Biochemistry

Slides

Lec No: 1

The Subject: **Visual transduction**

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00/00/2017

Visual transduction

Neuroscience, Biochemistry
Dr. Mamoun Ahram
Third year, 2017

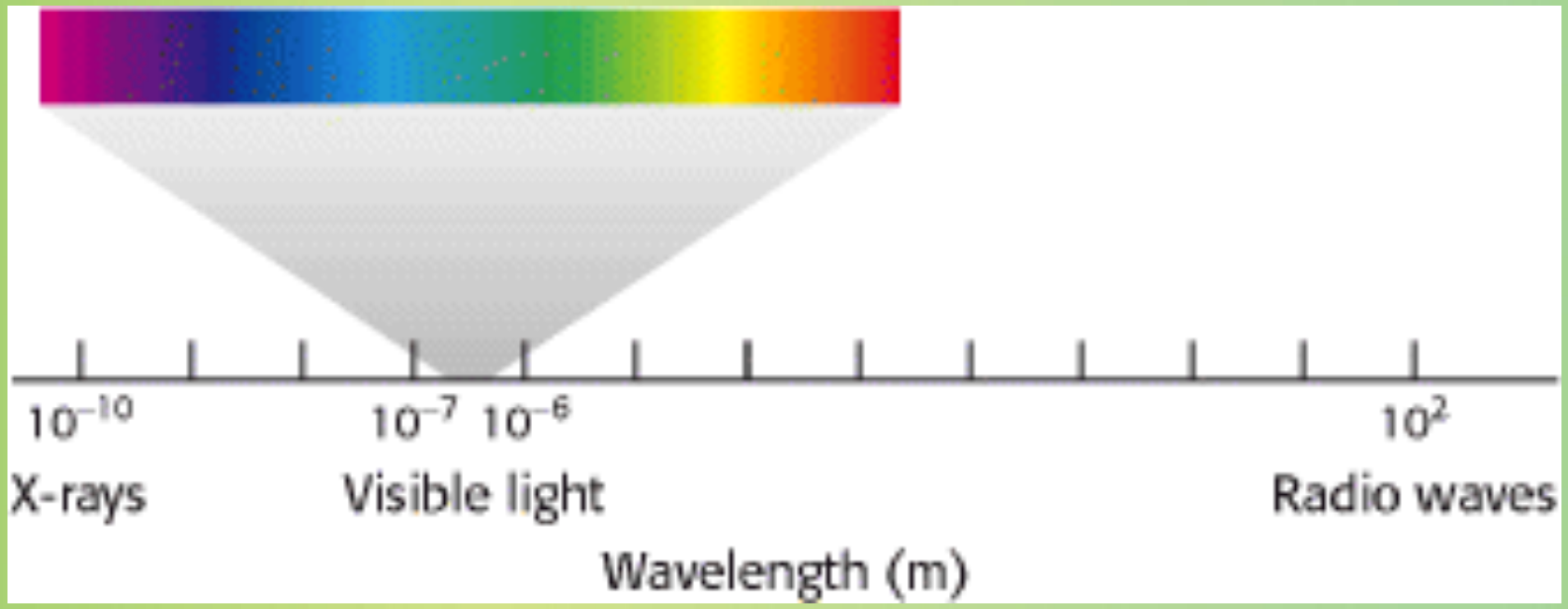
References

- 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)

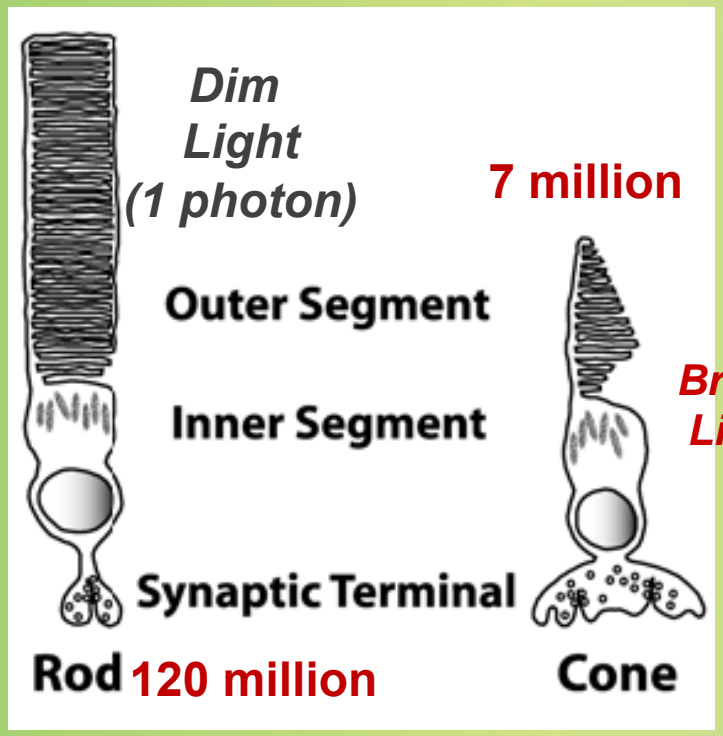
Lecture outline

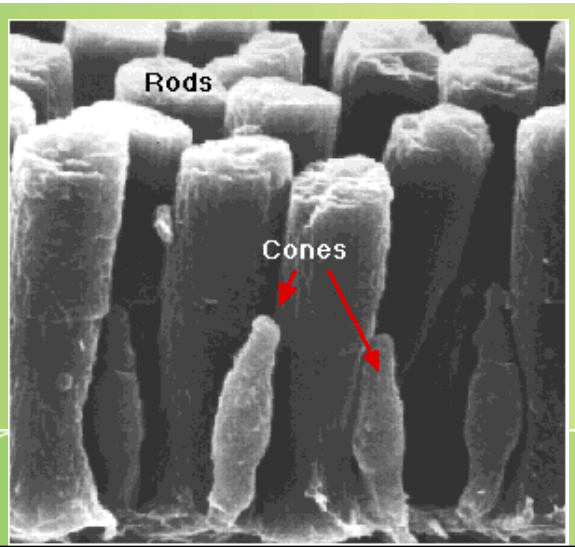
- Visual transduction (dim vs. bright light)
  - Components (cells and molecules)
  - Mechanisms of activation, amplification, and termination
- Color blindness

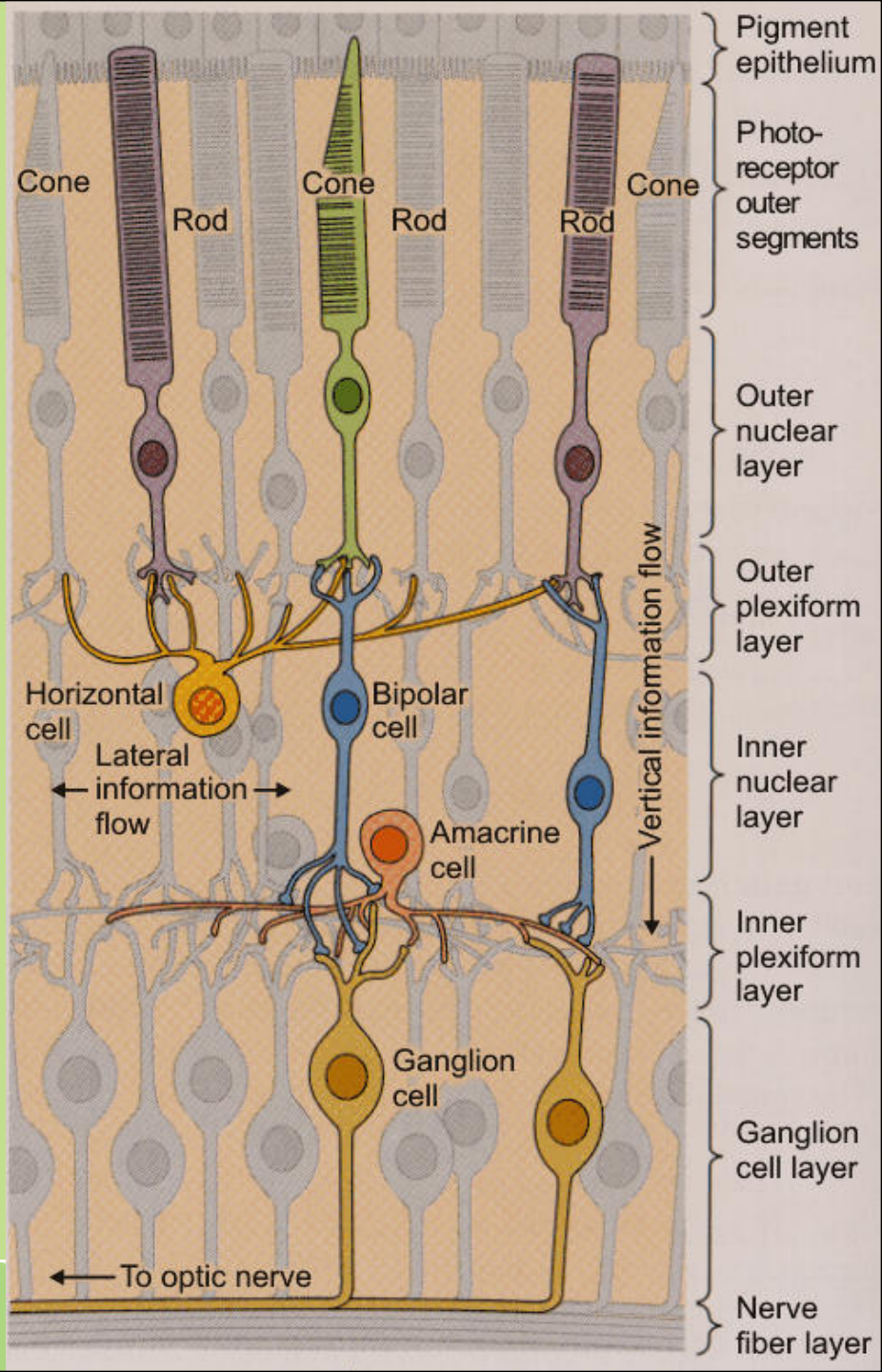
Basics of human vision



Rods and cones



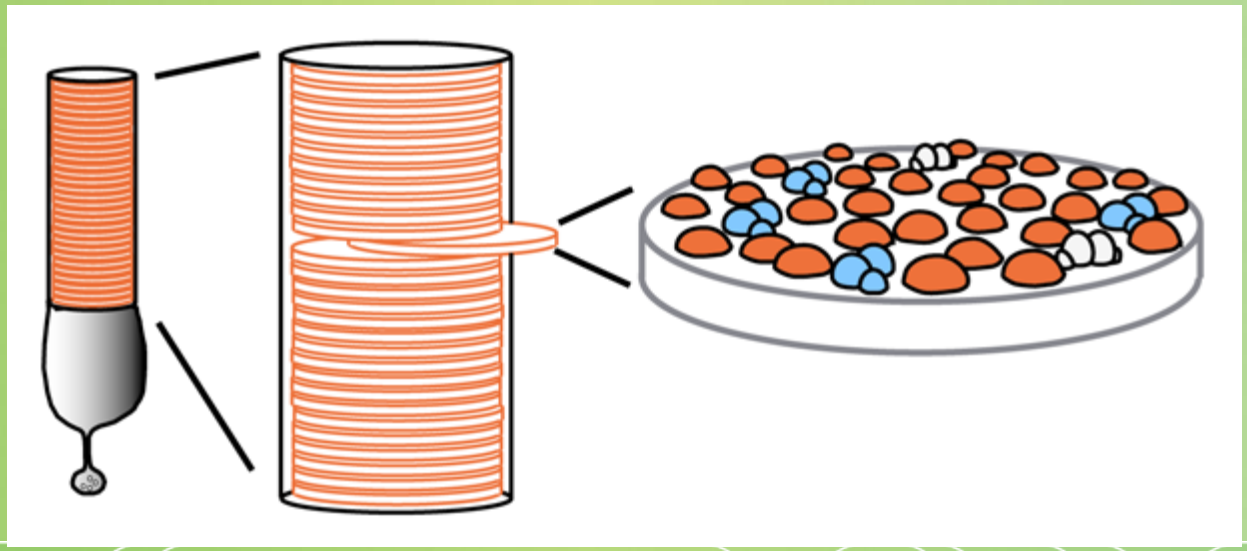


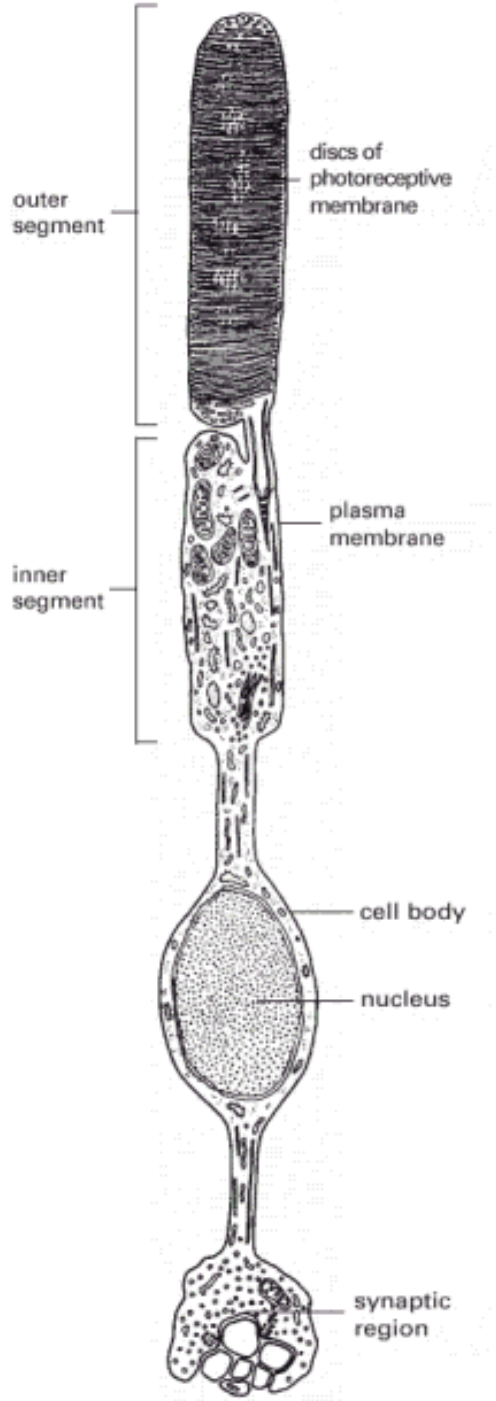


More on rod cells

1. The inner segment consists of the cell body and contains the cellular organelles found in other neurons, including a synaptic terminal.
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").

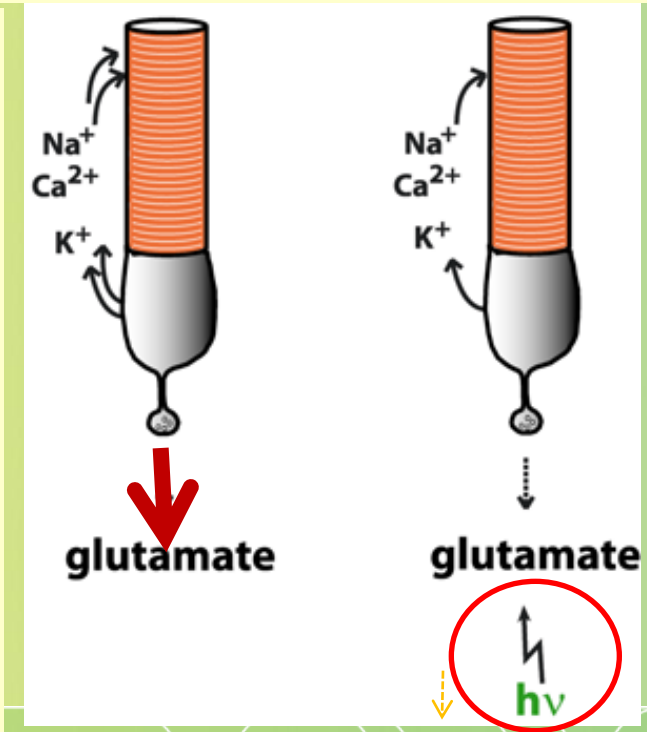




The dark current

1. Most neurons maintain a resting membrane potential of about -60 to -70 mV. When excited, they open cation channels causing depolarization and opening of voltage-gated Ca2+ channels at the synapse. Ca2+ flows in and promotes fusion of synaptic vesicles, which release neurotransmitter.
2. Rods and cones work “backwards”. At rest, that is in darkness, rods and cones are depolarized to -35 to -45 mV

1. No light, Na+ and a lesser amount of Ca2+ 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 depolarize.
4. The neurotransmitter glutamate is released continuously.



1. When excited, channels in the outer segment membrane close, the rod hyperpolarizes
2. Glutamate release decreases.

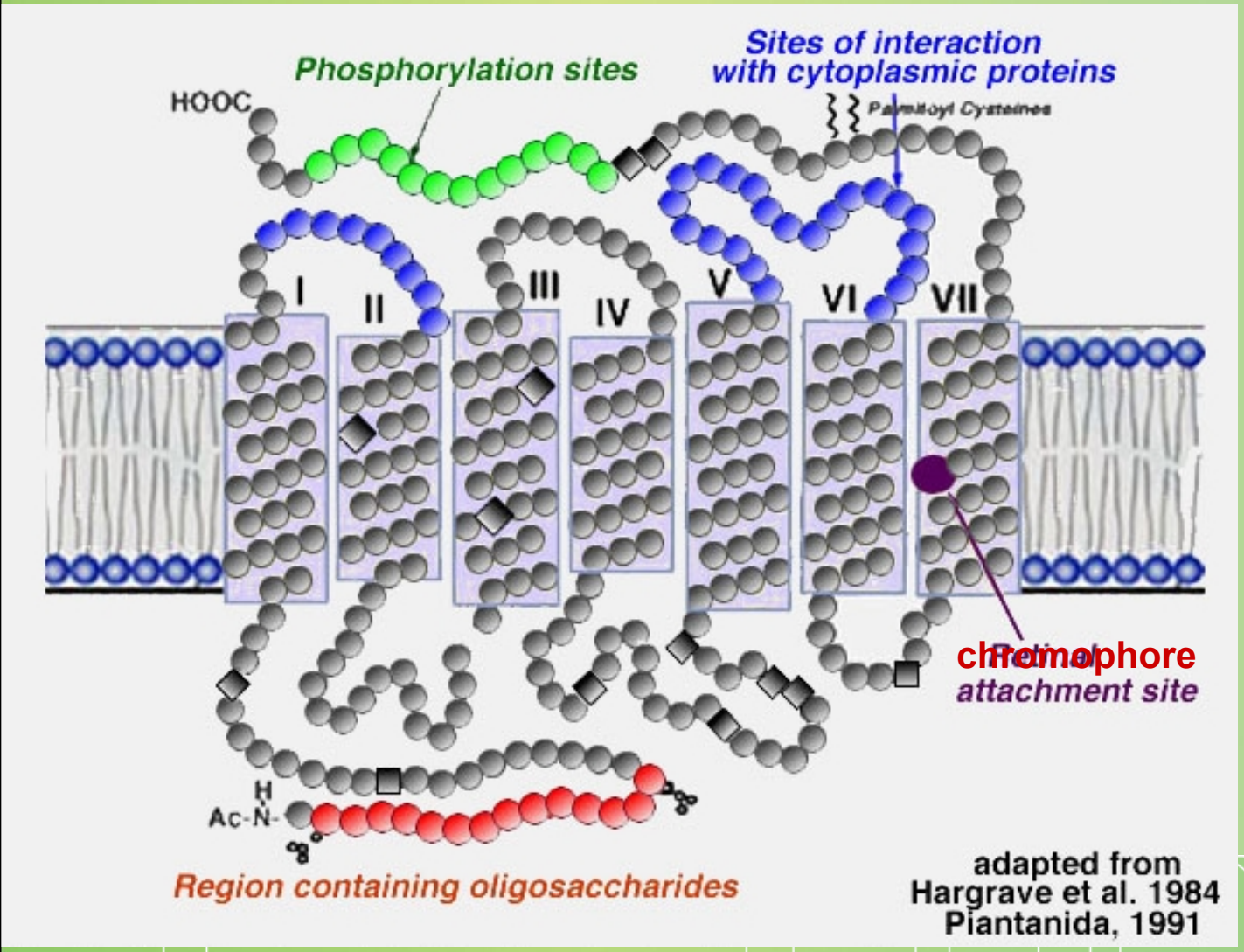
Generation of vision signals

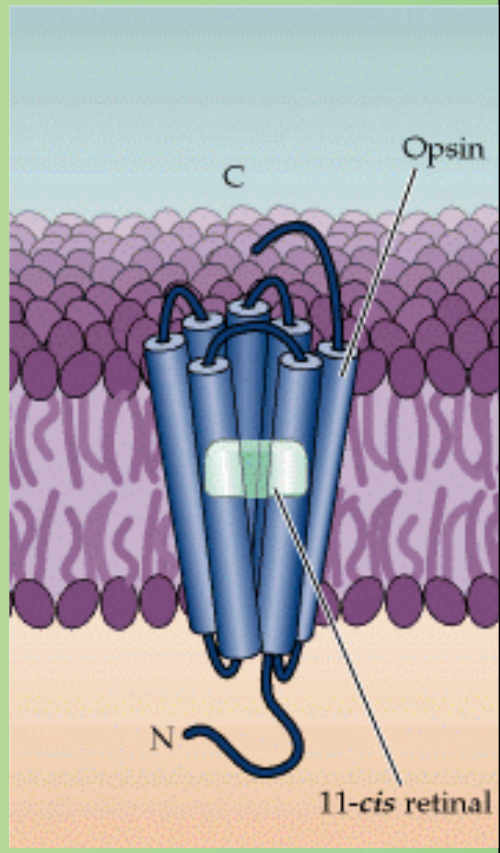
The players

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

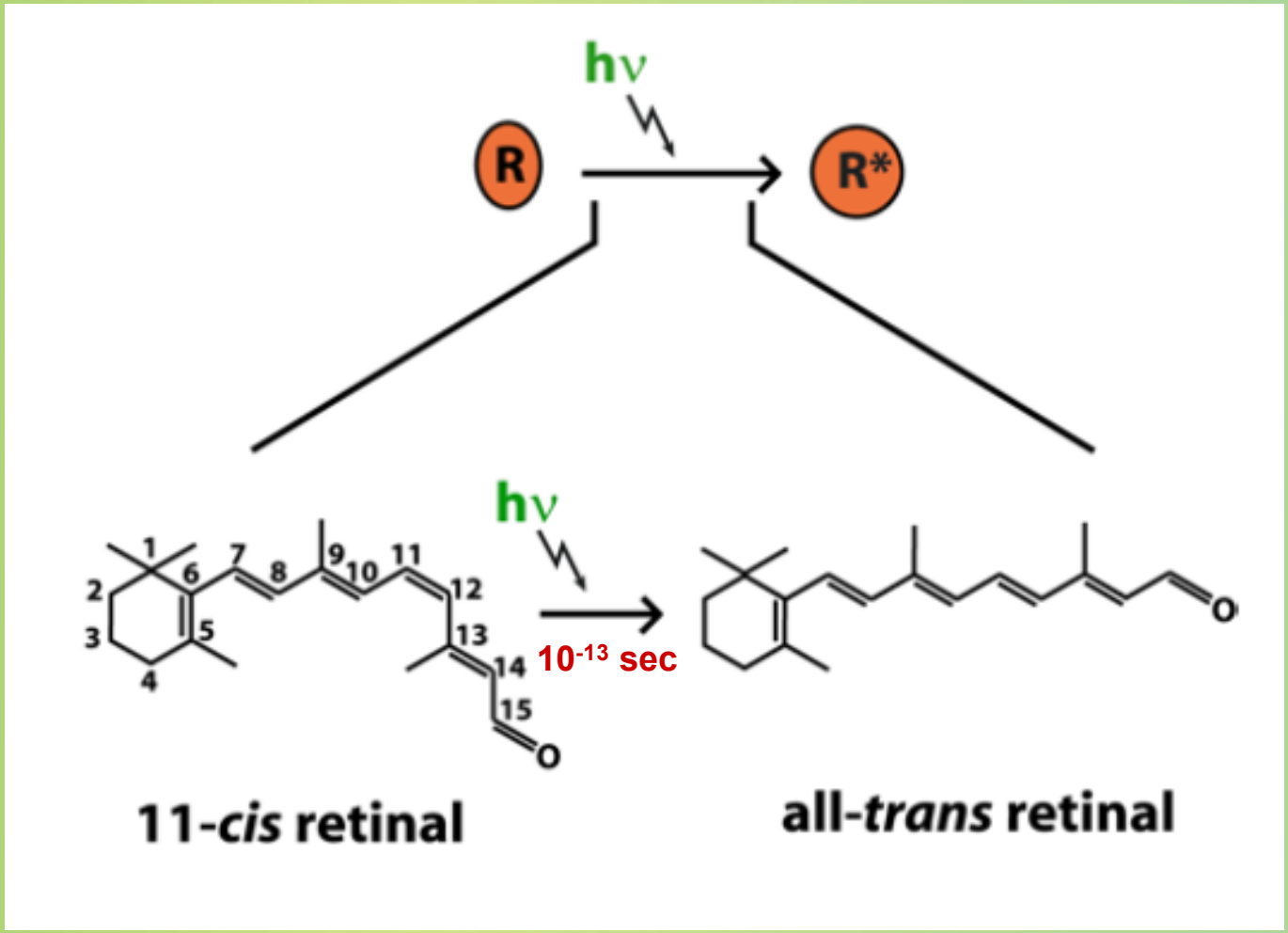
Rhodopsin

Opsin is a single polypeptide chain with 7 helical segments that span the membrane.

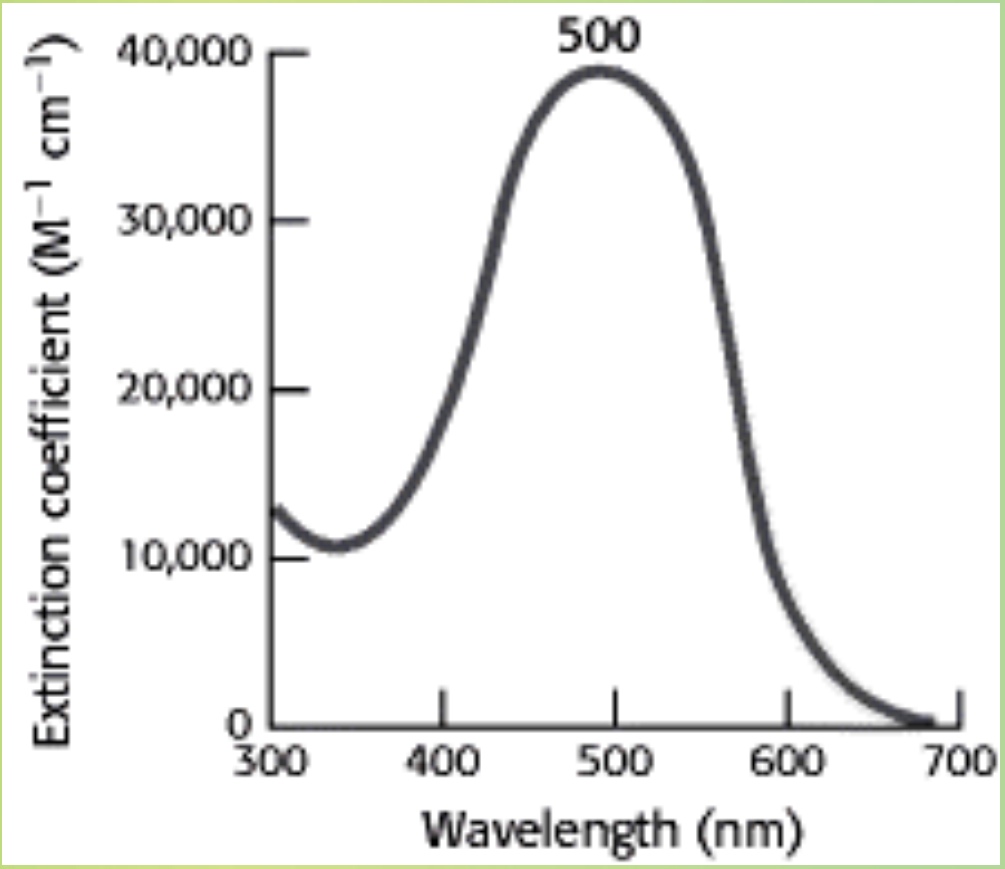




11-cis-retinal

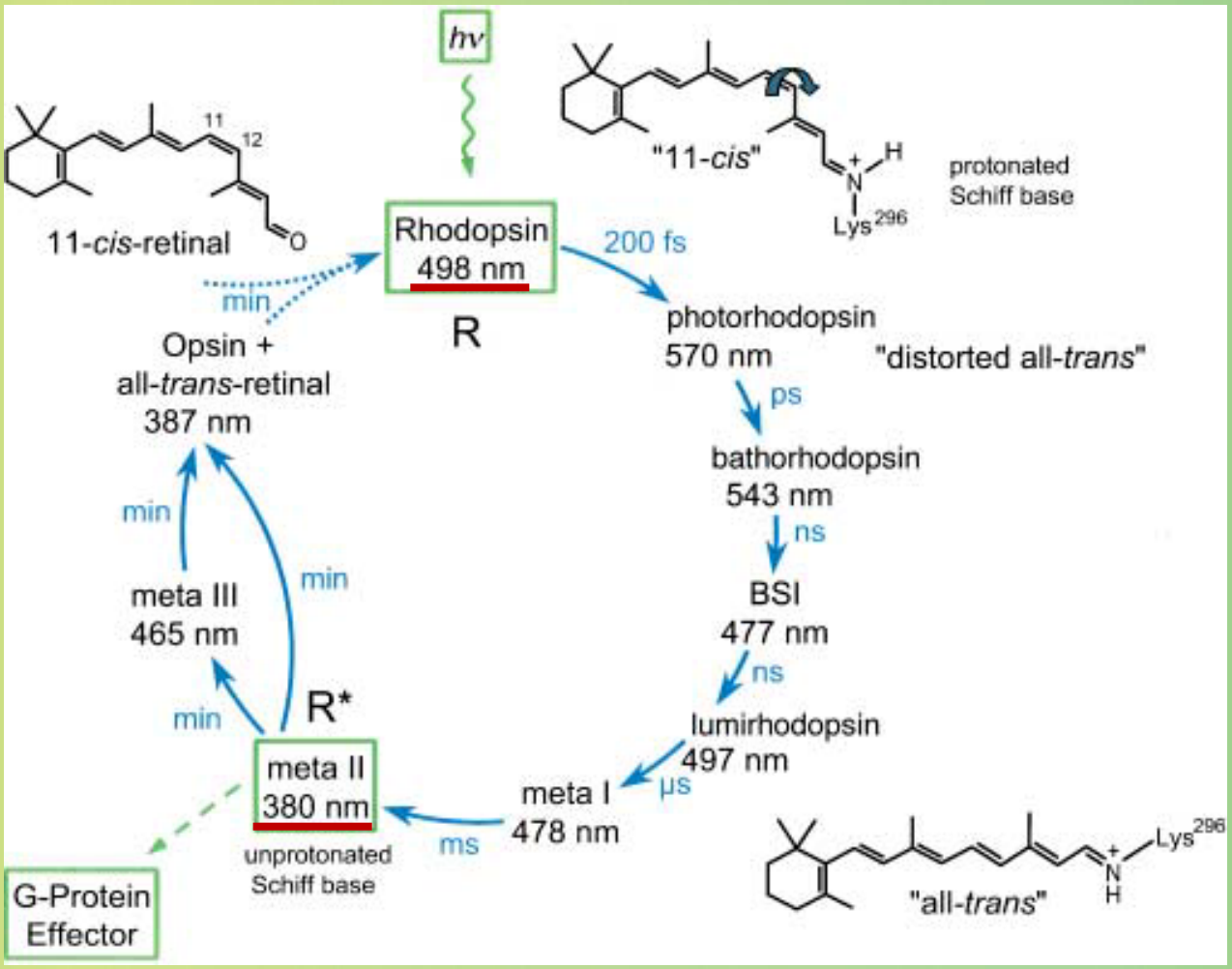


Light absorption by rhodopsin

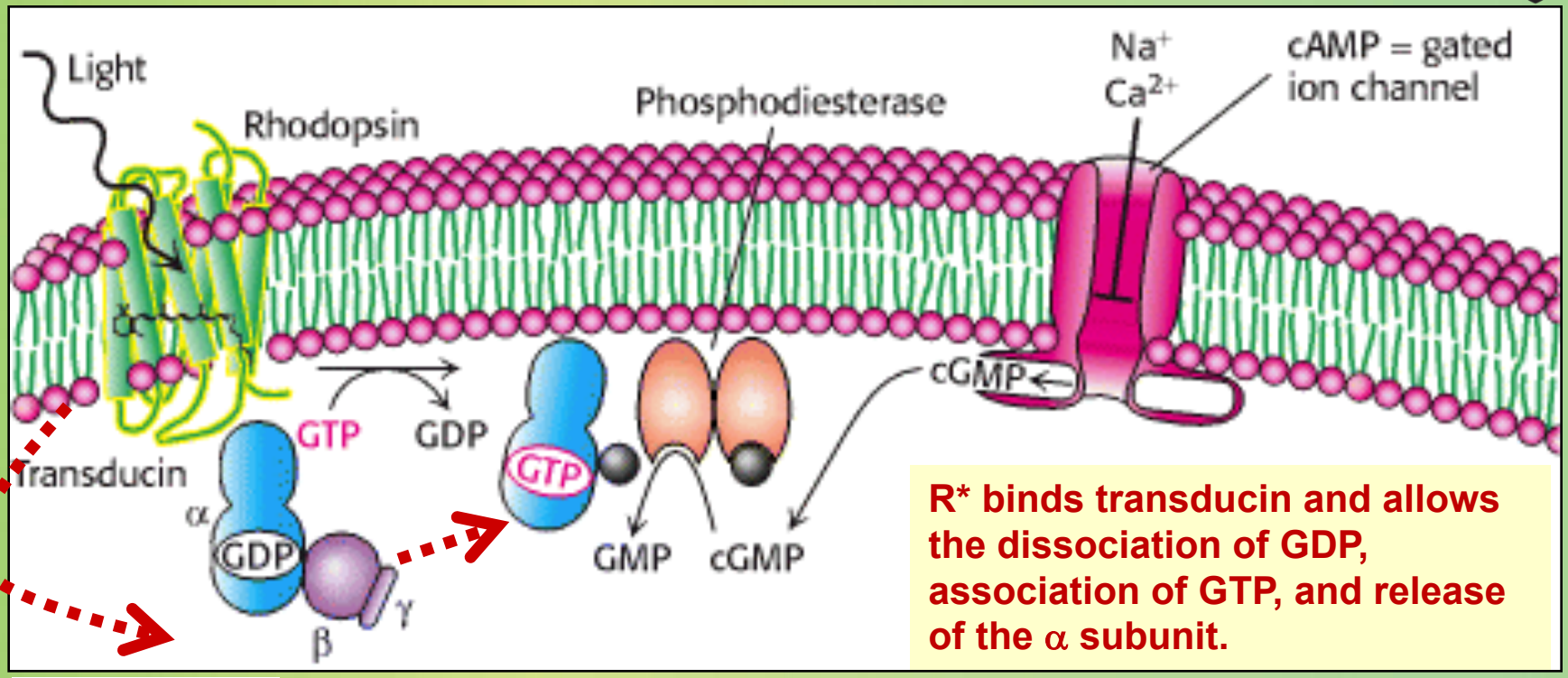


Rhodopsin intermediates

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



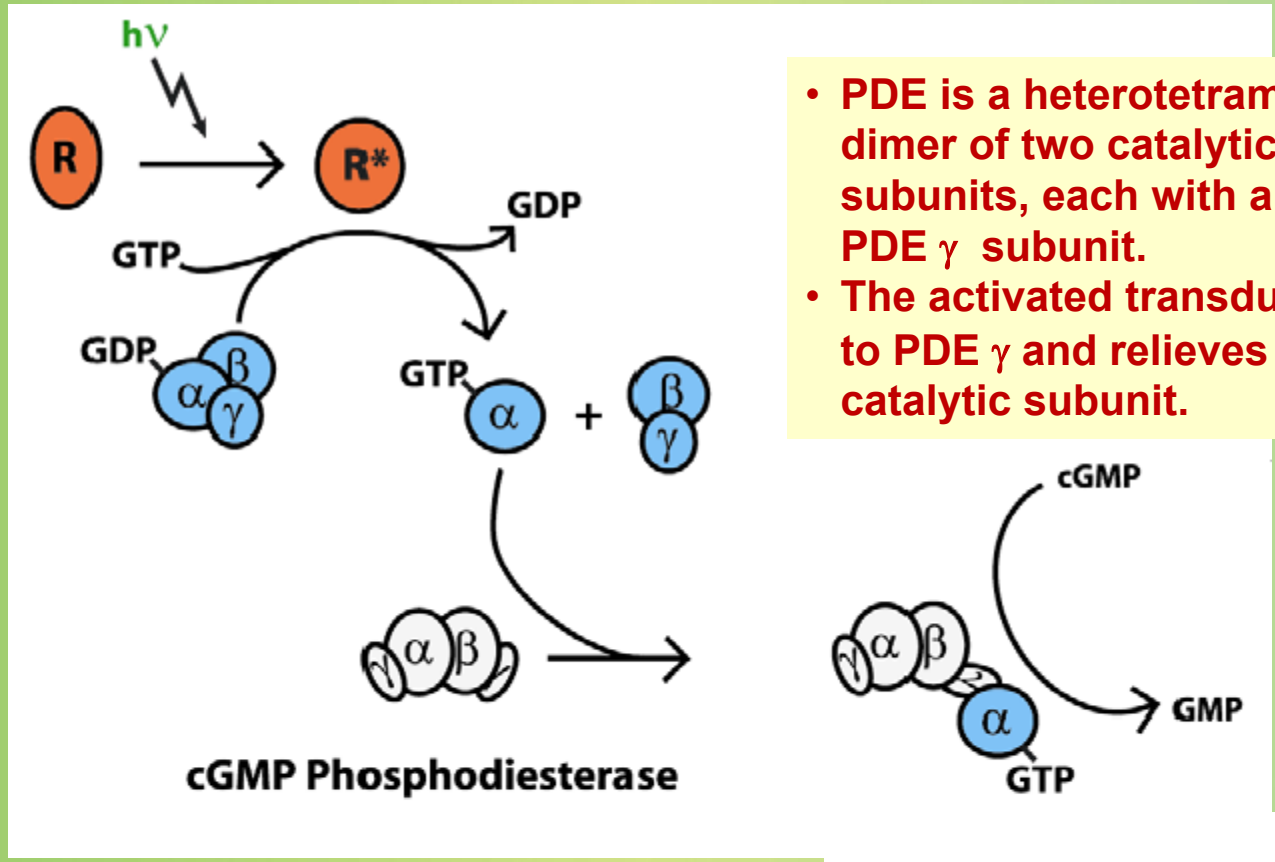
Transducin → Phosphodiesterase (PDE)



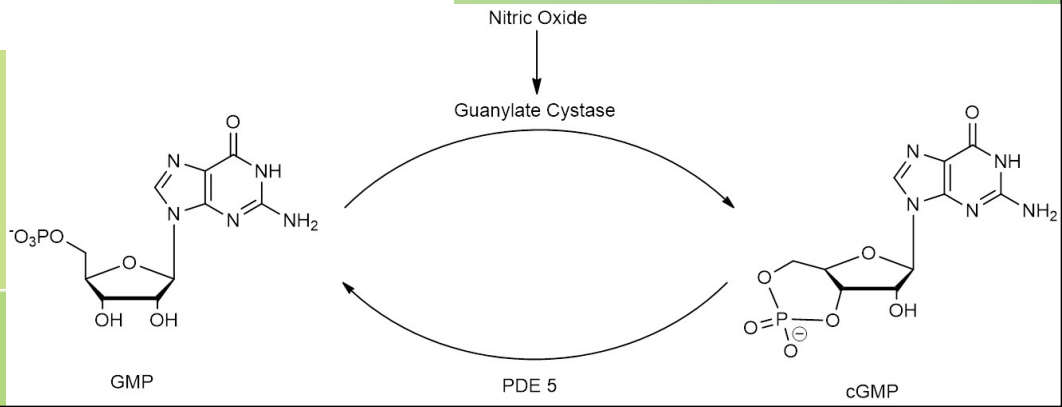
R* binds transducin and allows the dissociation of GDP, association of GTP, and release of the α subunit.

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.

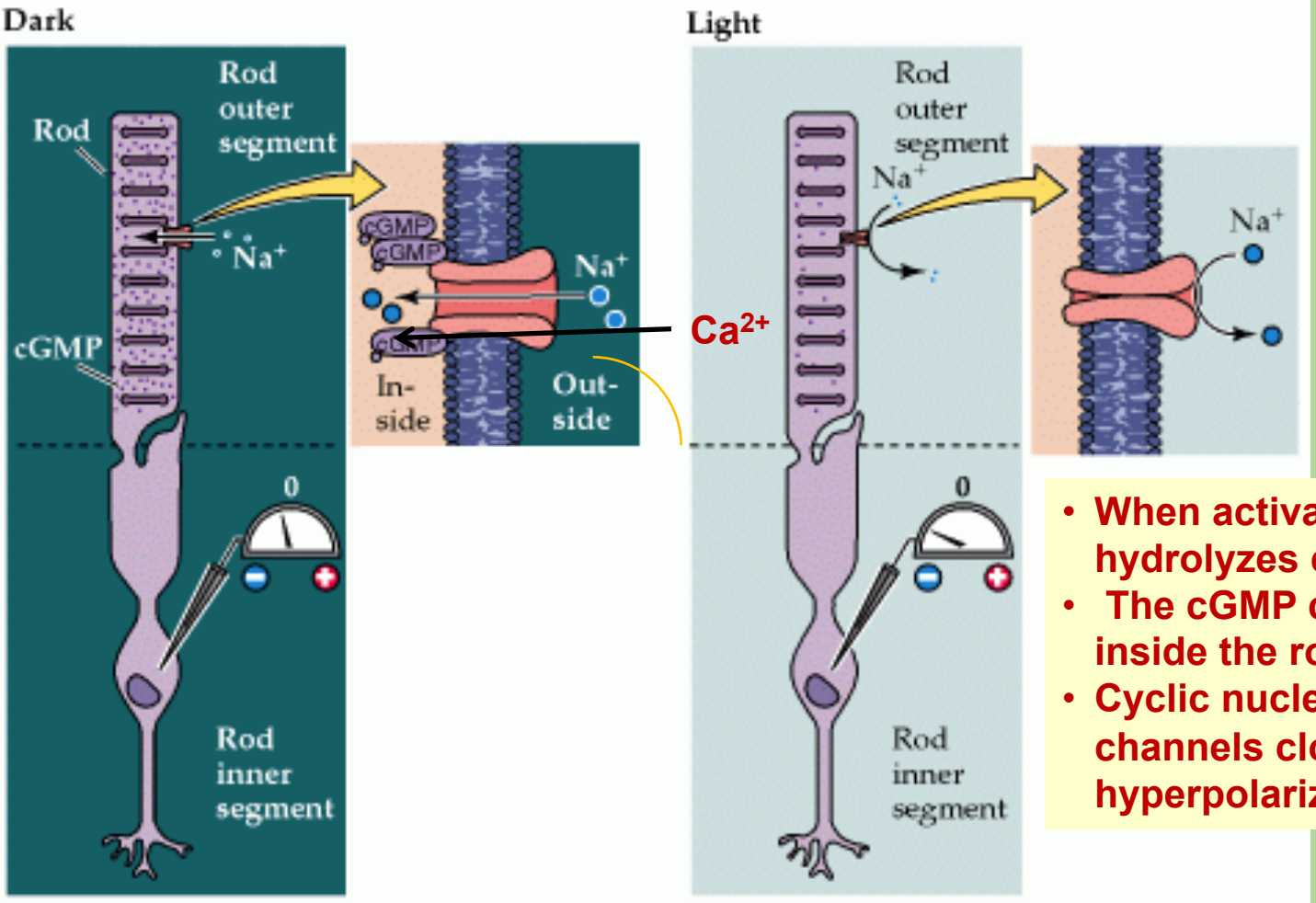
Activation of phosphodiesterase



- 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.



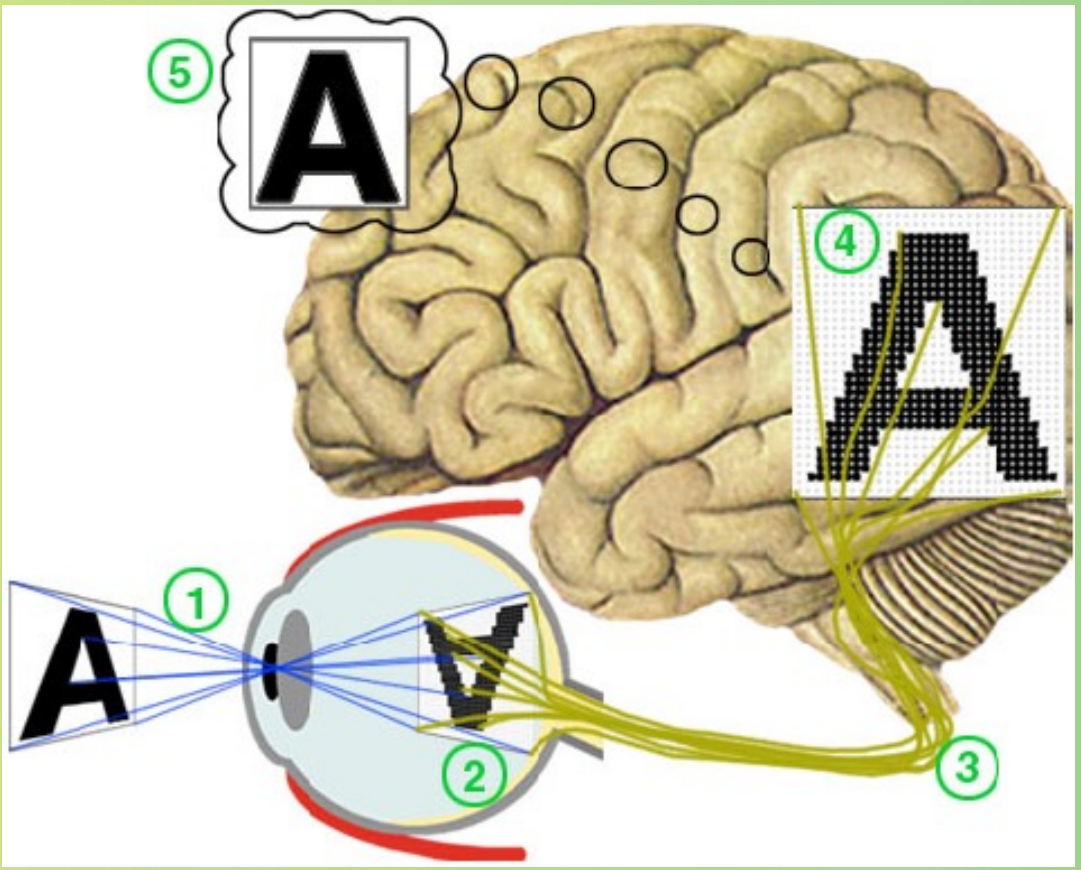
cGMP-gated channels



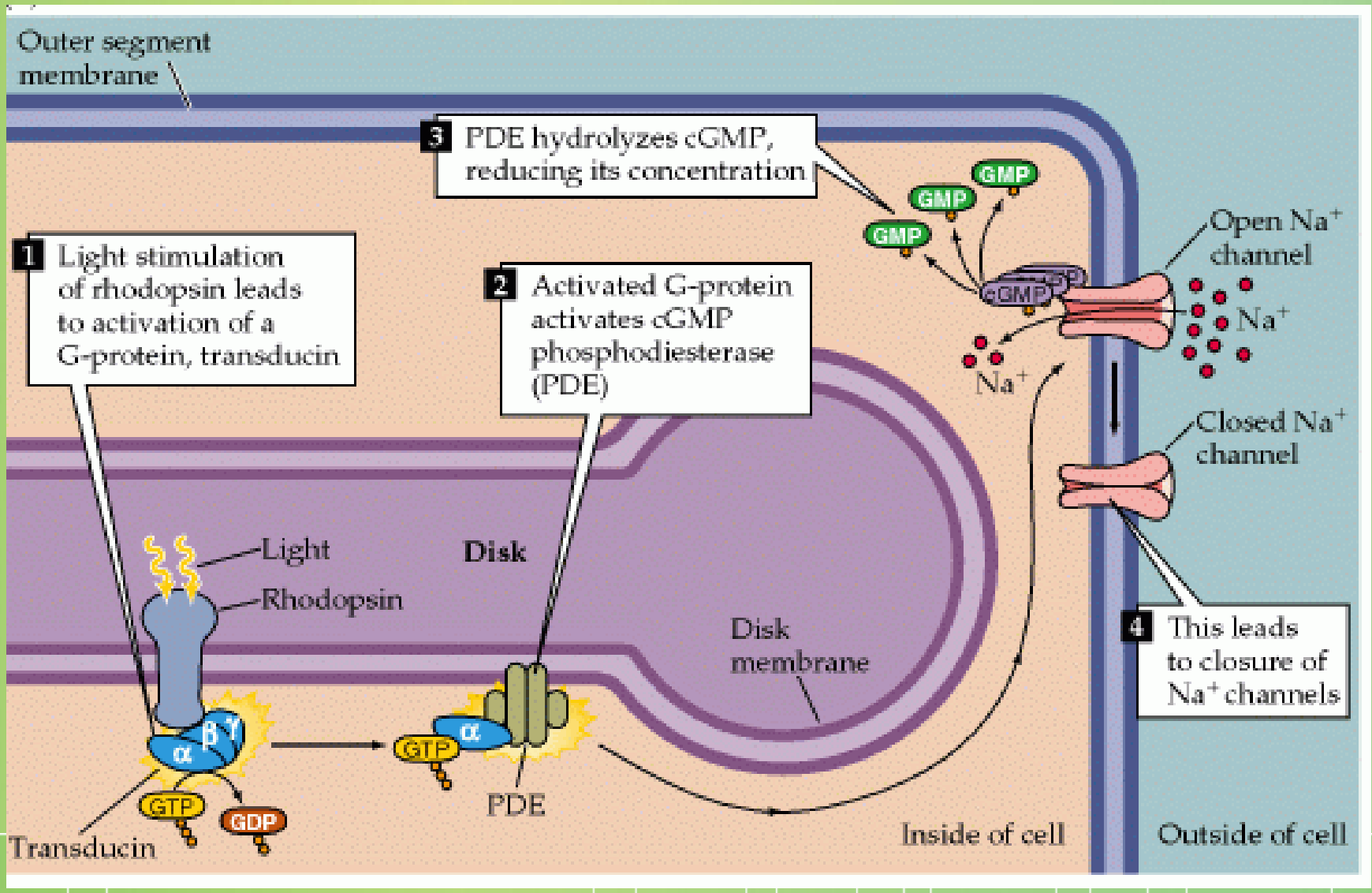
- When activated, PDE hydrolyzes cGMP to GMP.
- The cGMP concentration inside the rod decreases.
- Cyclic nucleotide-gated ion channels close leading to hyperpolarization.

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.



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

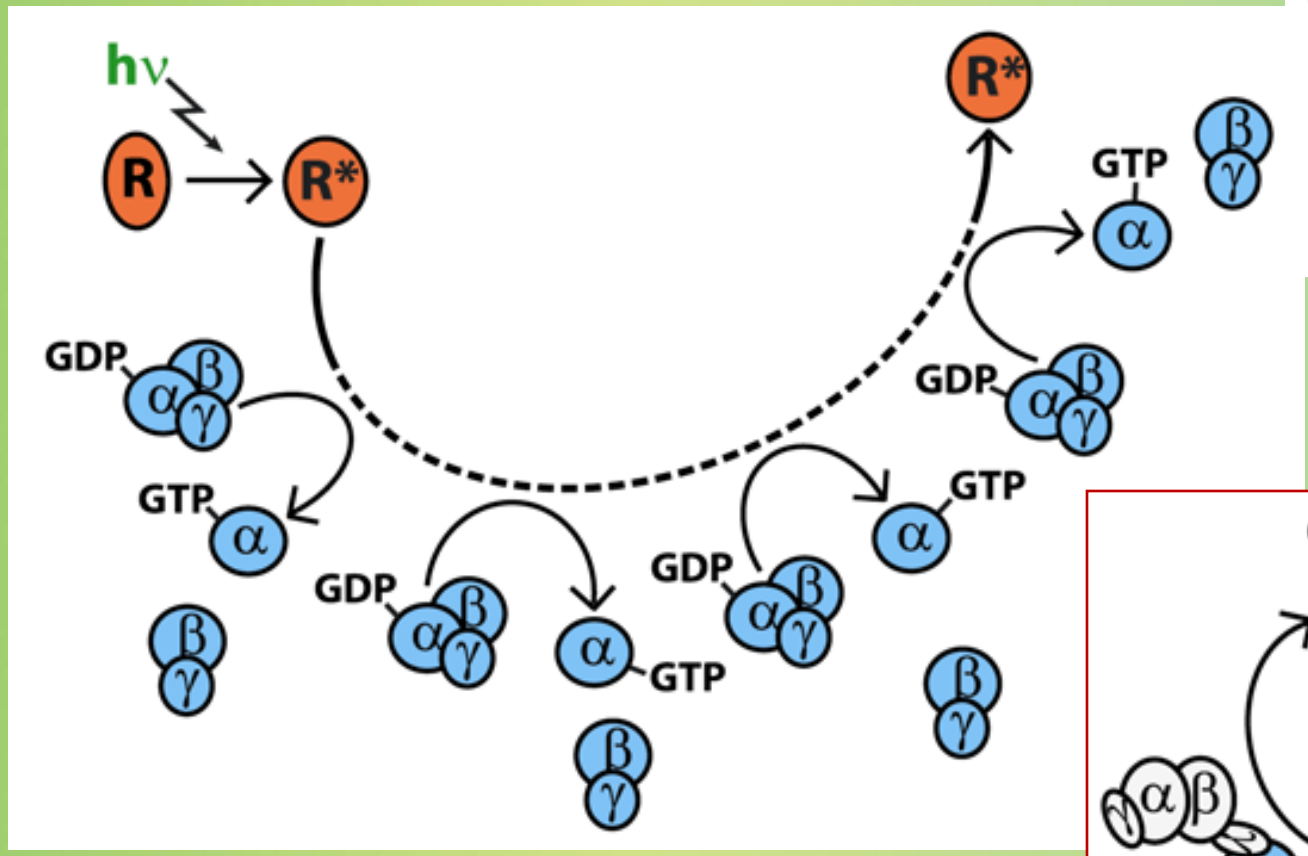


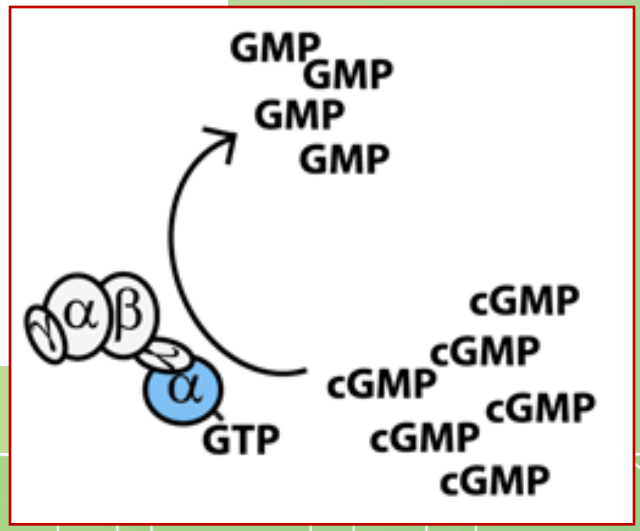
Signal amplification

Rhodopsin (1) → Transducin (500)

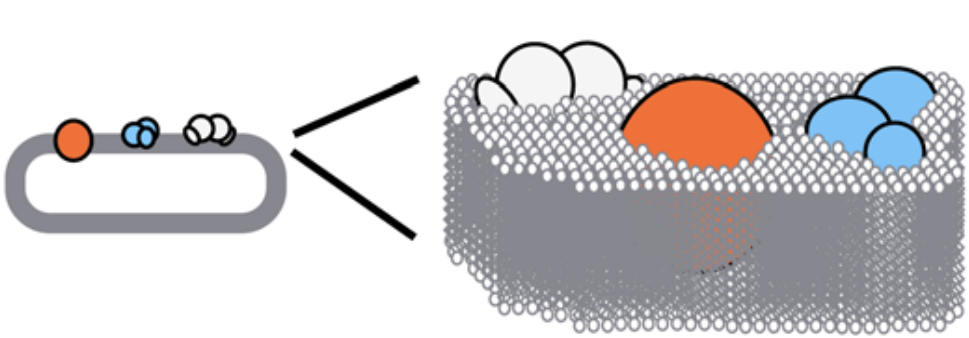
Transducin (1) → PDE (1)

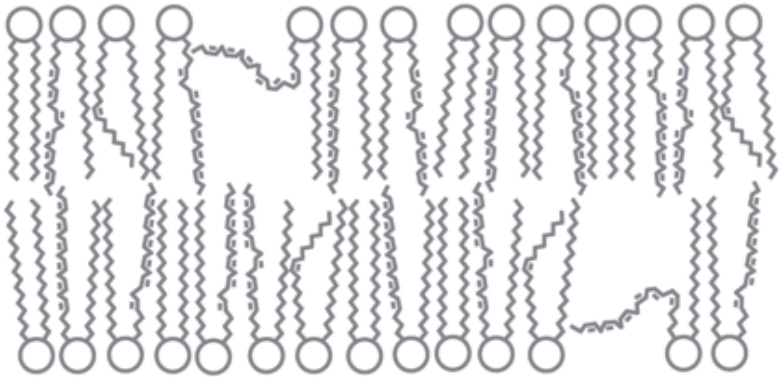
PDE (1) → cGMP (10^3)





Facilitation of transduction





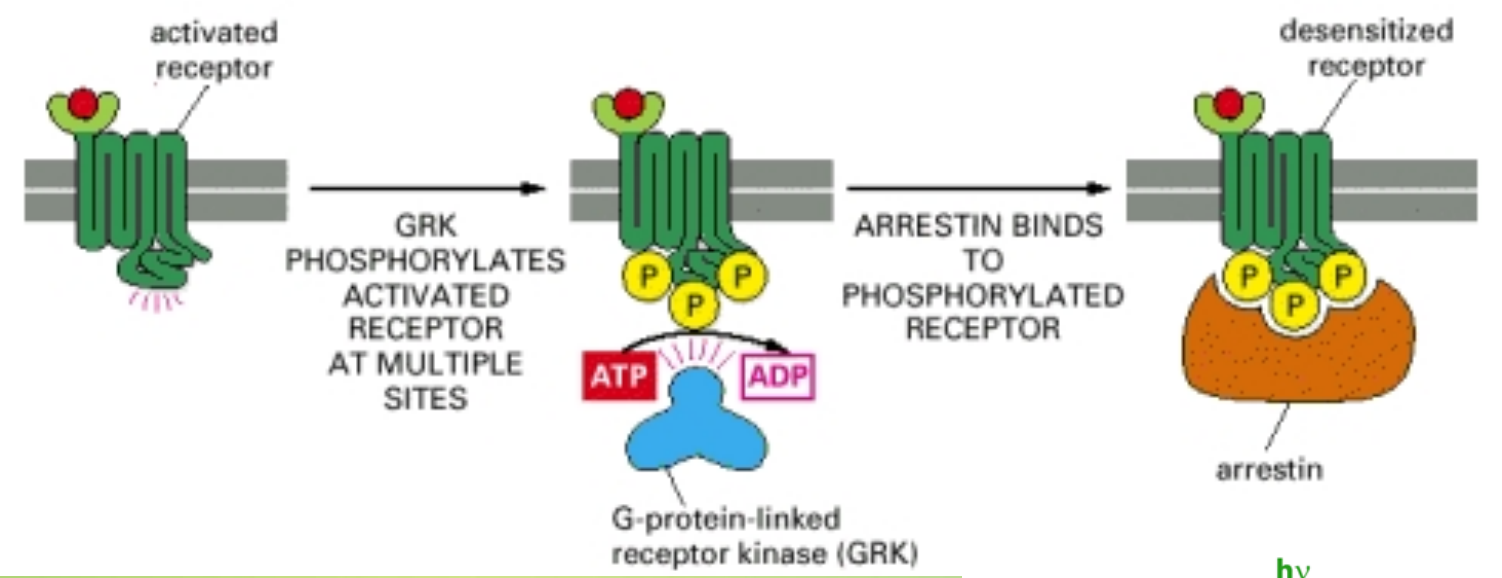
1. 2-dimensional surface
2. low in cholesterol and high content of unsaturated fatty acids
3. Cooperativity of binding: The binding of one cGMP enhances additional binding and channel opening (n = ~3)
4. since multiple cGMP molecules are required to open the channel, it will close when only one or two cGMP molecules leave the channel, making it easily shut down by absorption of light.

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

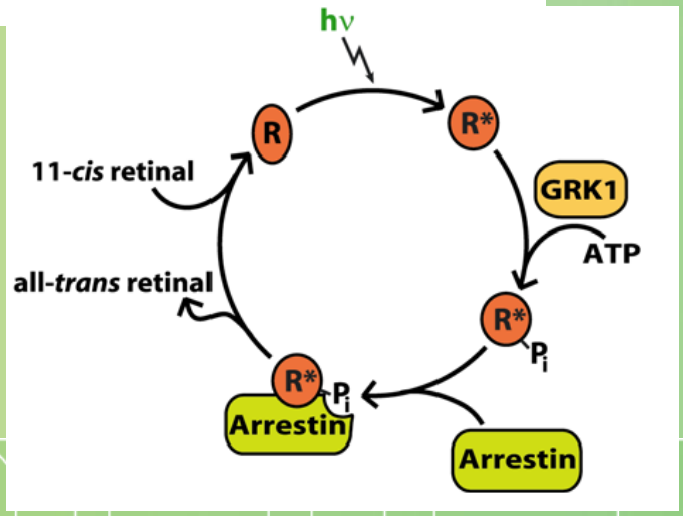
Signal termination

Mechanism I

Arrestin binding

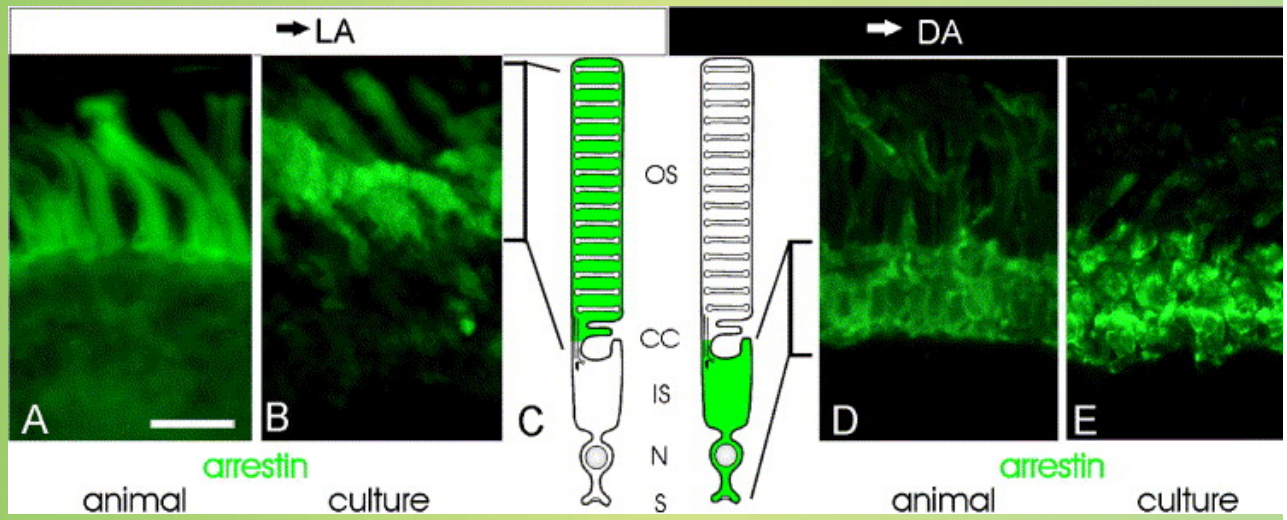


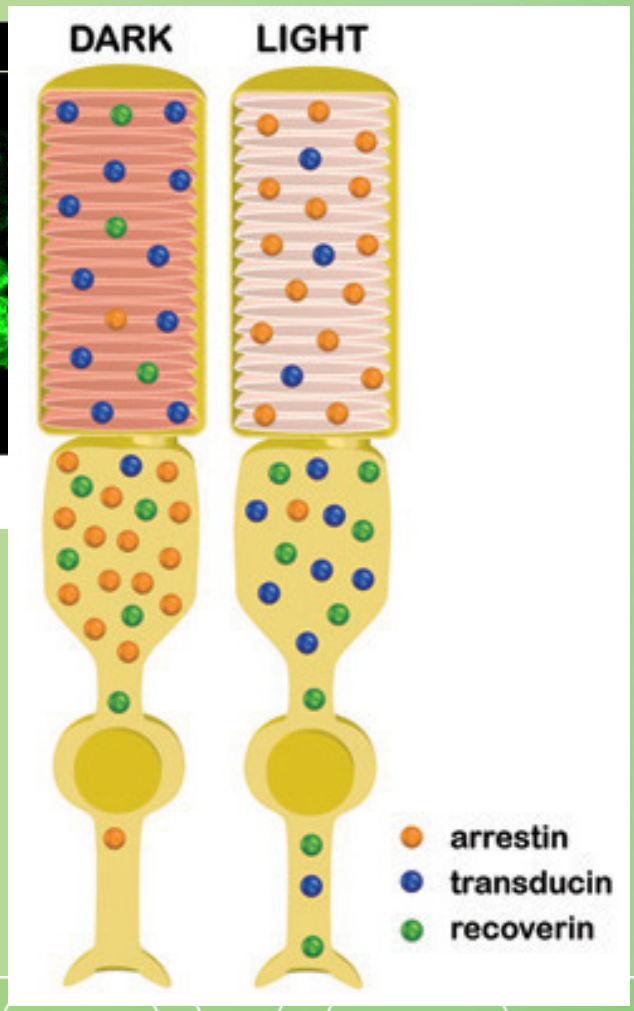
- **Rhodopsin kinase (GRK1) phosphorylates the C-terminus of R*.**
- **Phosphorylation of R* decreases transducin activation and facilitates binding to arrestin, which completely quenches its activity, and releases of the all *trans-retinal* regenerating rhodopsin.**



Mechanism II

Arrestin/transducin distribution

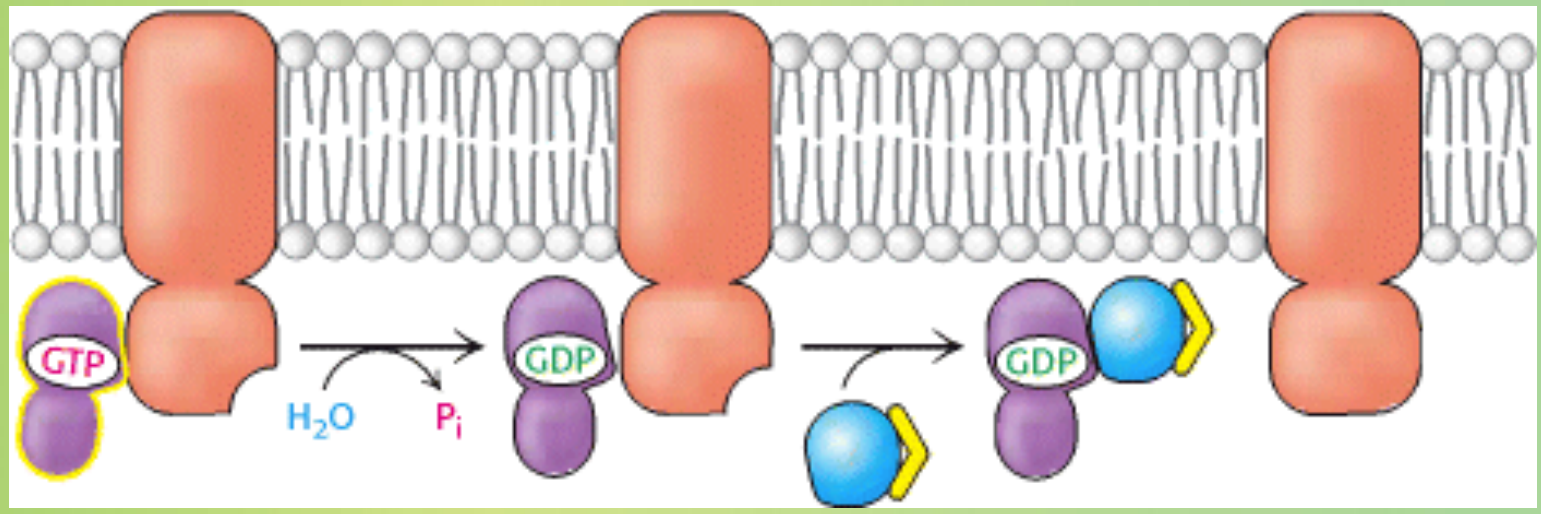




- In dark, the outer segment contains high levels of transducin and low levels of arrestin (low inhibition; ready to be activated).
- In light, it is the opposite (high inhibition; ready to be inactivated.

Mechanism III

Intrinsic GTPase activity of G protein

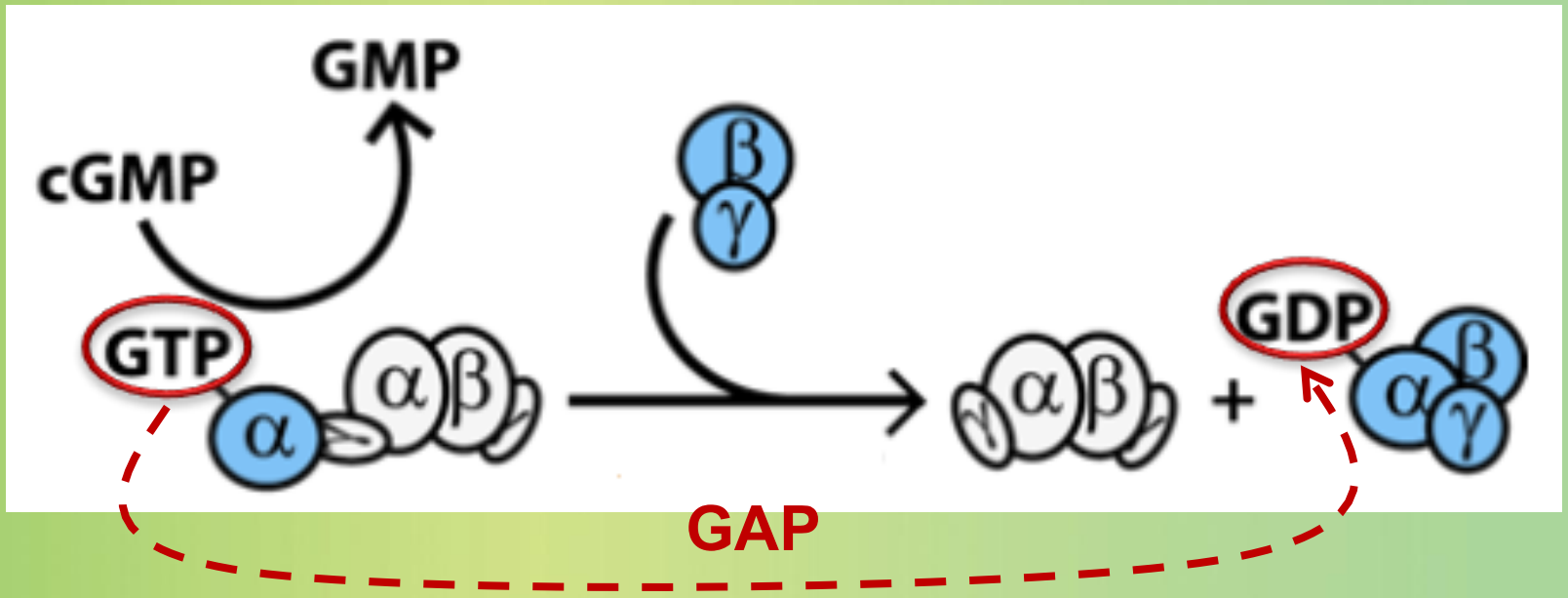


- Transducin has an intrinsic GTPase activity that hydrolyzes GTP to GDP.
- Upon hydrolysis of GTP to GDP, transducin α subunit releases the PDE γ subunit that re-inhibits the catalytic subunit.
- Transducin α-GDP eventually combines with transducin $\beta\gamma$

Mechanism IV

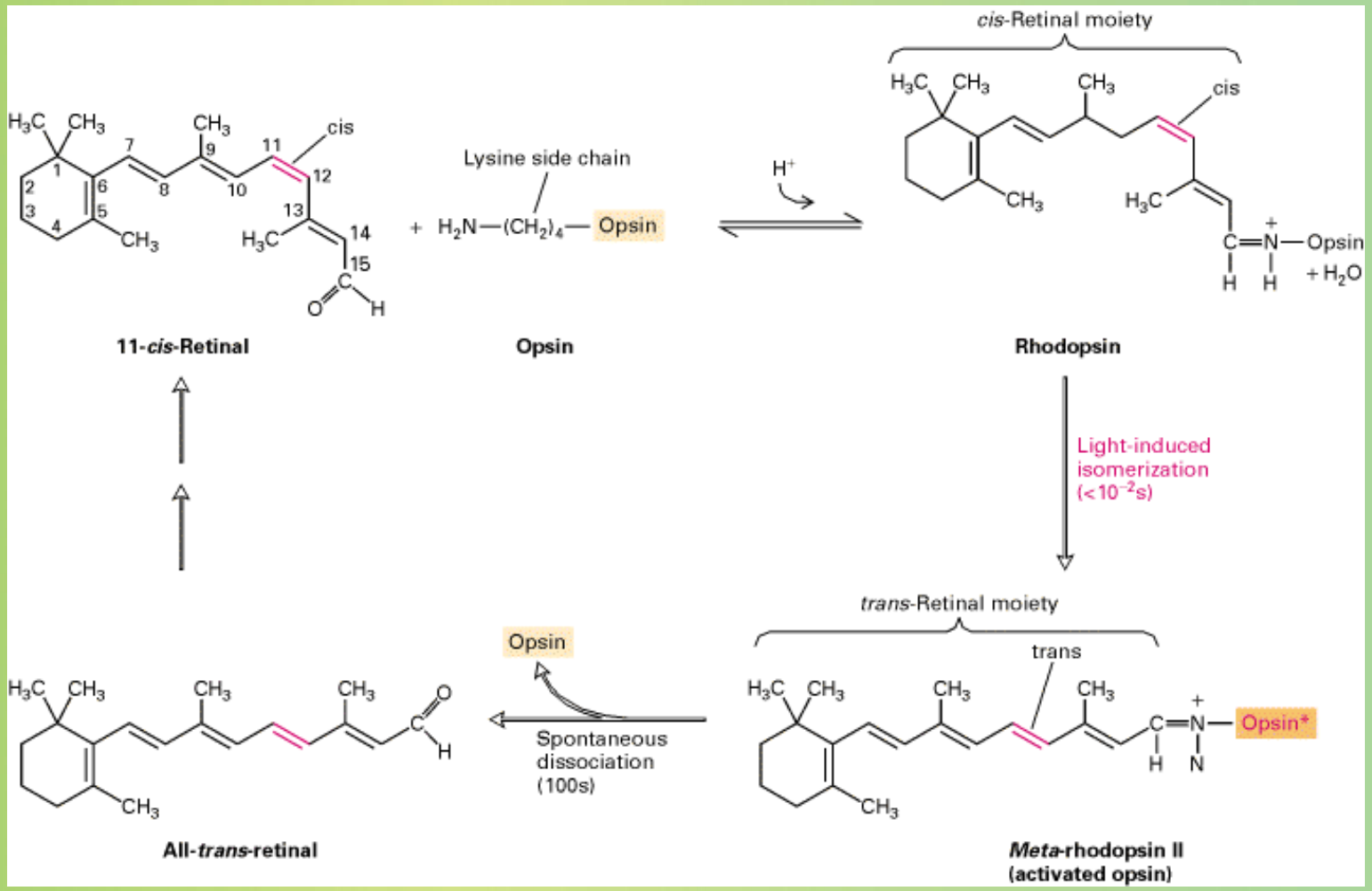
Facilitation of GTPase activity of G protein

- **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.**

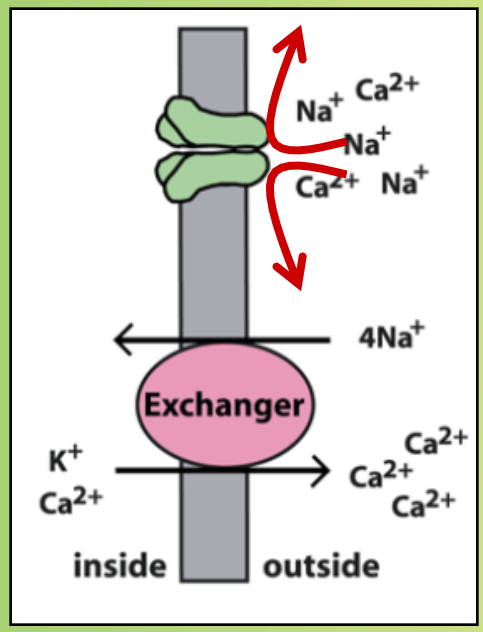


Mechanism V

Unstable all-trans rhodopsin complex



Feedback regulation by calcium ions



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

Activation

[cGMP]↓ → Ion channels closed →

Recovery

500 nM

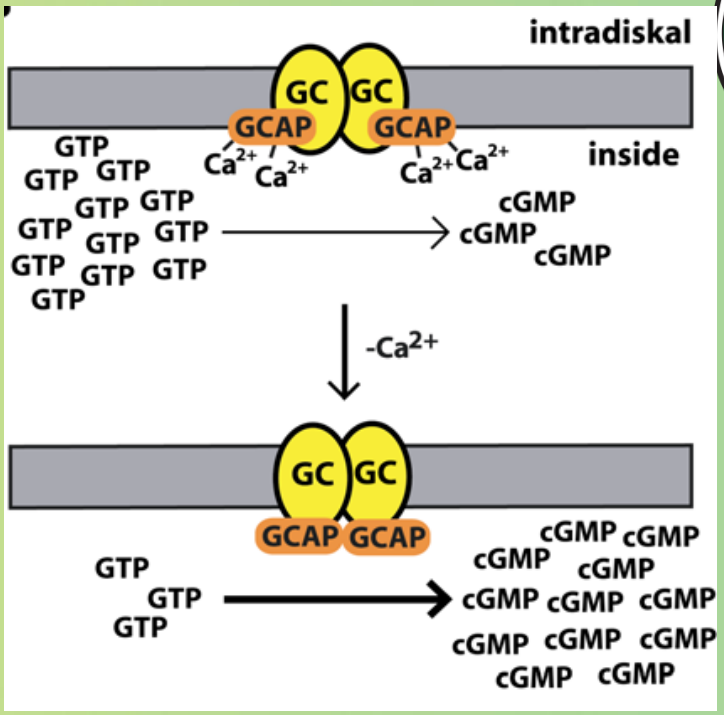
[Ca^{2+}]↓

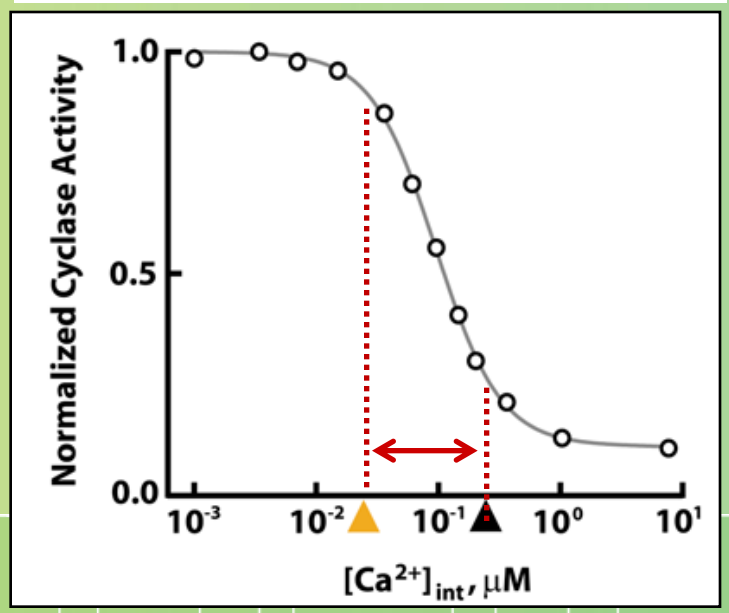
50 nM

→ Guanylate cyclase activity increased → [cGMP]↑

Mechanism VI
Guanylate cyclase

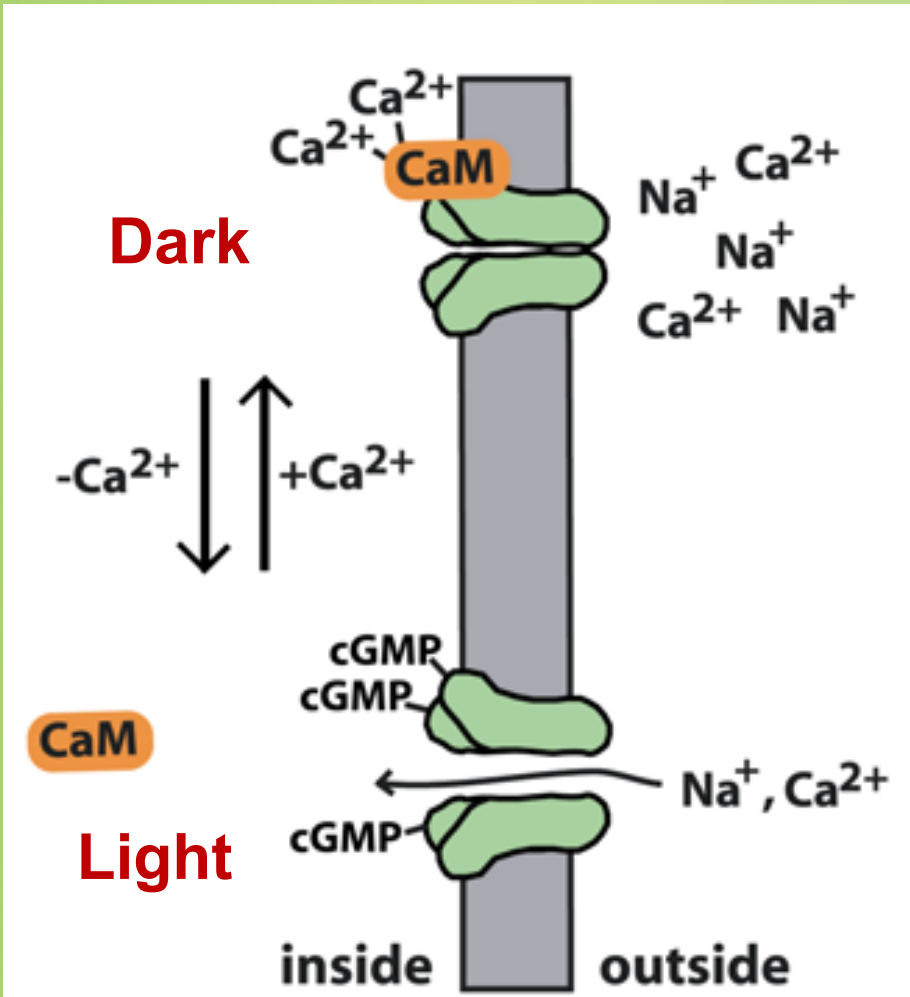
- In the dark, guanylate cyclase-activating proteins (GCAPs) bind Ca^{2+} blocking their activation of guanylate cyclase.
- A decrease in intracellular [Ca^{2+}] causes Ca^{2+} to dissociate from GCAPs leading to full activation of guanylate cyclase subunits, and an increase in the rate of cGMP synthesis.





Mechanism VII

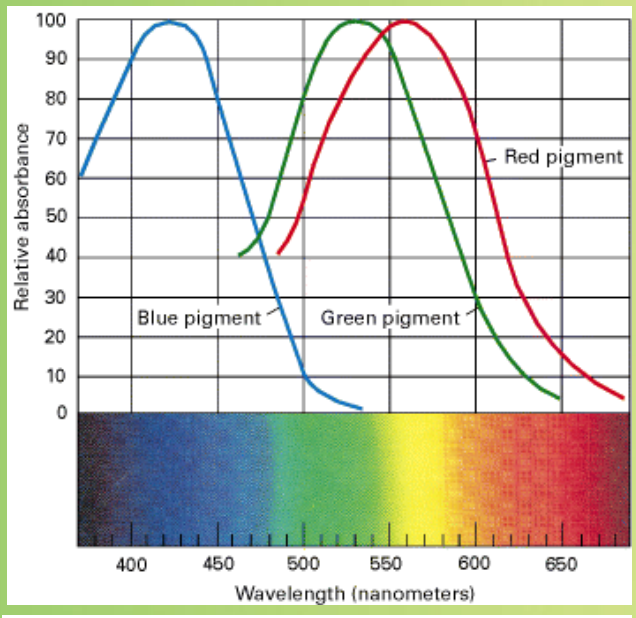
Ca-calmodulin

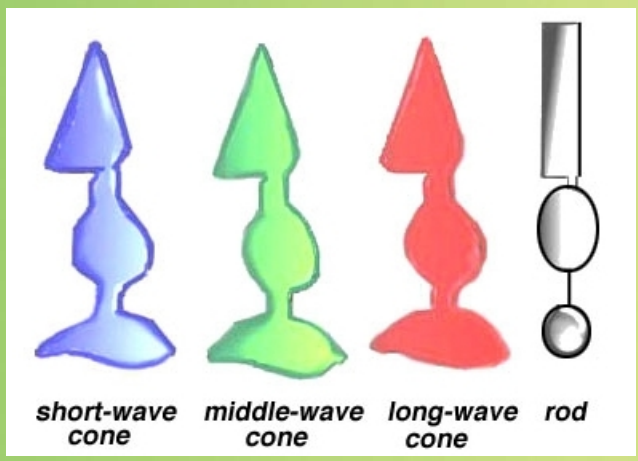


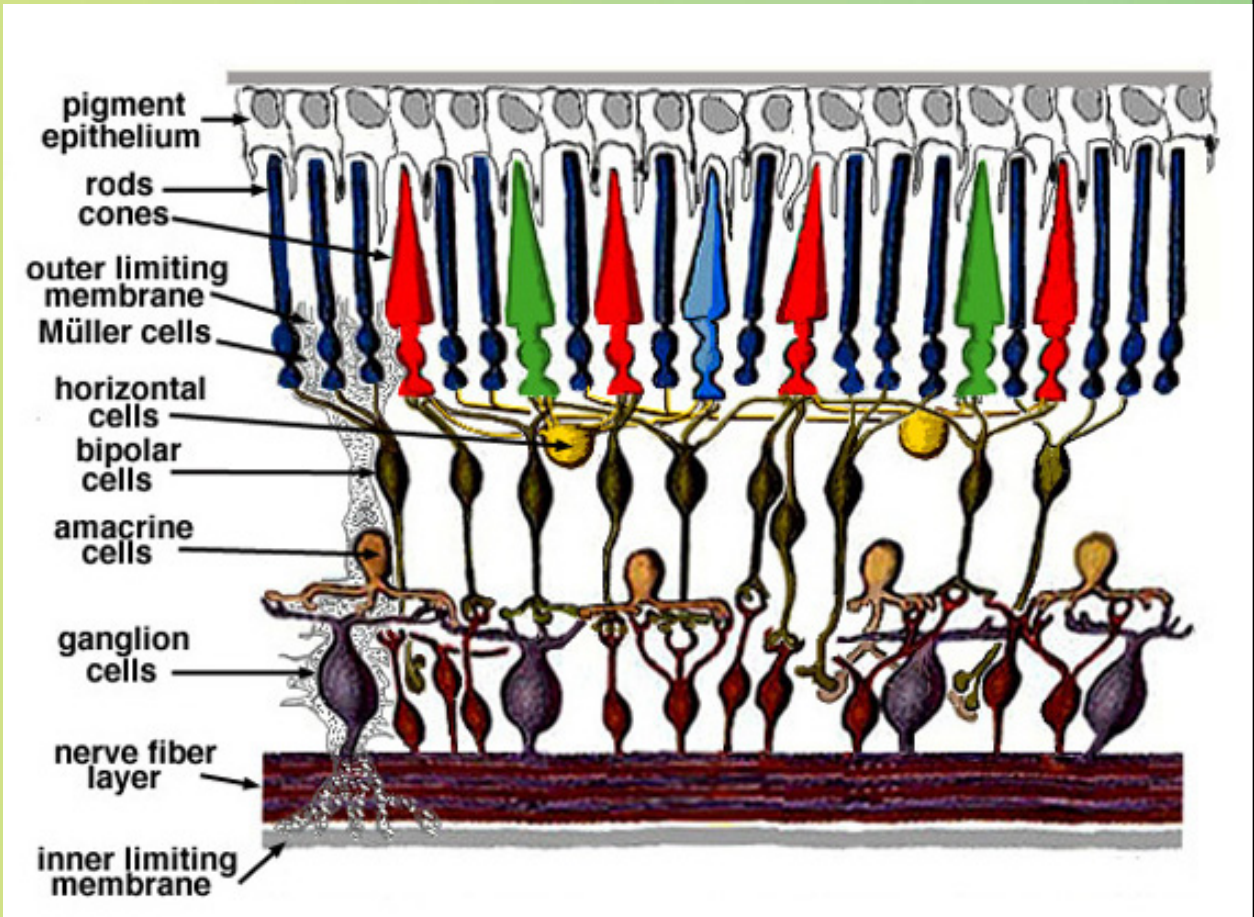
- In the dark, Ca^{2+}-Calmodulin (CaM) binds the channel and shuts it down.
- During visual transduction, the decrease in intracellular [Ca^{2+}] causes CaM to be released, and the channel reopens at lower levels of cGMP.

Color vision

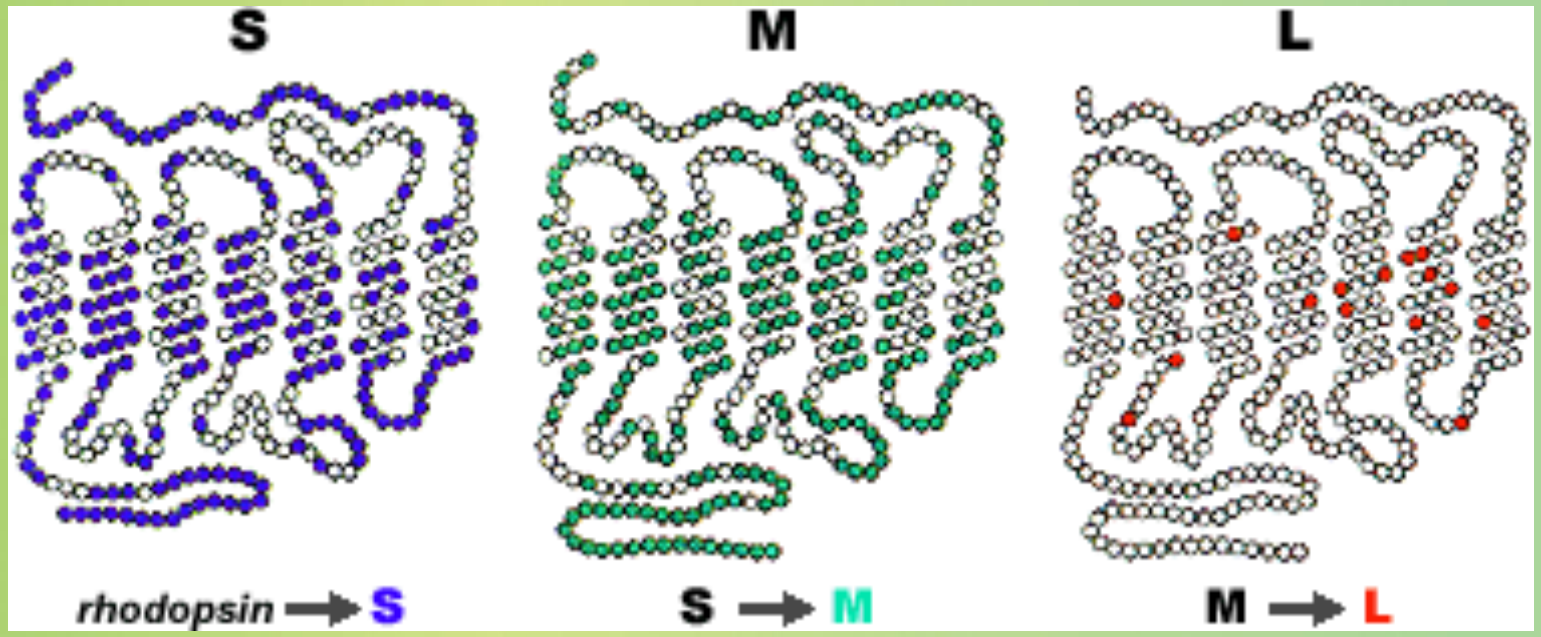
Cone photoreceptor proteins





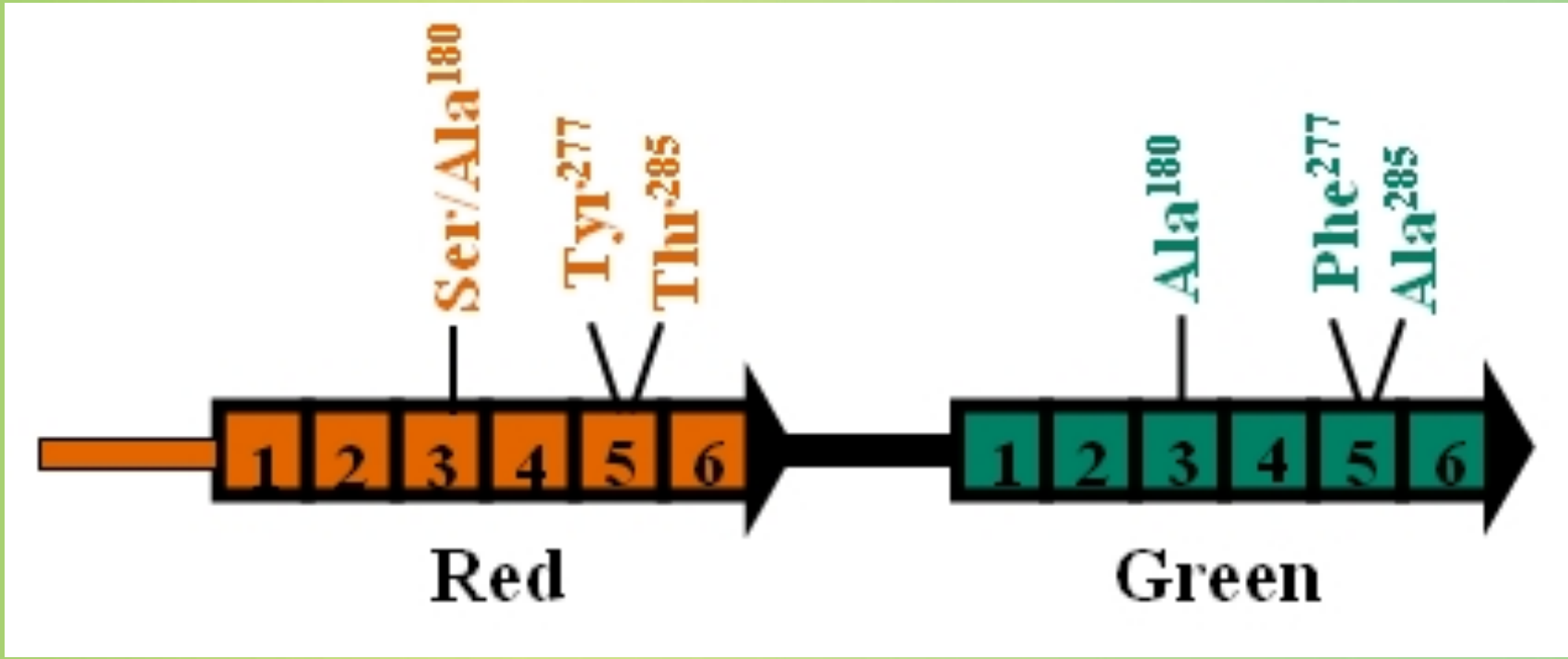


How different are they?



- 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.

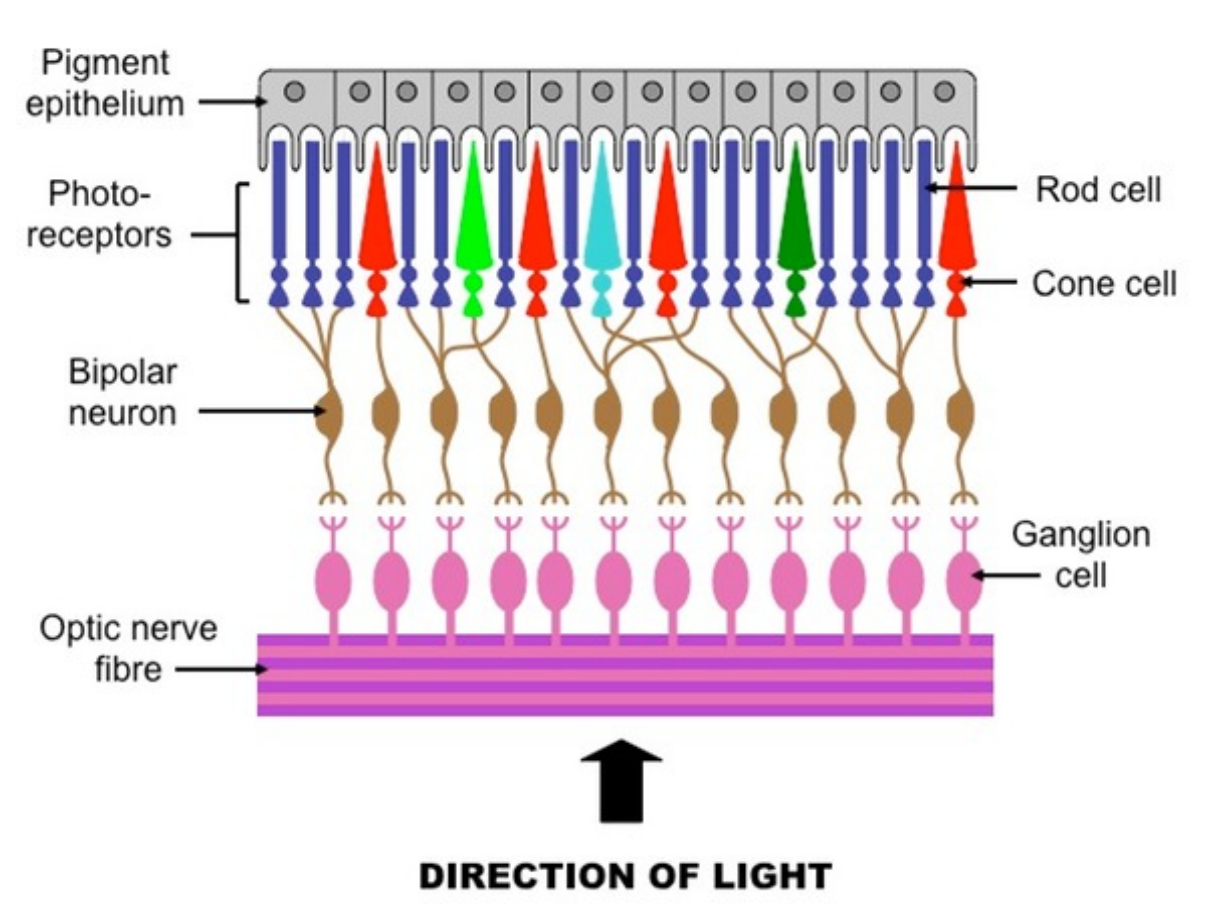
Three important aa residues



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**



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.

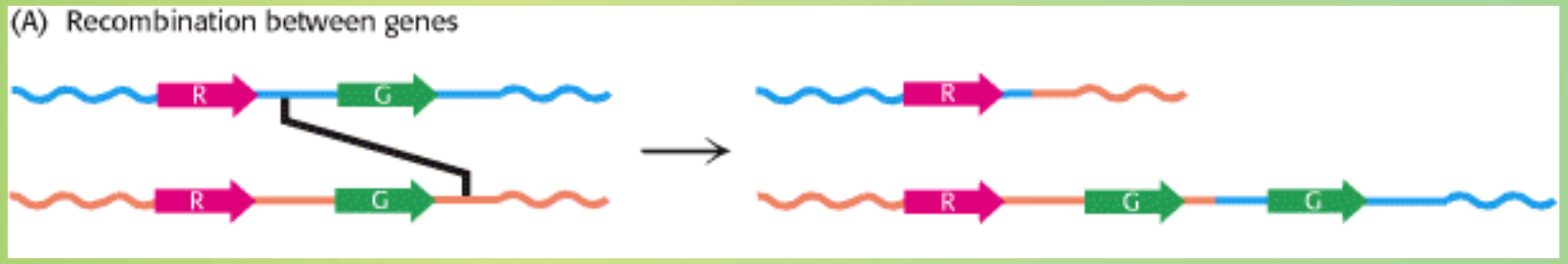
Color blindness

Chromosomal locations

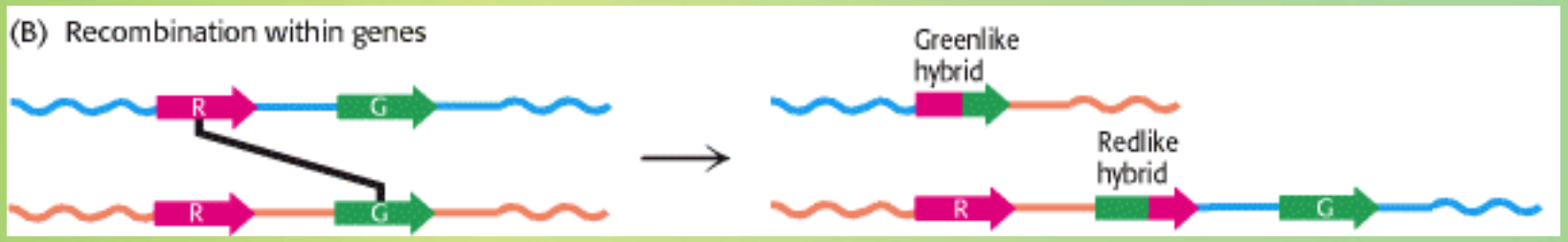
- The "blue" opsin gene: chromosome 7
- The "red" and "green" opsin genes: X chromosome
- The X chromosome normally carries a cluster of from 2 to 9 opsin genes.
- Multiple copies of these genes are fine.

Red-green homologous recombination

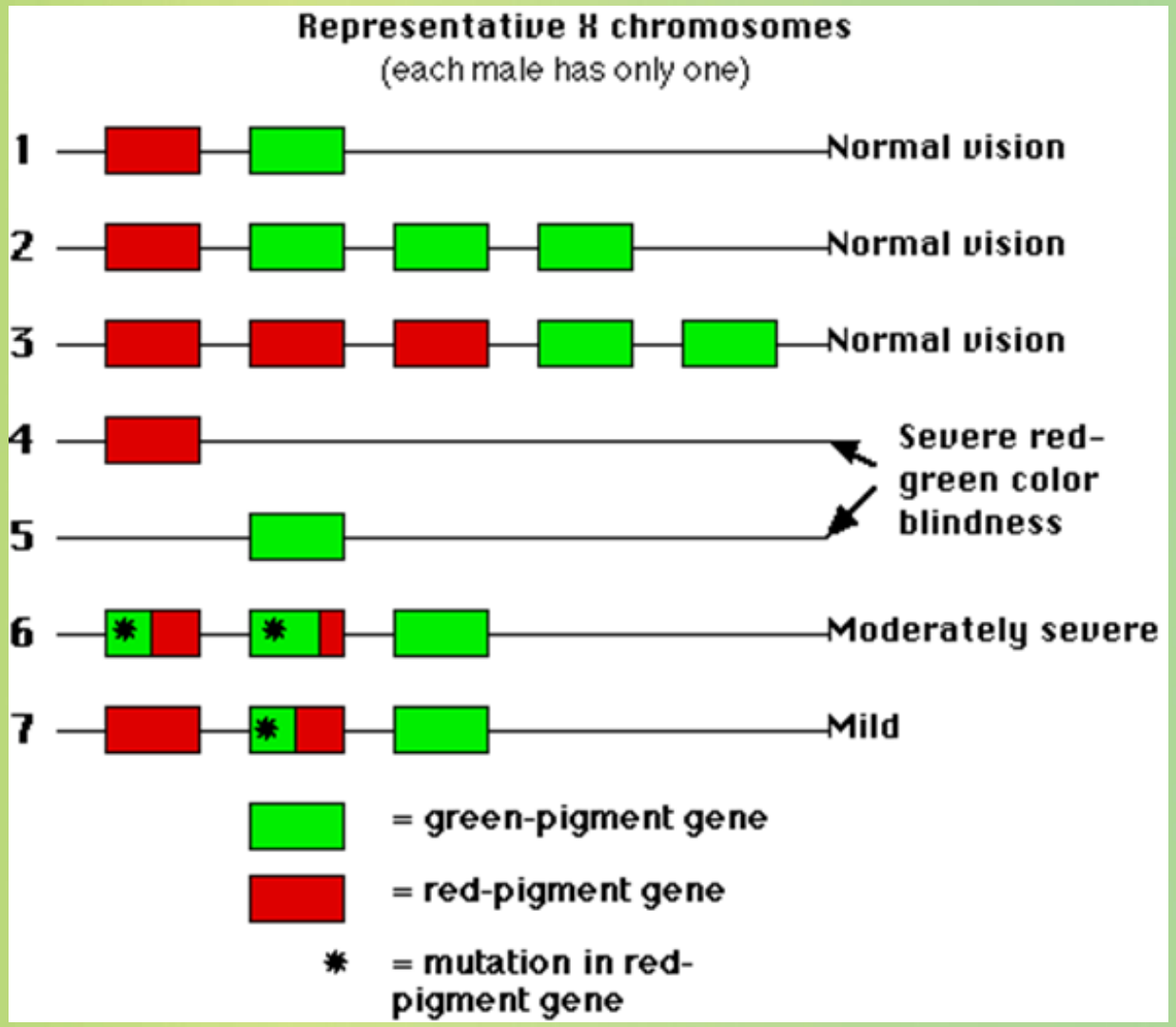
- **Between transcribed regions of the gene (inter-genic)**

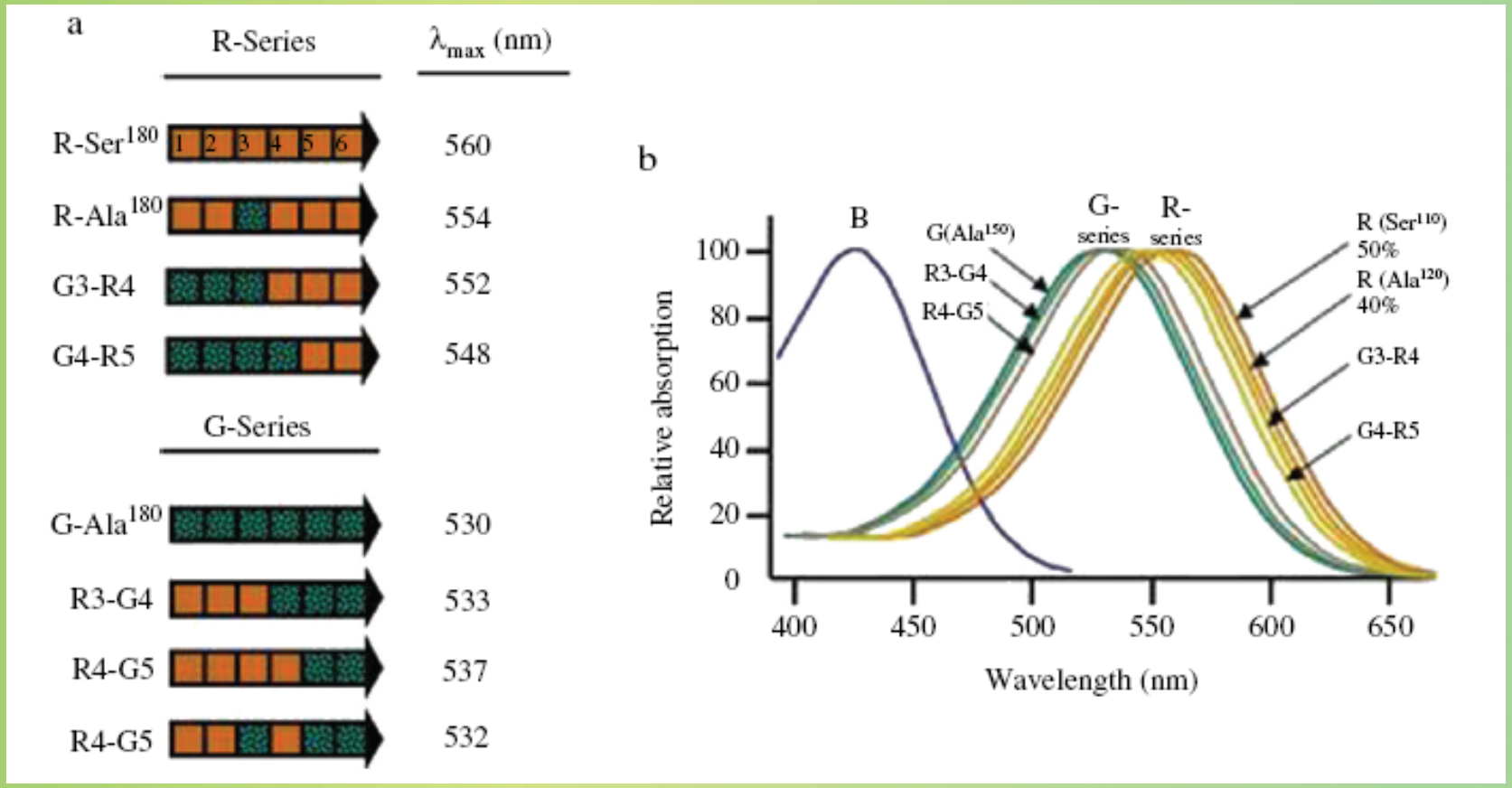


- **Within transcribed regions of the gene (intra-genic)**

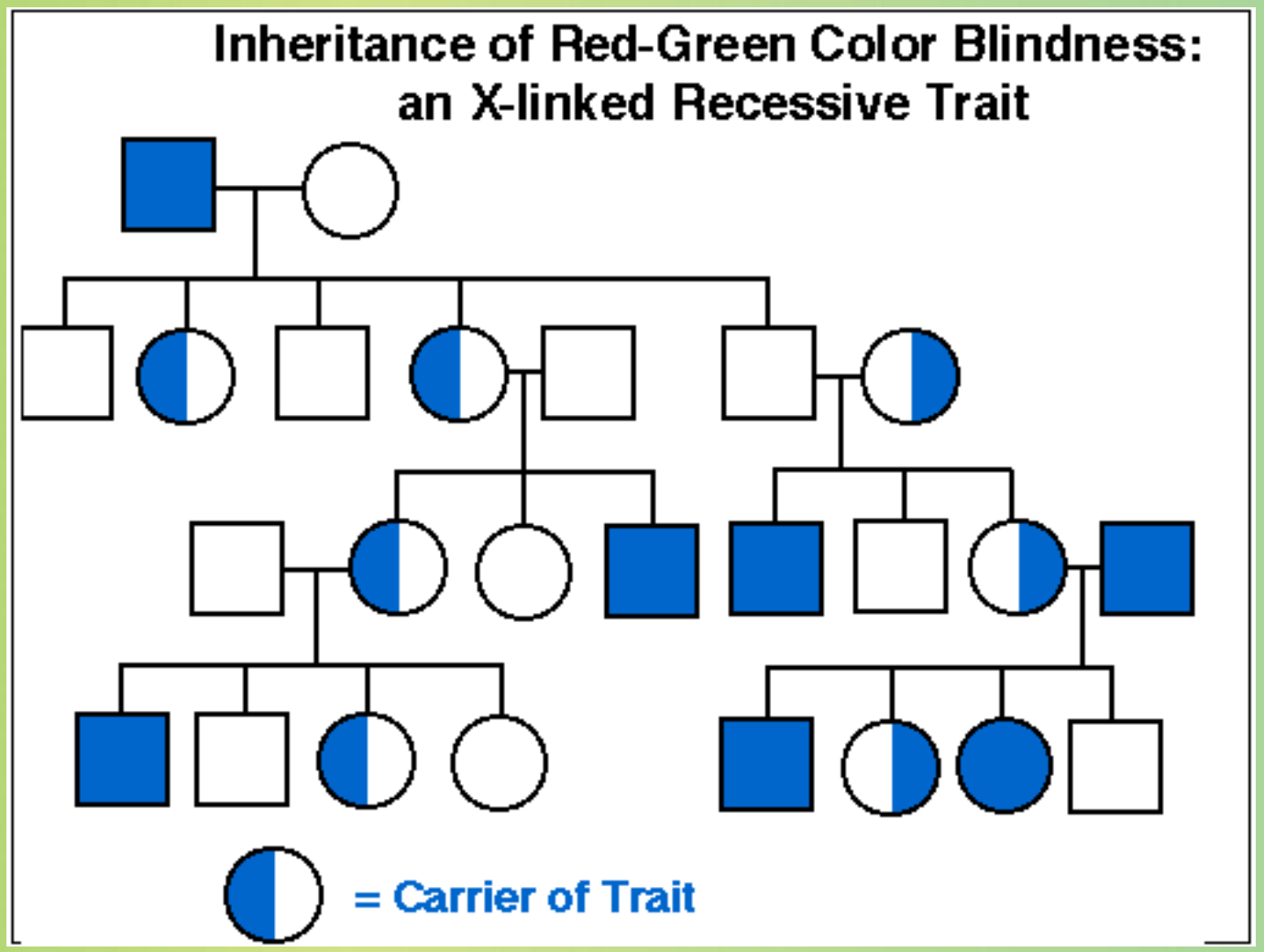


Genetic probabilities




a
R-Series
λmax (nm)
R-Ser180
1 2 3 4 5 6
560
R-Ala180
554
G3-R4
552
G4-R5
548
G-Series
G-Ala180
530
R3-G4
533
R4-G5
537
R4-G5
532
b
B
G(Ala150)
R3-G4
R4-G5
G-series
R-series
R (Ser110) 50%
R (Ala120) 40%
G3-R4
G4-R5
Relative absorption
100
80
60
40
20
0
400
450
500
550
600
650
Wavelength (nm)

Pedigree


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

Examples

Red blindness

Green blindness