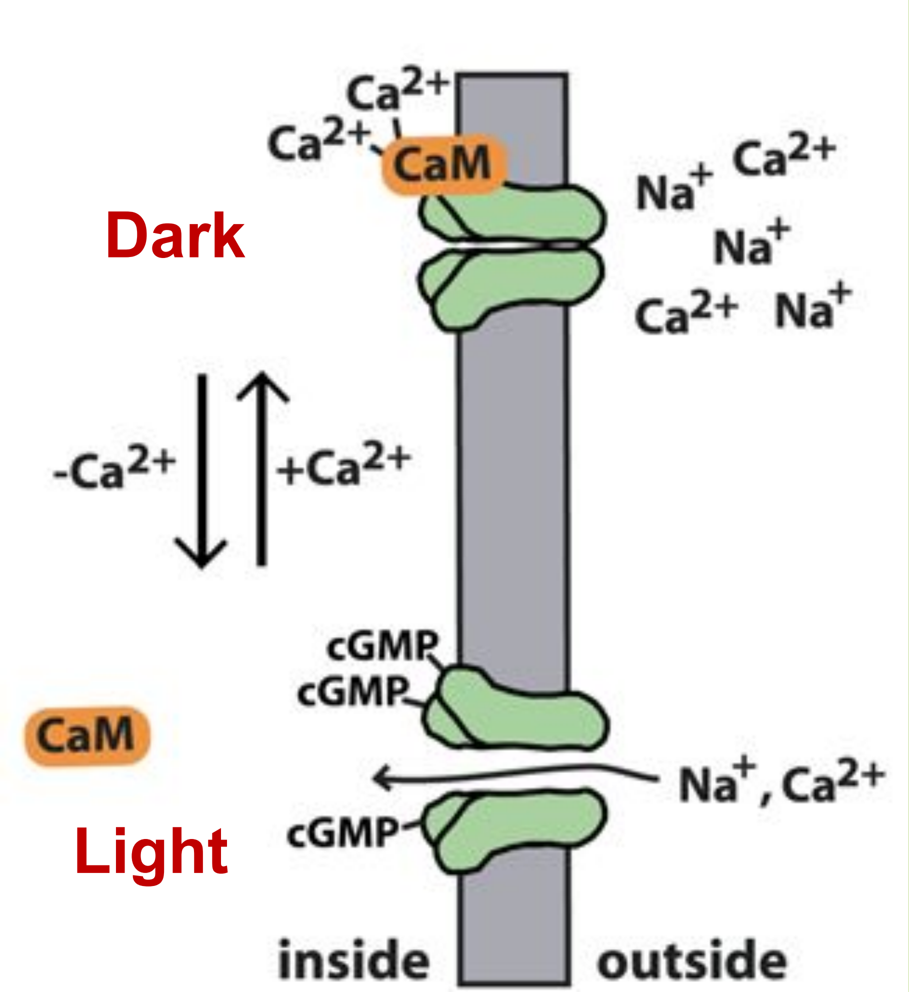
- In the dark, guanylate cyclase-activating proteins (GCAPs) bind Ca^{2+} blocking their activation of guanylate cyclase.
- A decrease in intracellular [Ca^{2+}] causes Ca^{2+} to dissociate from GCAPs leading to full activation of guanylate cyclase subunits, and an increase in the rate of cGMP synthesis.





Mechanism VI

Ca-calmodulin *and cGMP-gated channels*

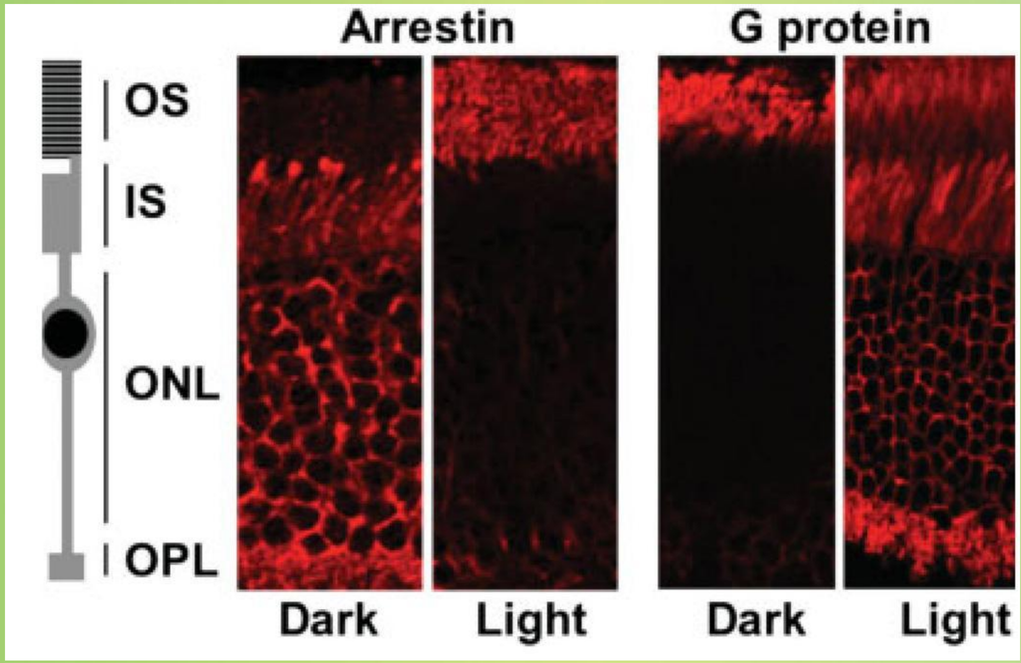


- In the dark, Ca^{2+}-Calmodulin (CaM) binds the channel and reduces its affinity to cGMP and shuts it down.
- During visual transduction, the decrease in intracellular $[Ca^{2+}]$ causes CaM to be released, the affinity towards cGMP increases, and the channel reopens in response to the slightest increase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

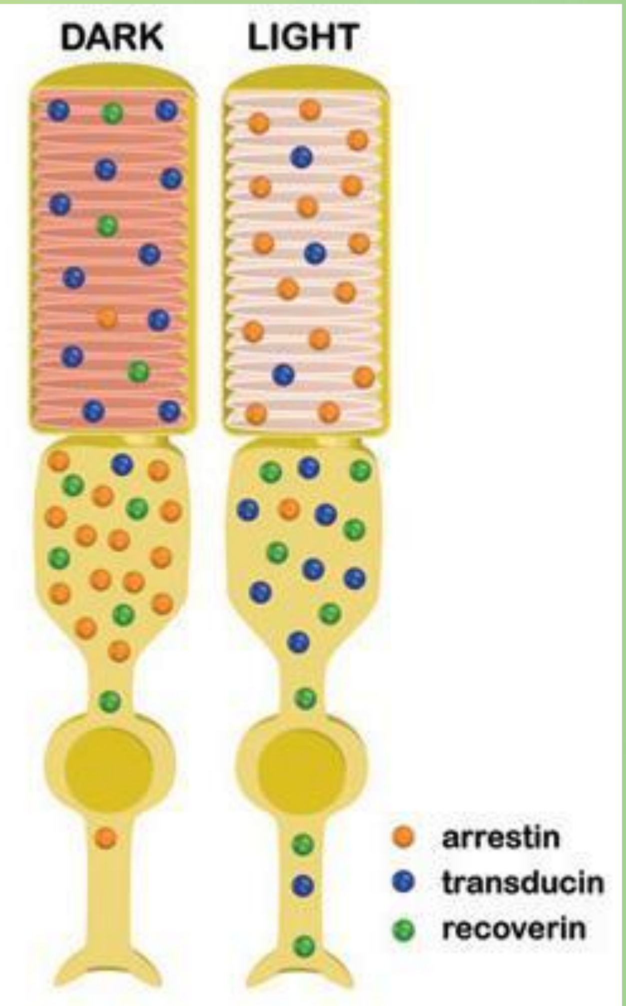
- *Note: Ca^{2+}-Calmodulin (CaM) also binds to GRK1 and inhibits it.*

Adaptation to light/dark conditions

Arrestin/recoverin/transducin distribution

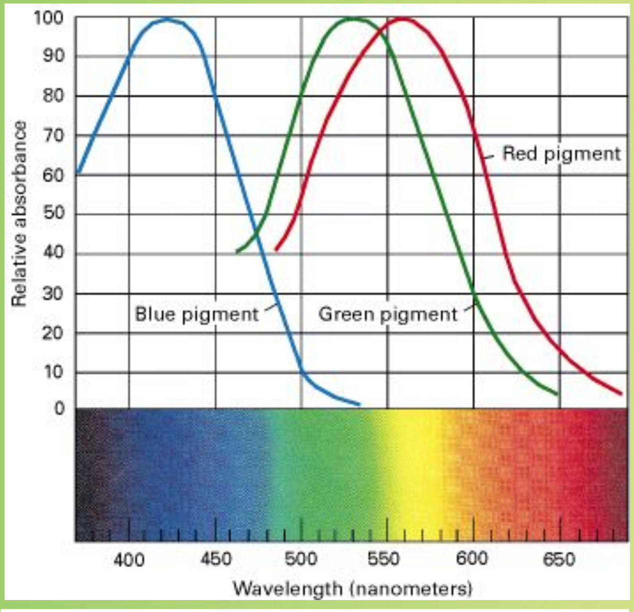


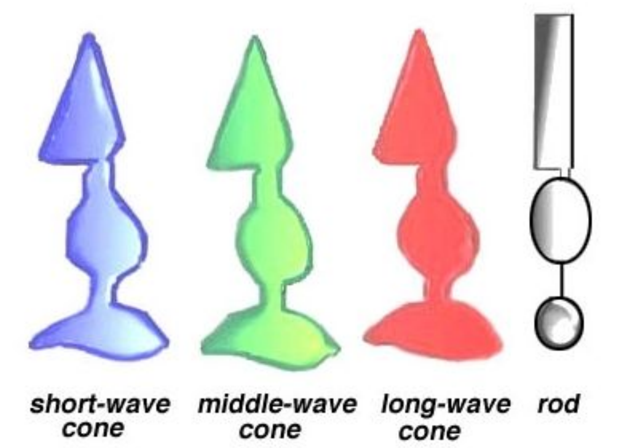
- In dark, the outer segment contains high levels of transducin and recoverin and low levels of arrestin (low inhibition; ready to be activated).
- In light, it is the opposite (high inhibition; ready to be inactivated).

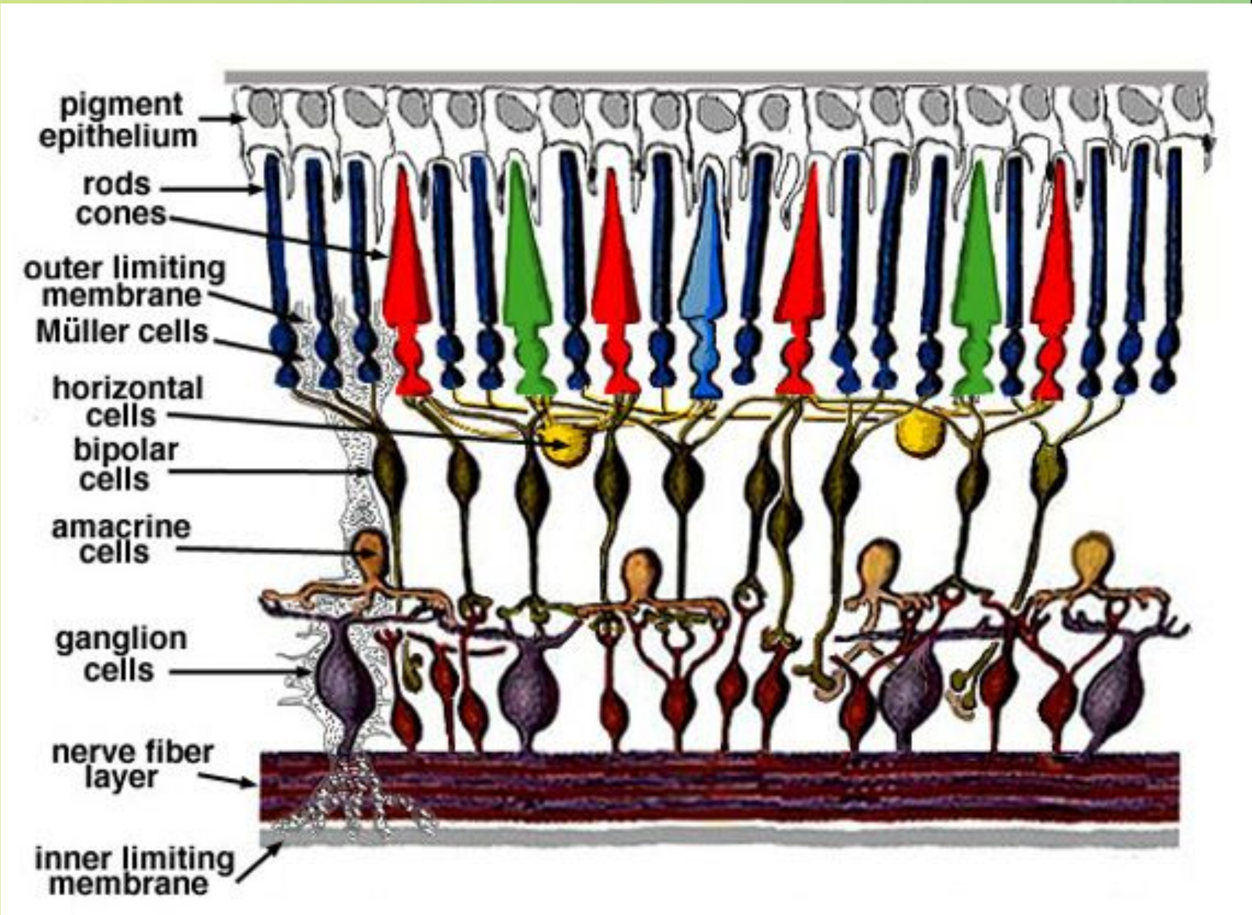


Color vision

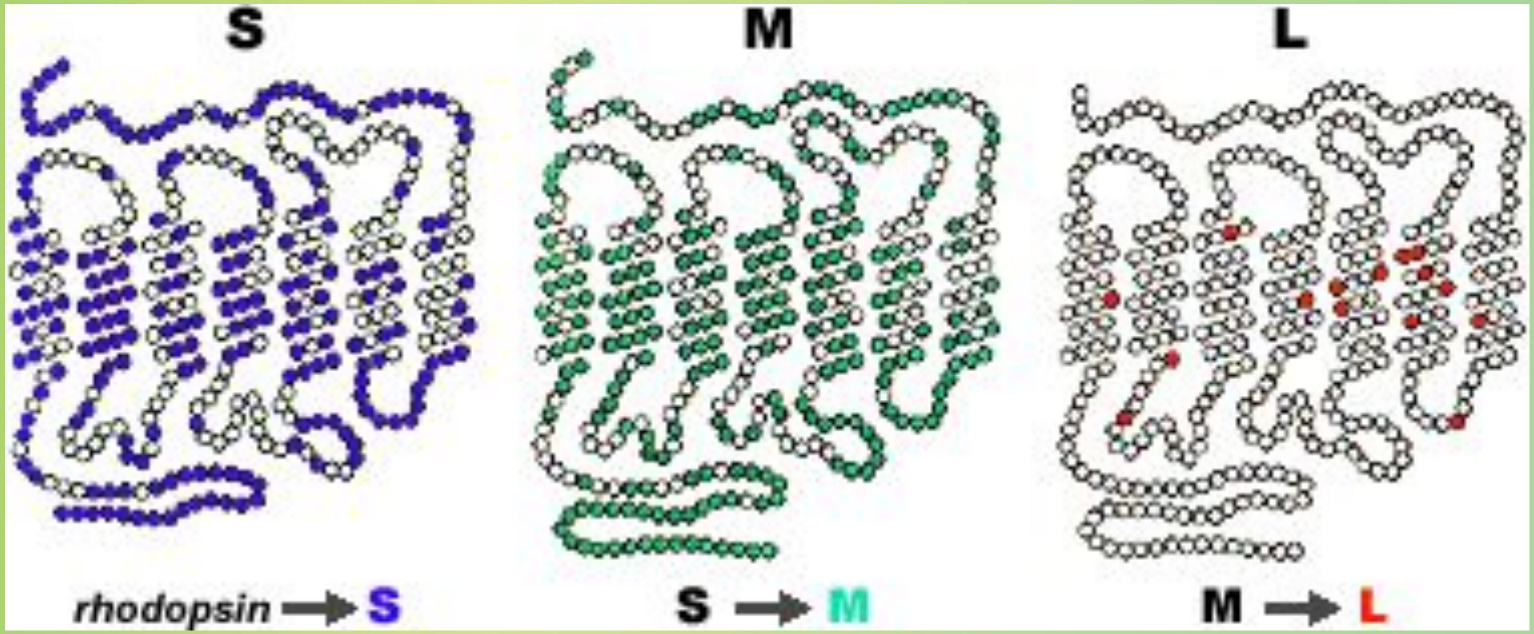
Cone photoreceptor proteins





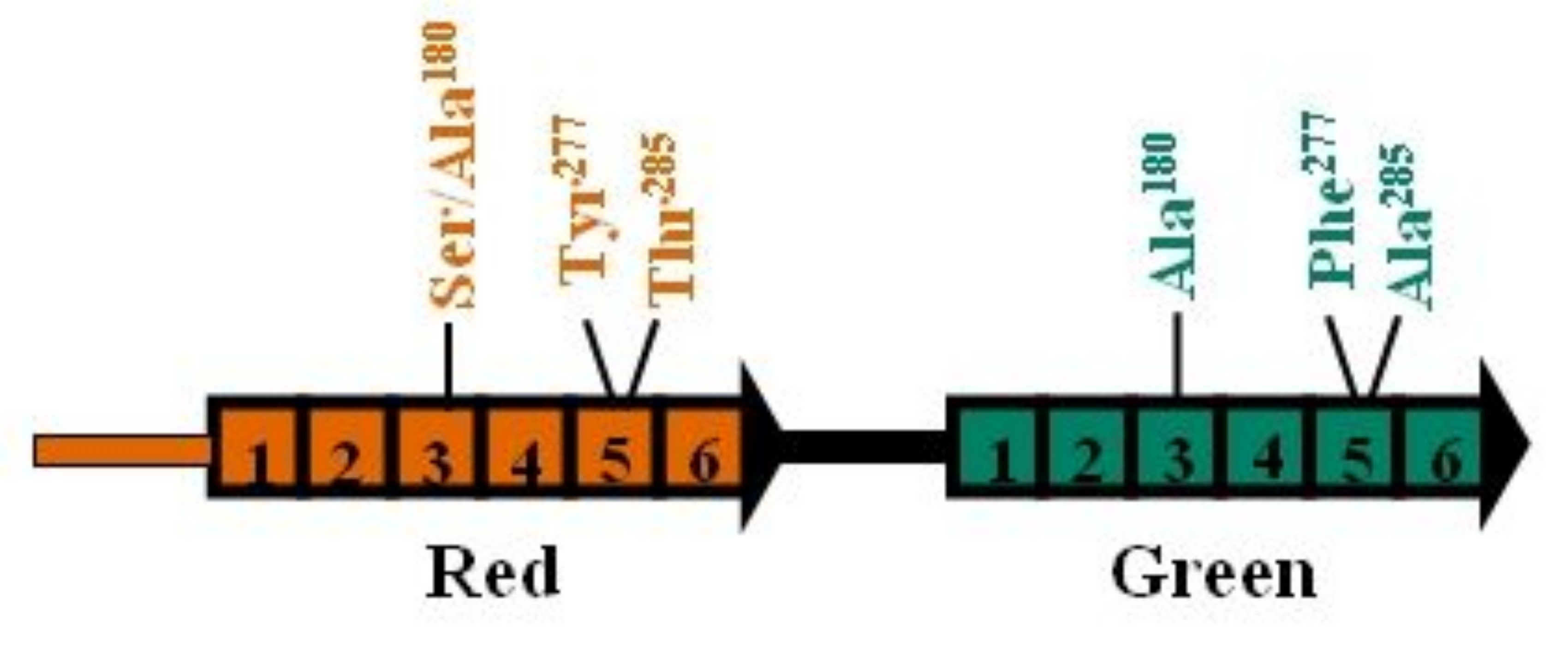


How different are they?



•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.

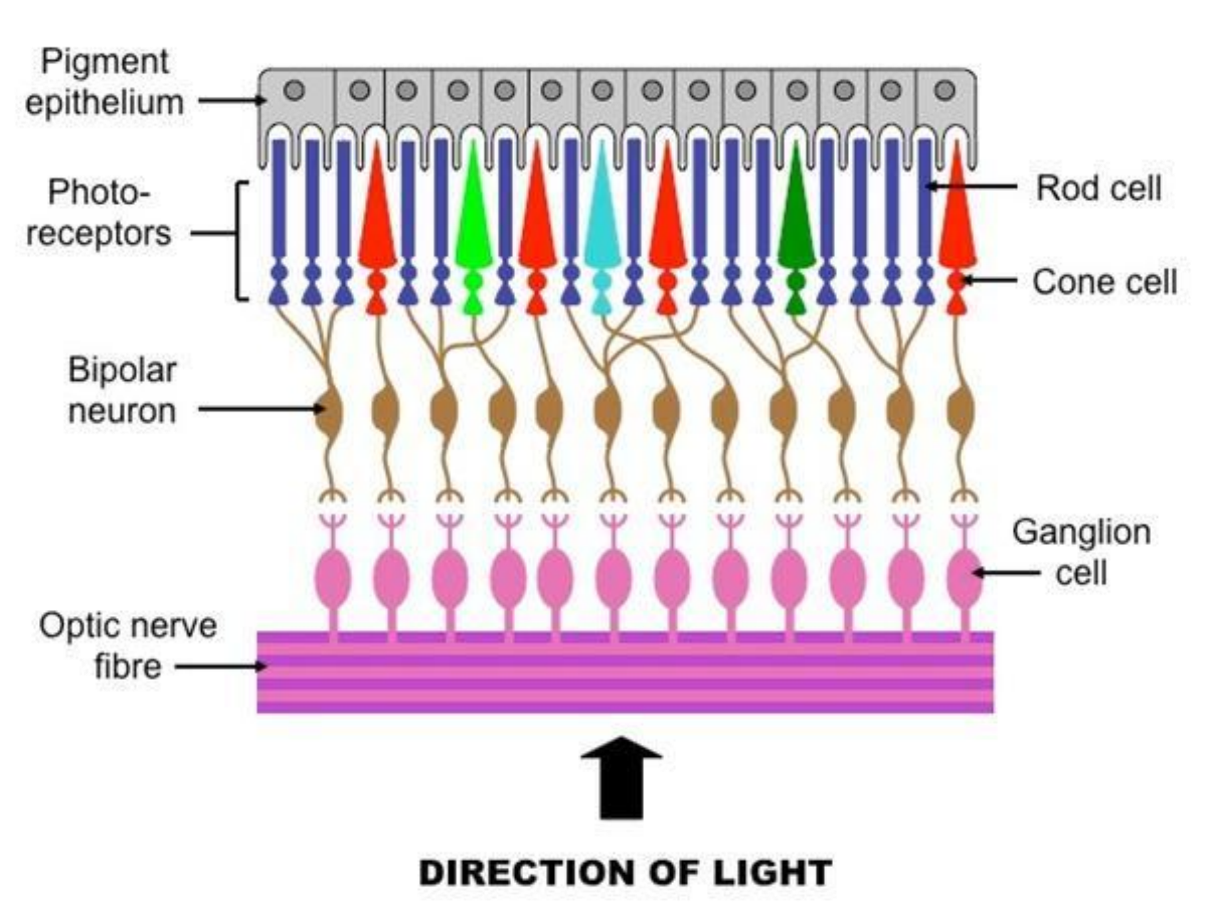
Three important aa residues



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).

Rods vs. cones

- Light absorption, number, structure, photoreceptors, chromophores, image sharpness, 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 nerve fiber.

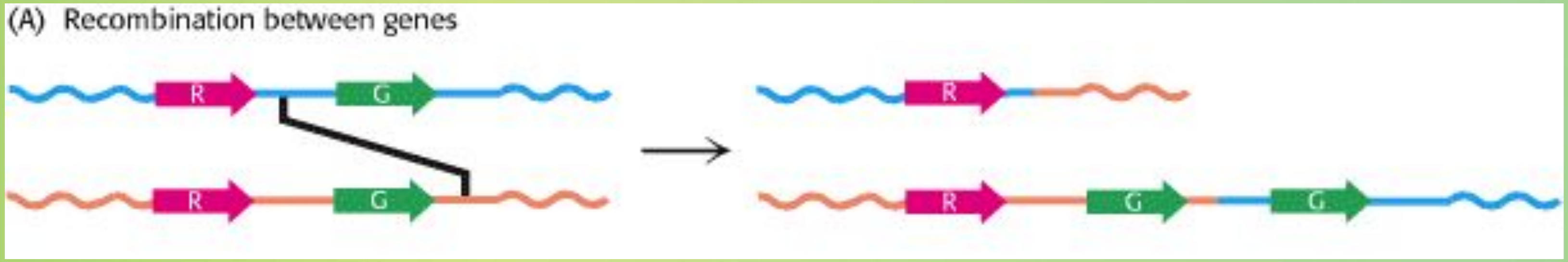
Color blindness

Chromosomal locations

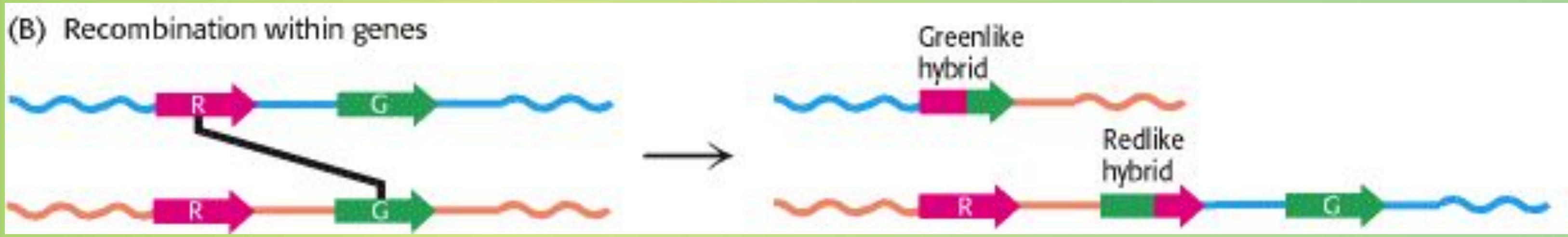
- The "blue" opsin gene: chromosome 7
- The "red" and "green" opsin genes: X chromosome
- The X chromosome normally carries a cluster of from 2 to 9 opsin genes.
- Multiple copies of these genes are fine.

Red-green homologous recombination

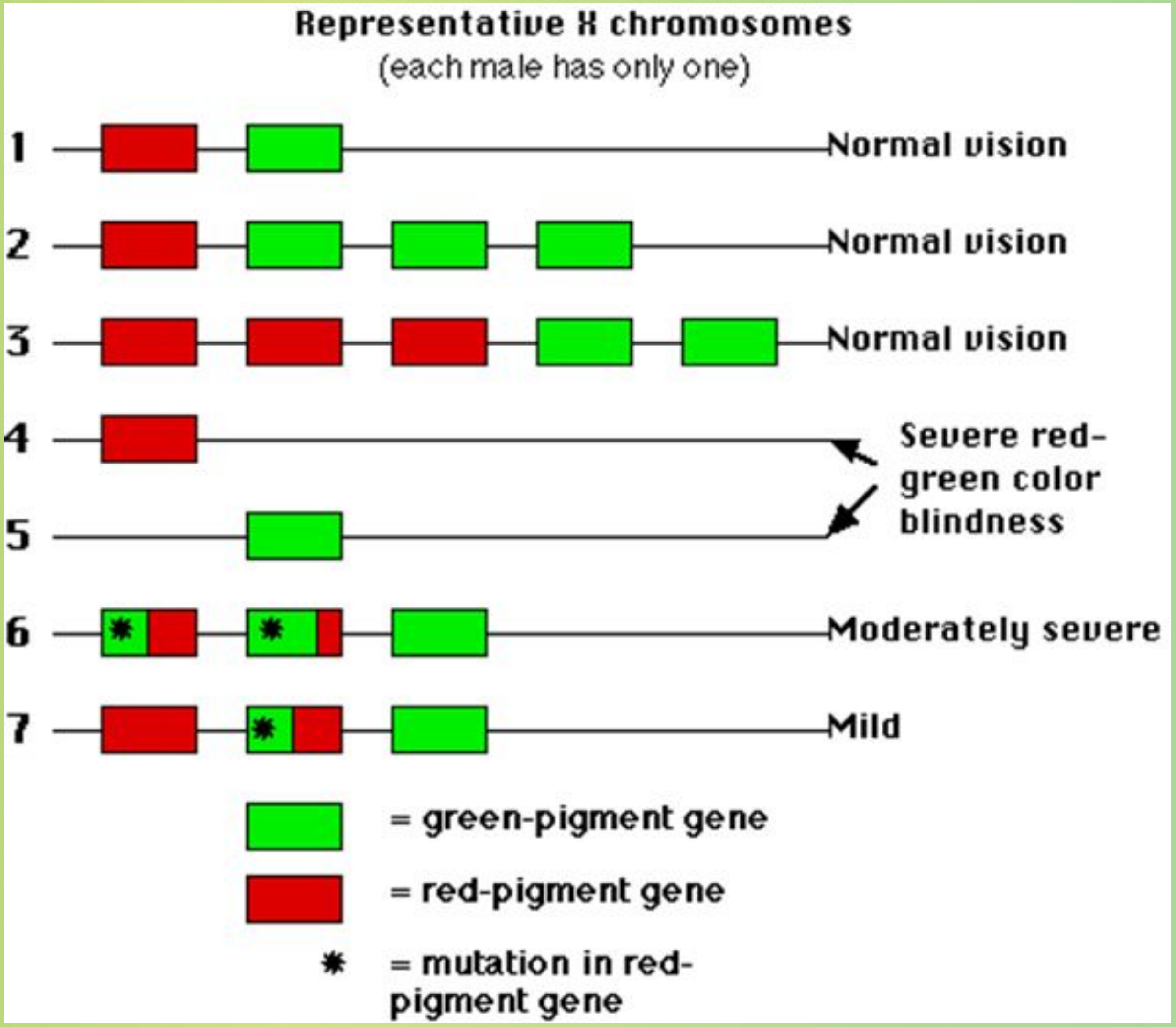
- **Between transcribed regions of the gene (inter-genic)**



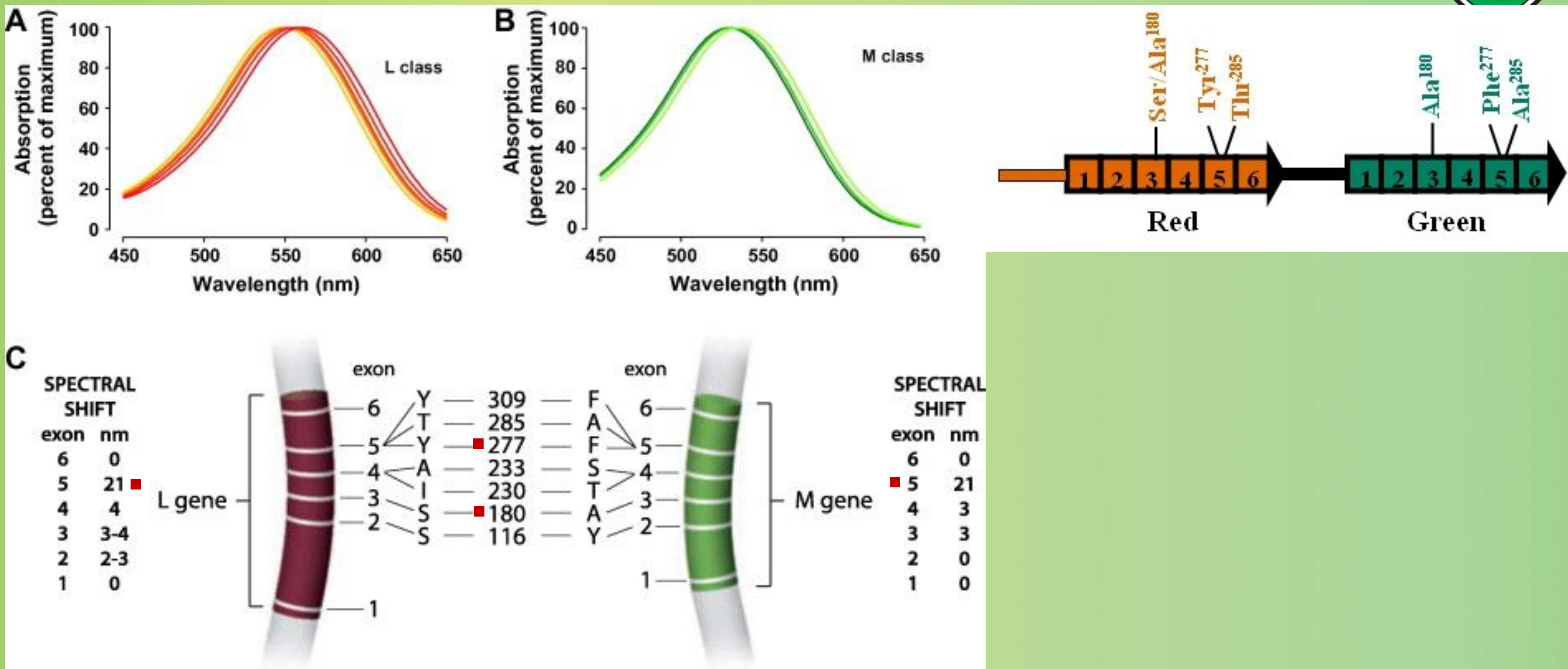
- **Within transcribed regions of the gene (intra-genic)**



Genetic probabilities

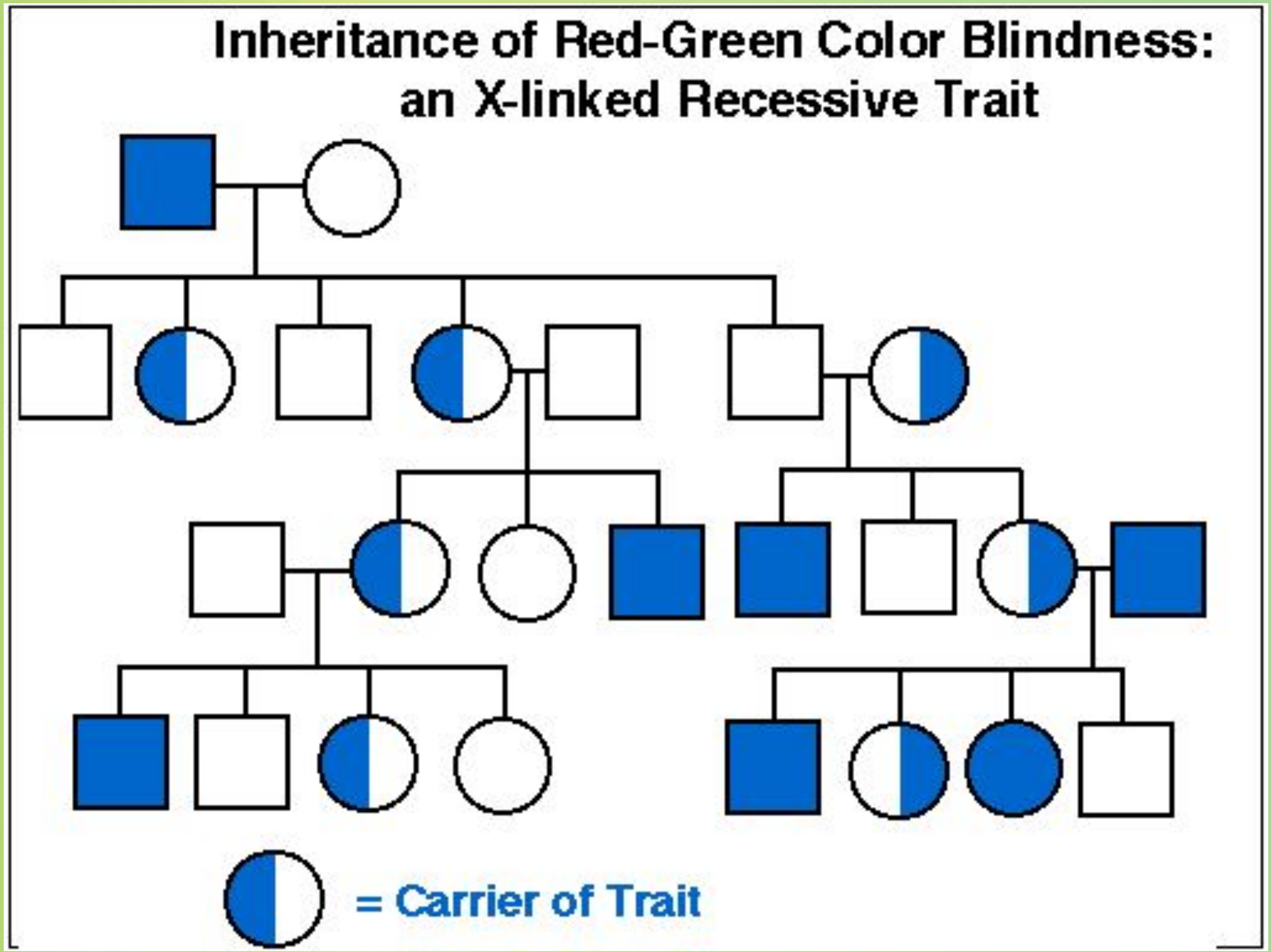


Spectral tuning



- **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


Inheritance of Red-Green Color Blindness:
an X-linked Recessive Trait
= Carrier of Trait

Examples

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