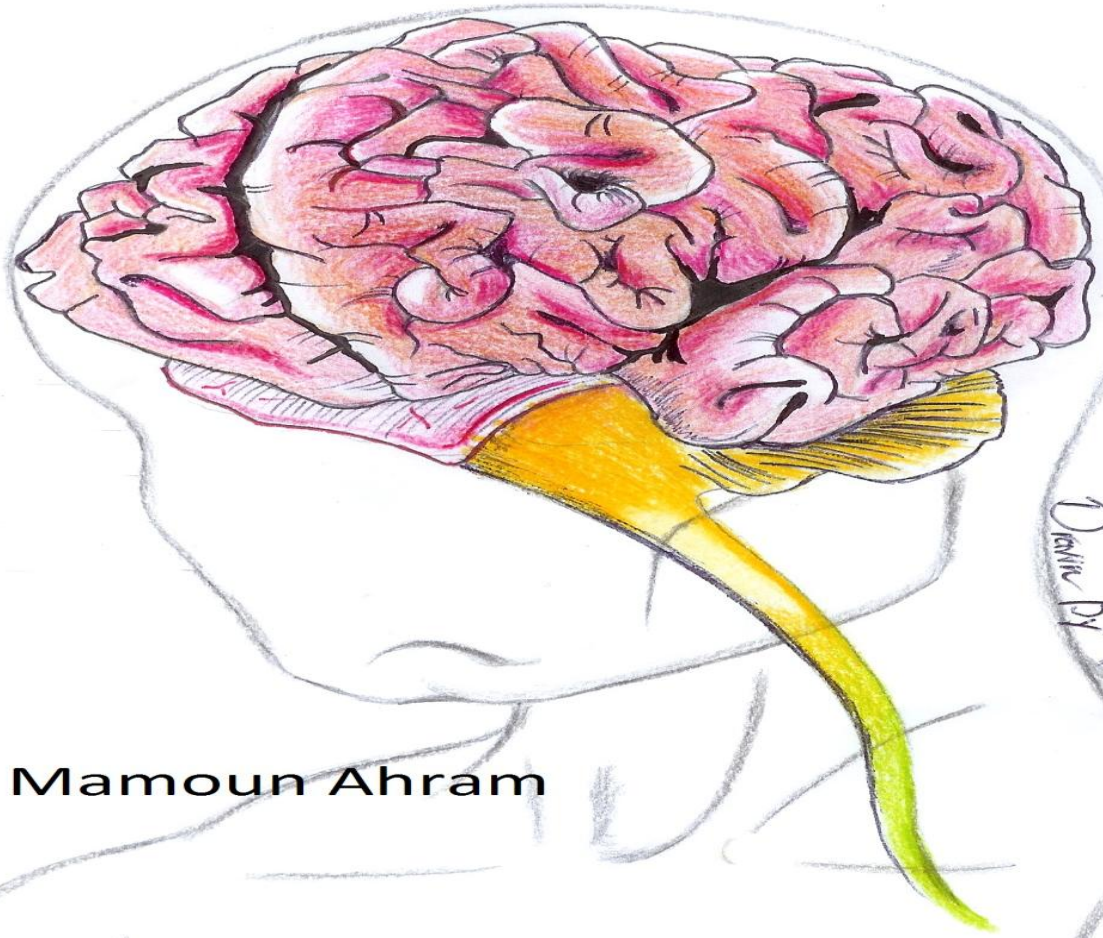
CENTRAL NERVOUS SYSTEM

- ○ Handout
- ○ Sheet
- ● Slide

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



Drawn By Tariq Bushnaq...

Done By :

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Lec # : 2

Visual pigments

Neuroscience, Biochemistry
Dr. Mamoun Ahram
Third year, 2016

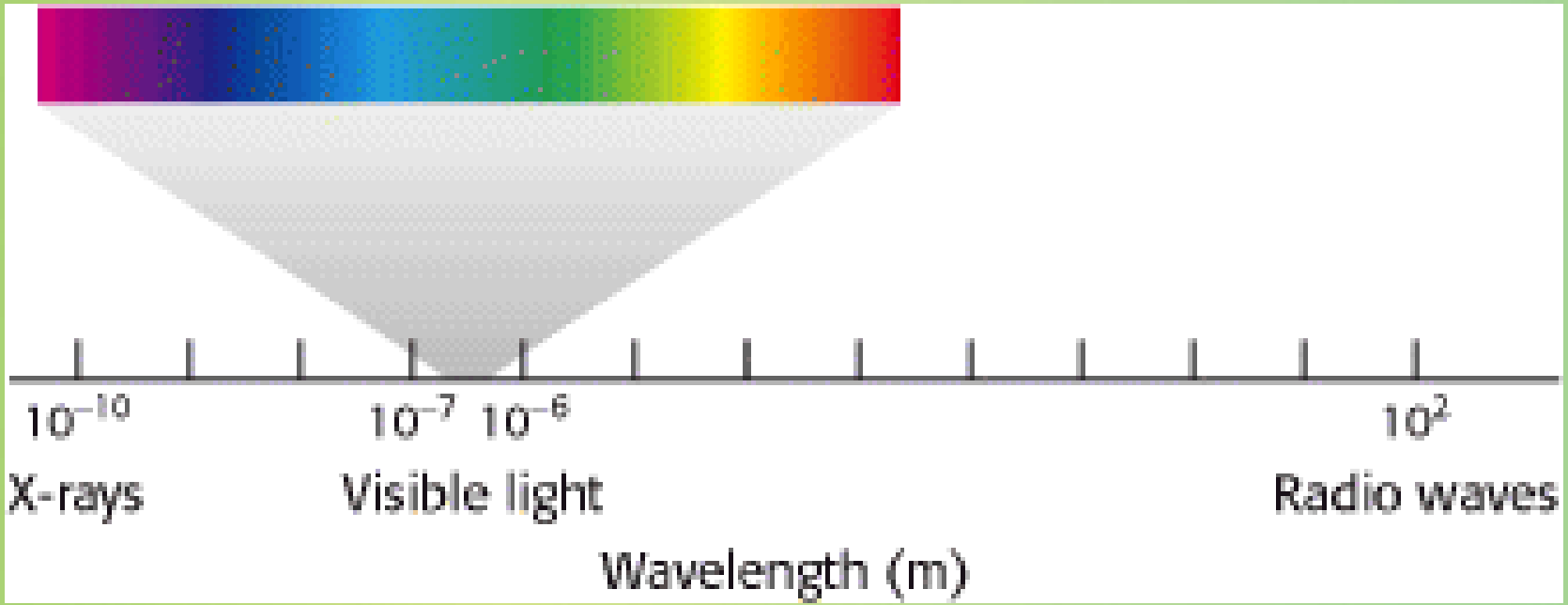
References

- 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

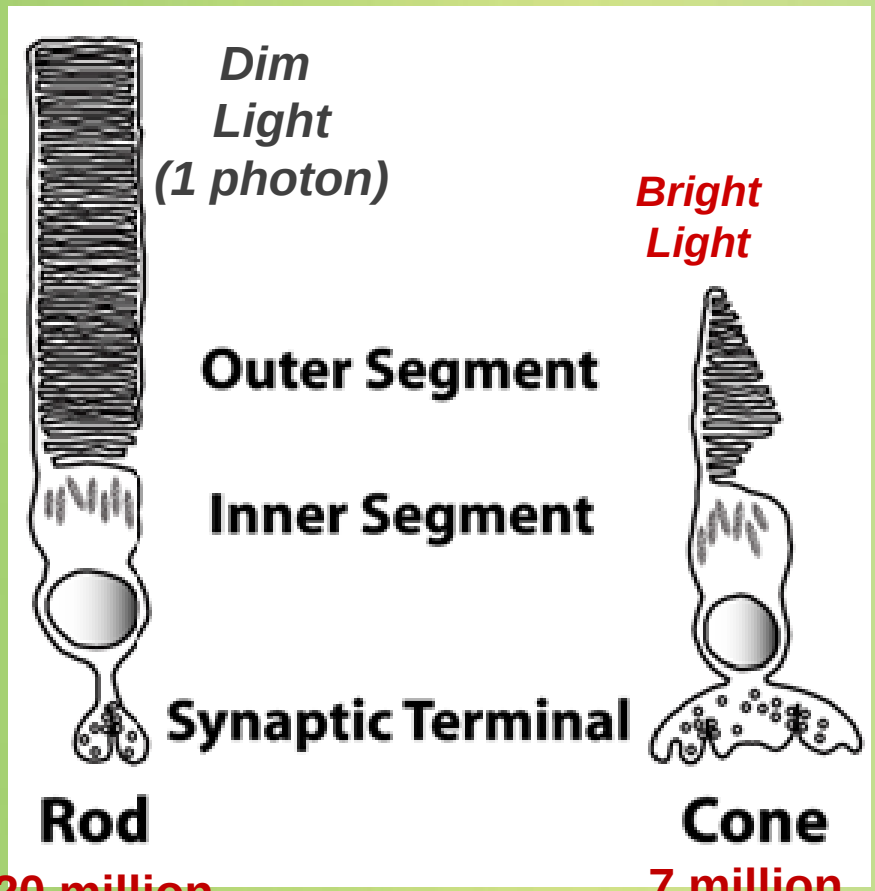
Lecture outline

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

Basics of human vision

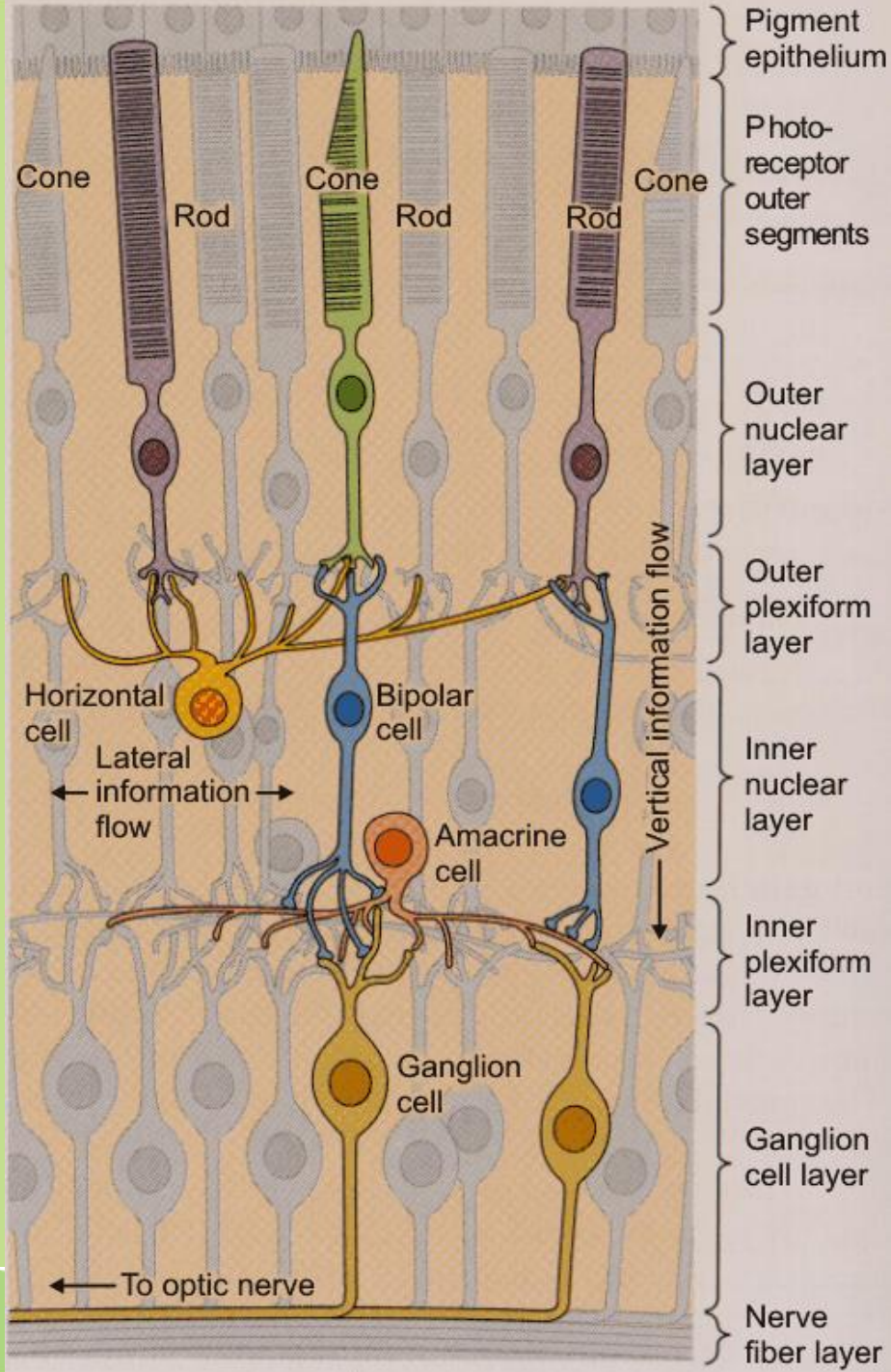


Rods and cones

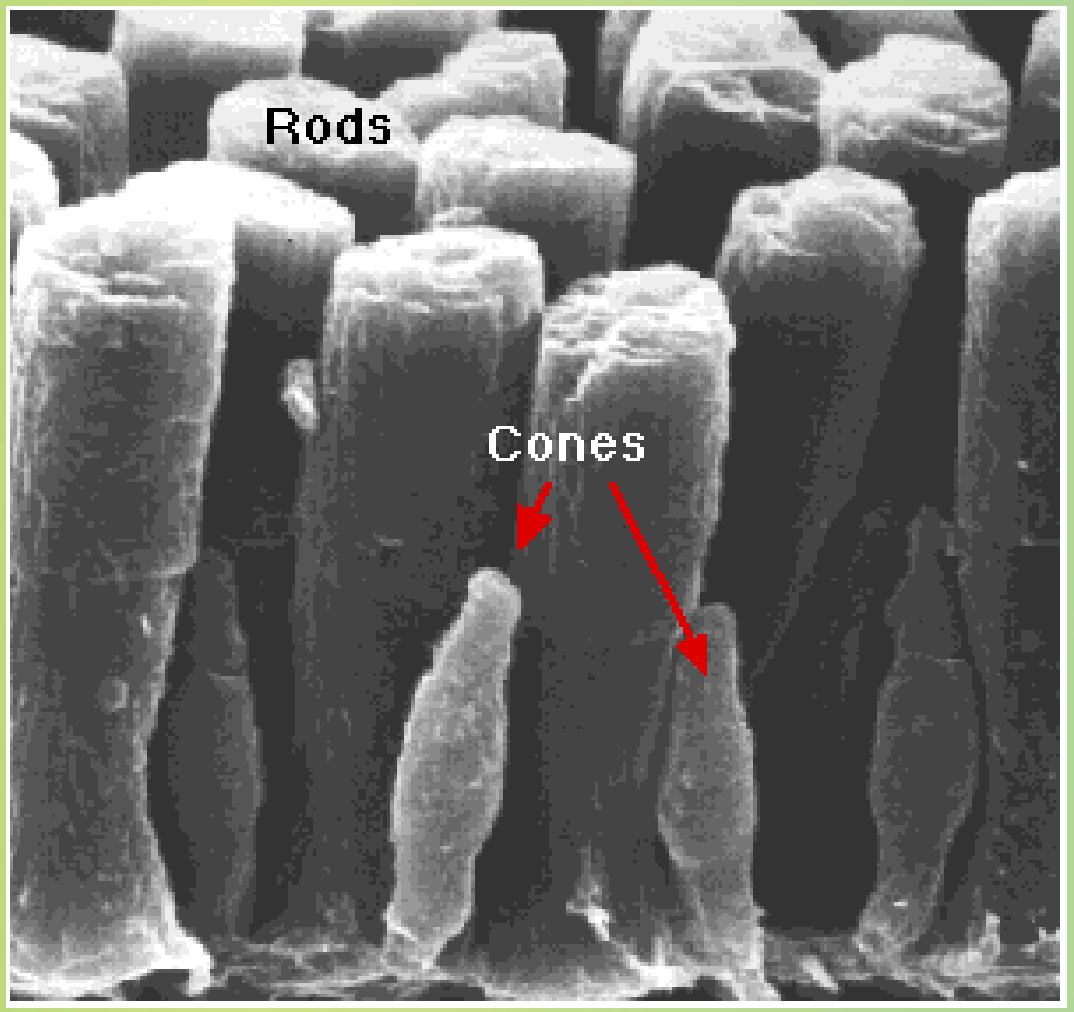


120 million

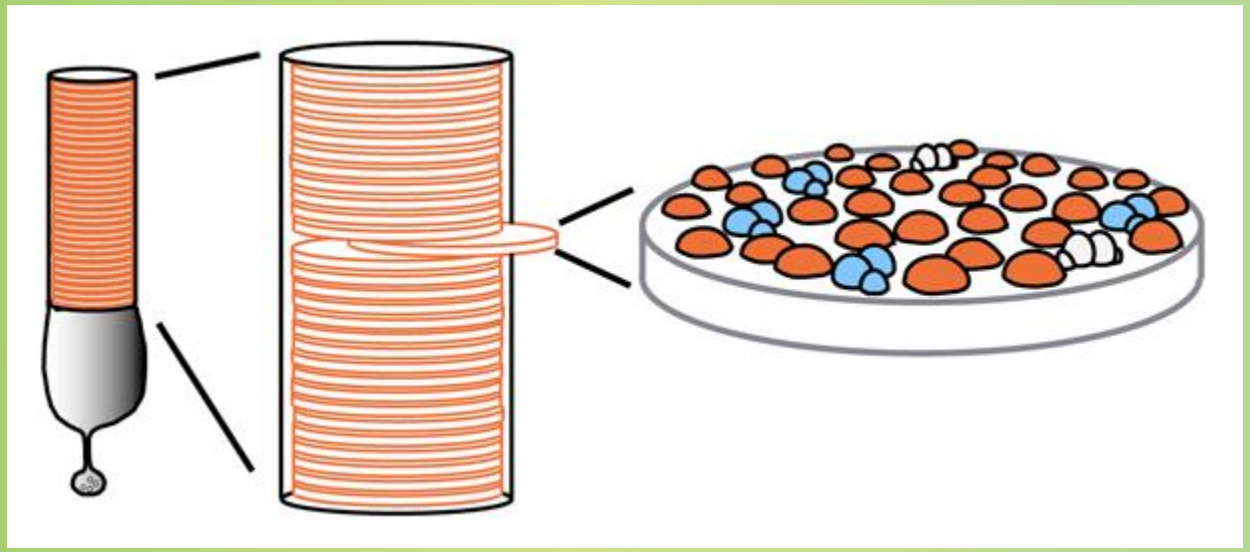
7 million

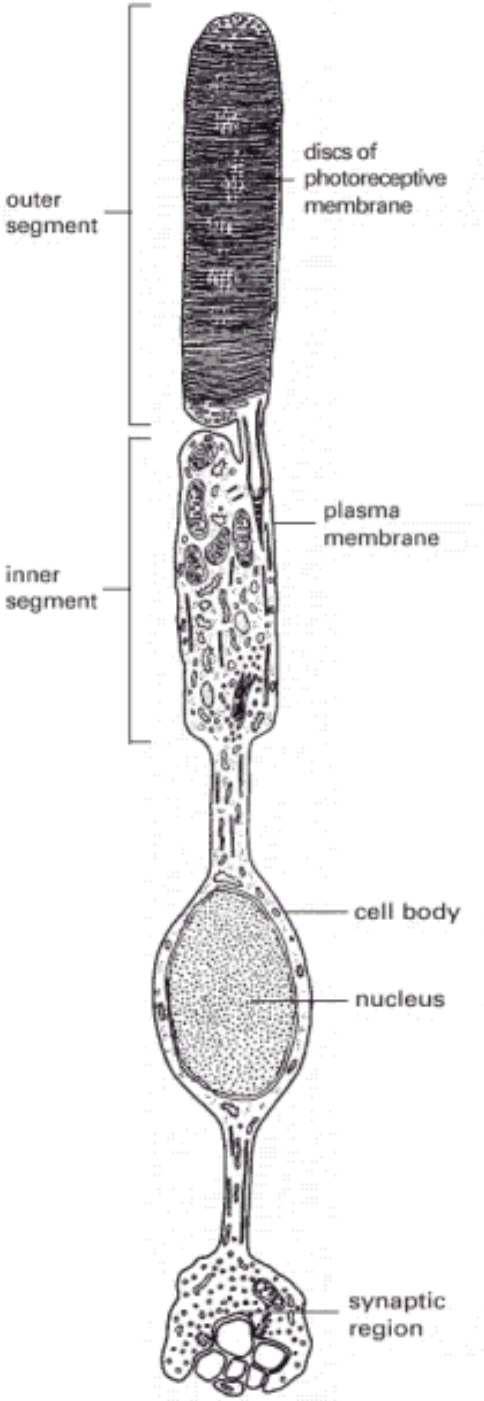


How they really look like…



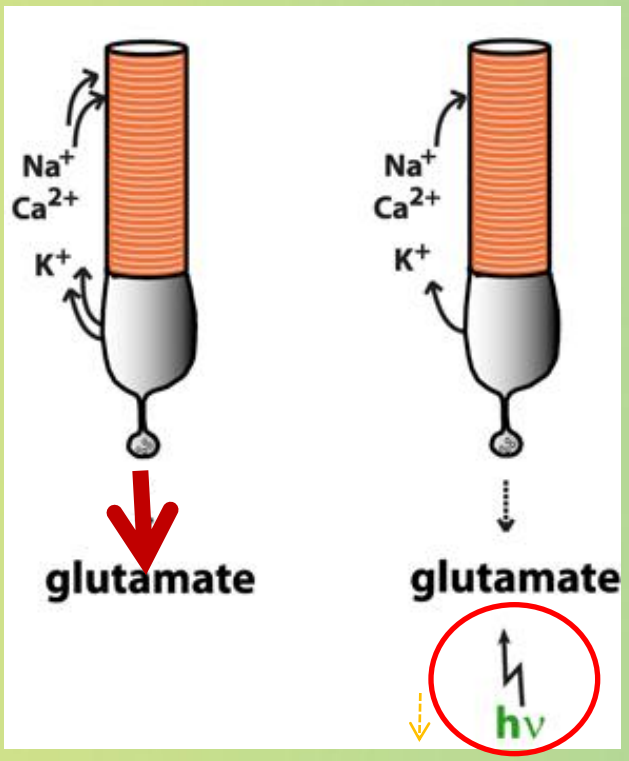
More on rod cells





The dark current

1. 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.



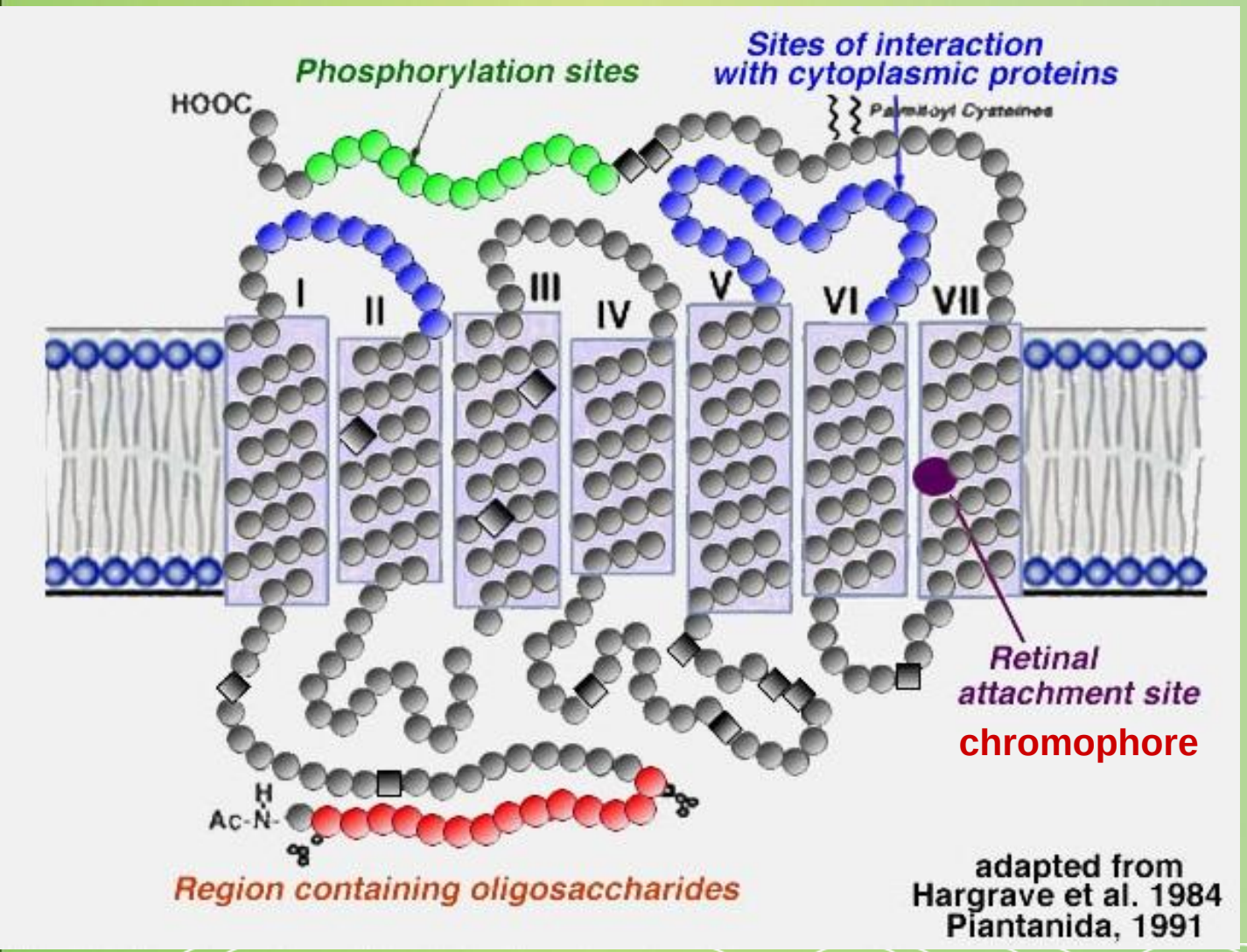
1. Channels in the outer segment membrane close, the rod hyperpolarizes
2. Glutamate release decreases.

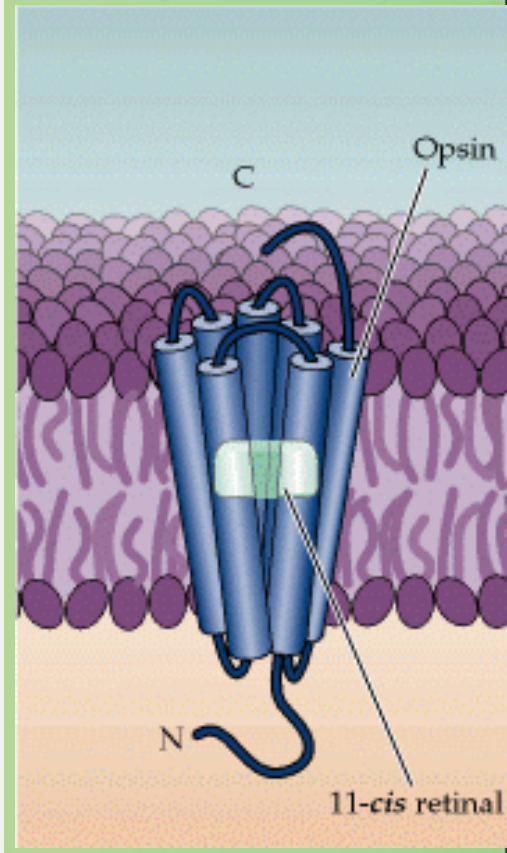
Generation of vision signals

The players

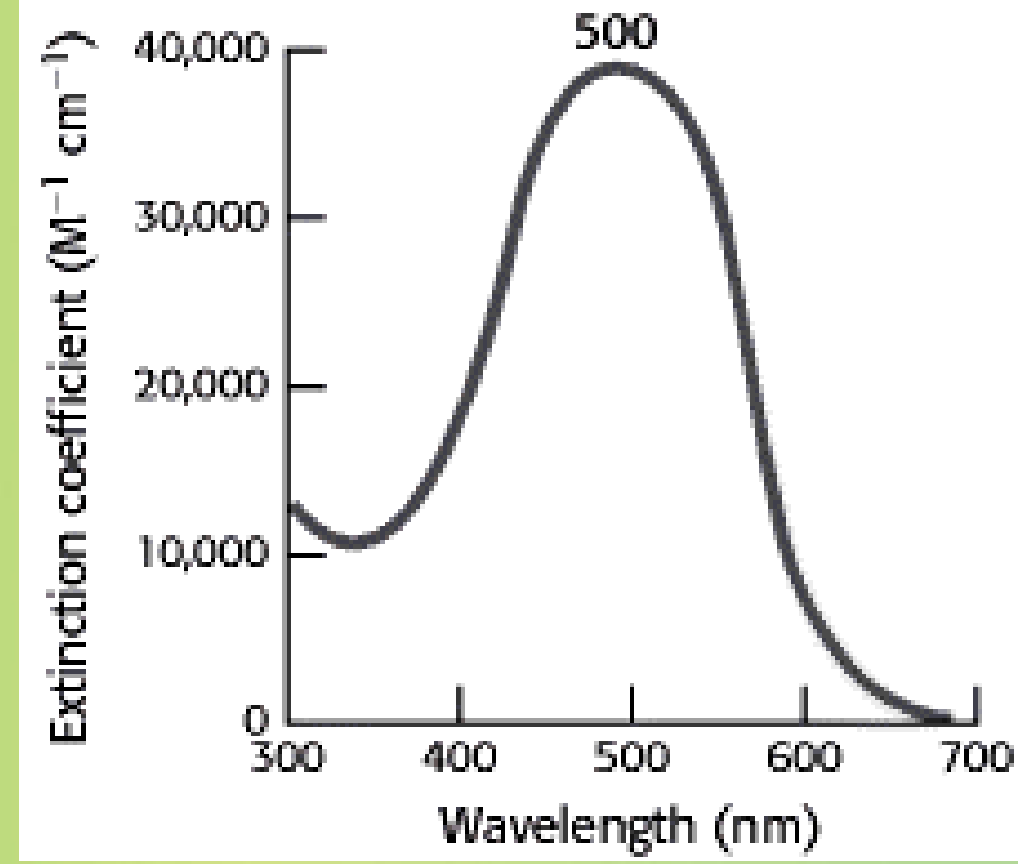
- Rhodopsin
- Transducin
- Phosphodiesterase
- Na^+-gated channels
- Regulatory proteins

Rhodopsin

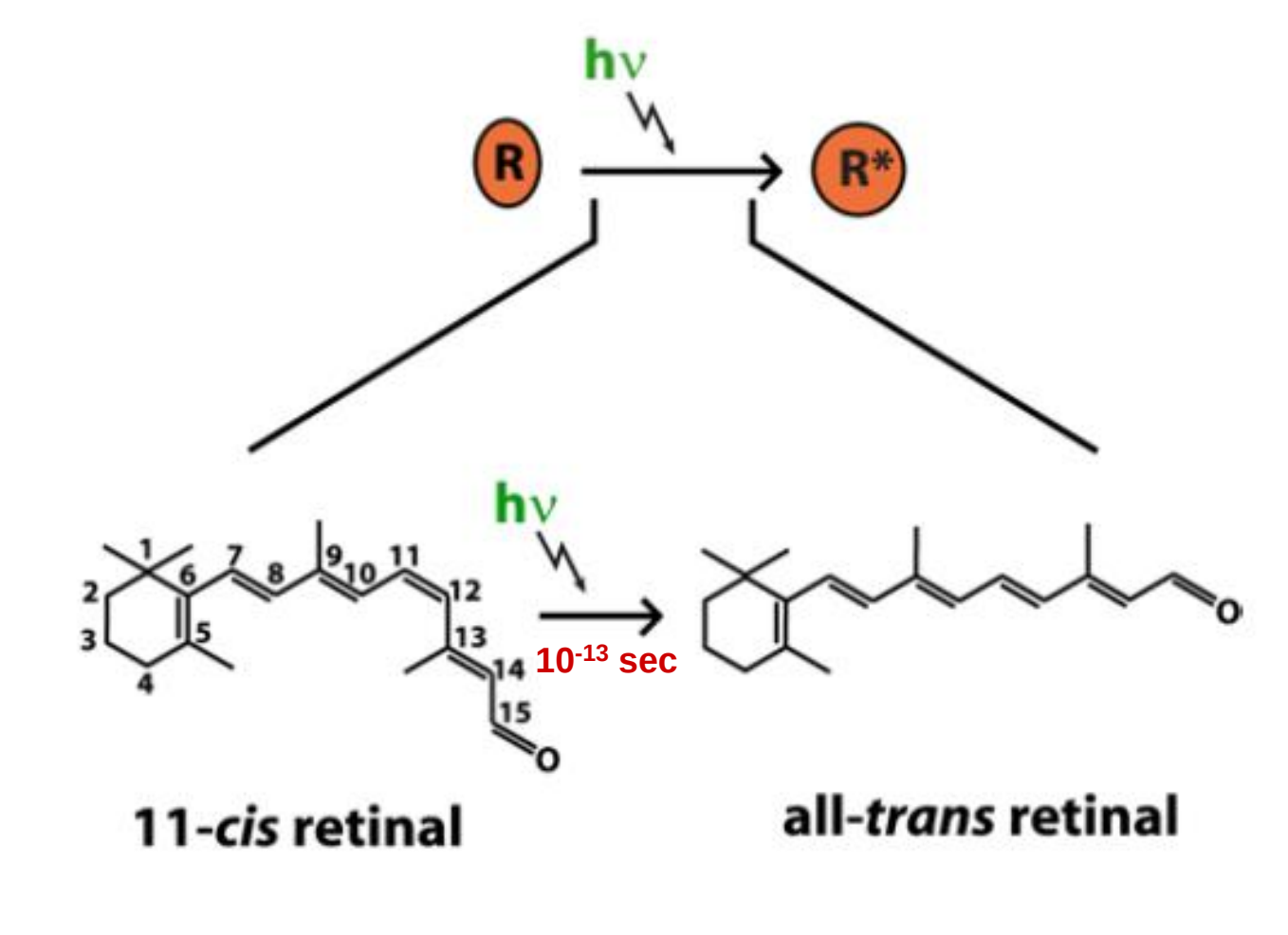

Phosphorylation sites
Sites of interaction with cytoplasmic proteins
HOOC
Palmitoyl Cysteines
I
II
III
IV
V
VI
VII
Retinal attachment site
chromophore
Ac-N-
H
Region containing oligosaccharides
adapted from Hargrave et al. 1984 Piantanida, 1991


Opsin
C
N
11-cis retinal

Light absorption by rhodopsin

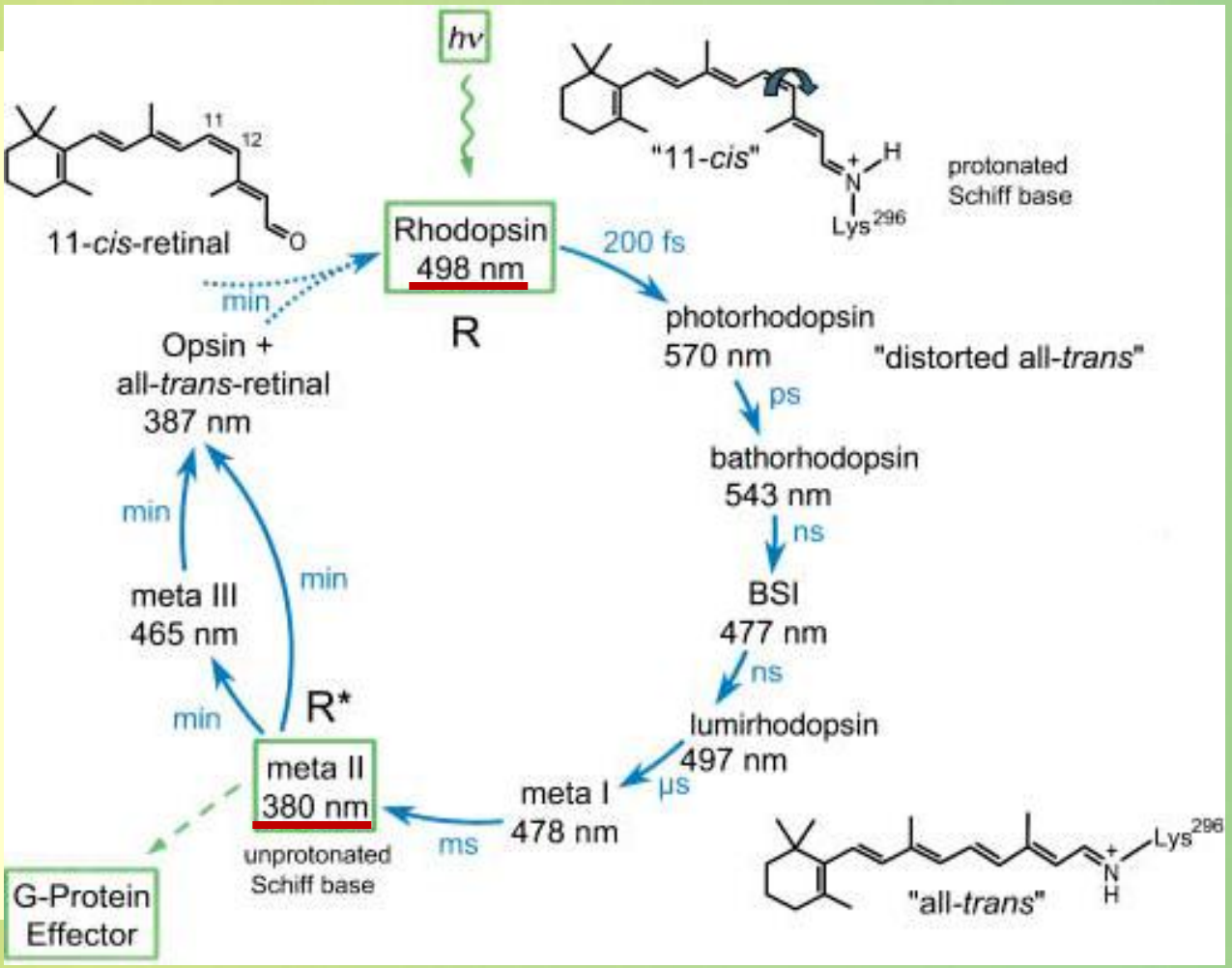


11-cis-retinal

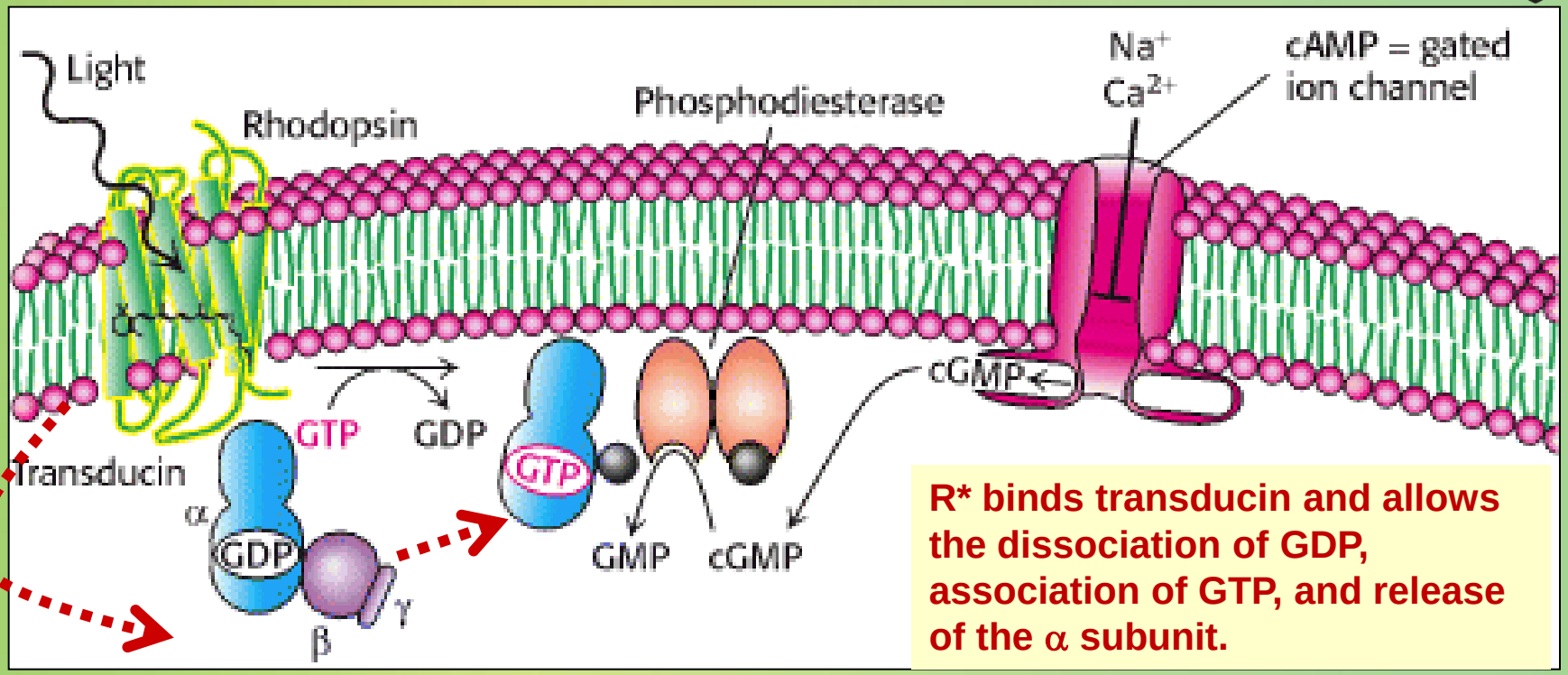


Rhodopsin intermediates

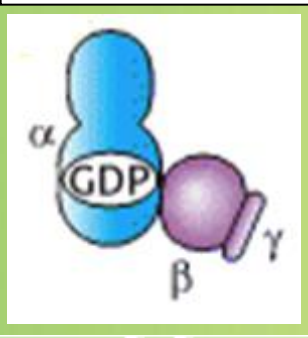
- By itself, 11-cis retinal absorbs near UV light. But opsin perturbs the distribution of the electrons exiting its electrons with less energy (i.e., longer wavelength light).
- The chromophore converts the energy of a photon into a conformational change in protein structure.
- Rearrangements in the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



Transducin → Phosphodiesterase (PDE)

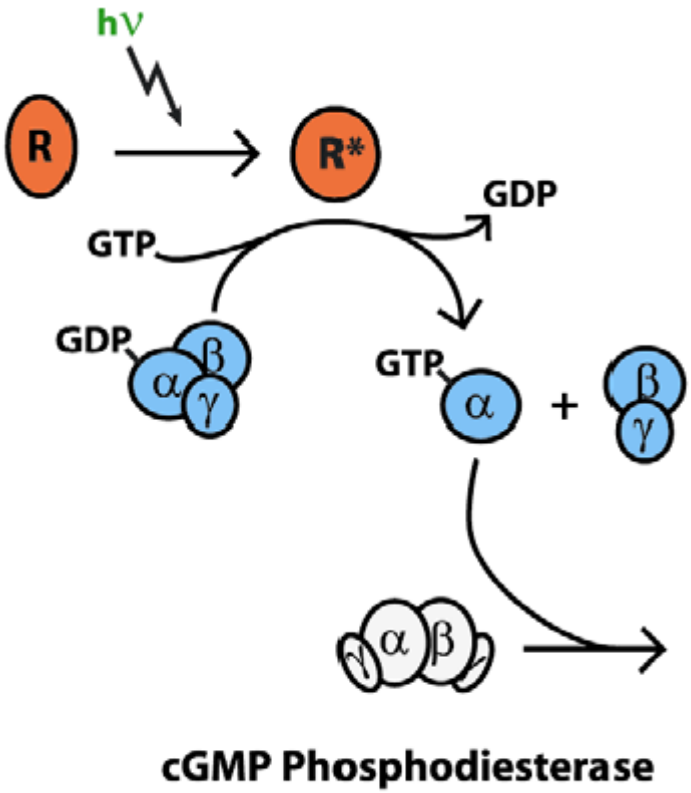


R* binds transducin and allows the dissociation of GDP, association of GTP, and release of the α subunit.

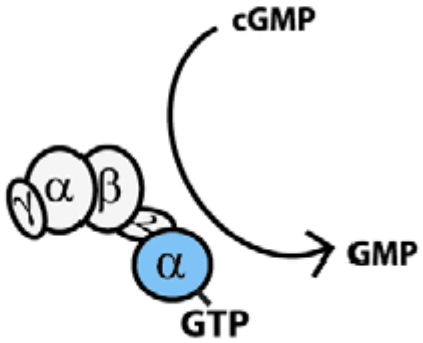


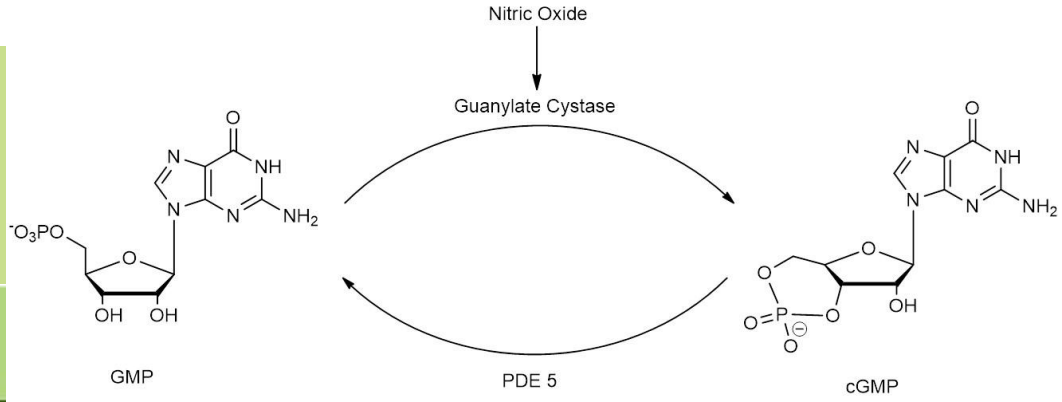
G proteins are heterotrimeric, consisting of α, β, and γ subunits. In its inactive state, transducin's α subunit has a GDP bound to it.

Activation of phosphodiesterase

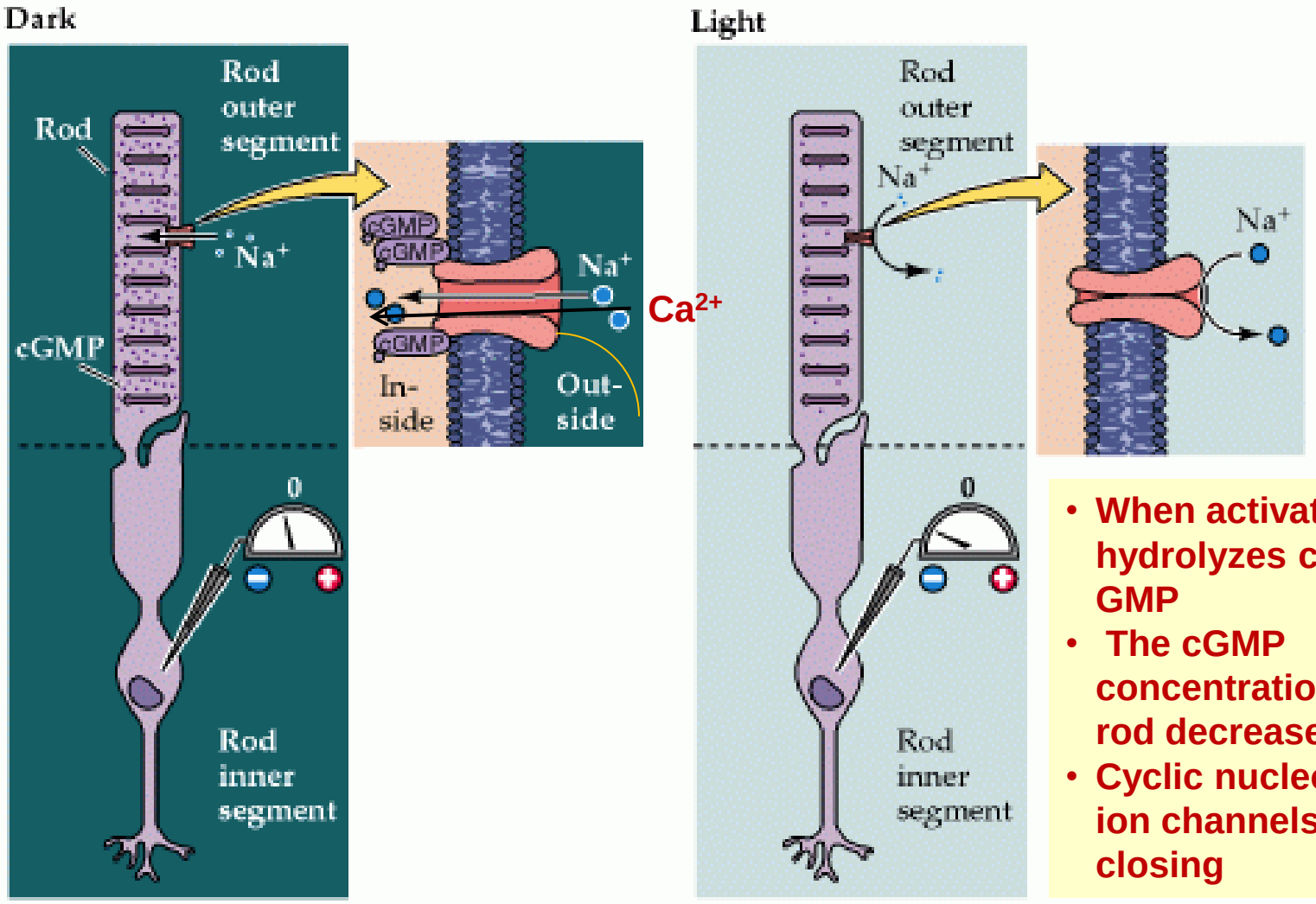


- PDE is a heterotetramer that consists of a dimer of two catalytic subunits, α and β subunits, each with an active site inhibited by a PDE γ subunit.
- The activated transducin α subunit-GTP binds to PDE γ and relieves the inhibition on a catalytic subunit.

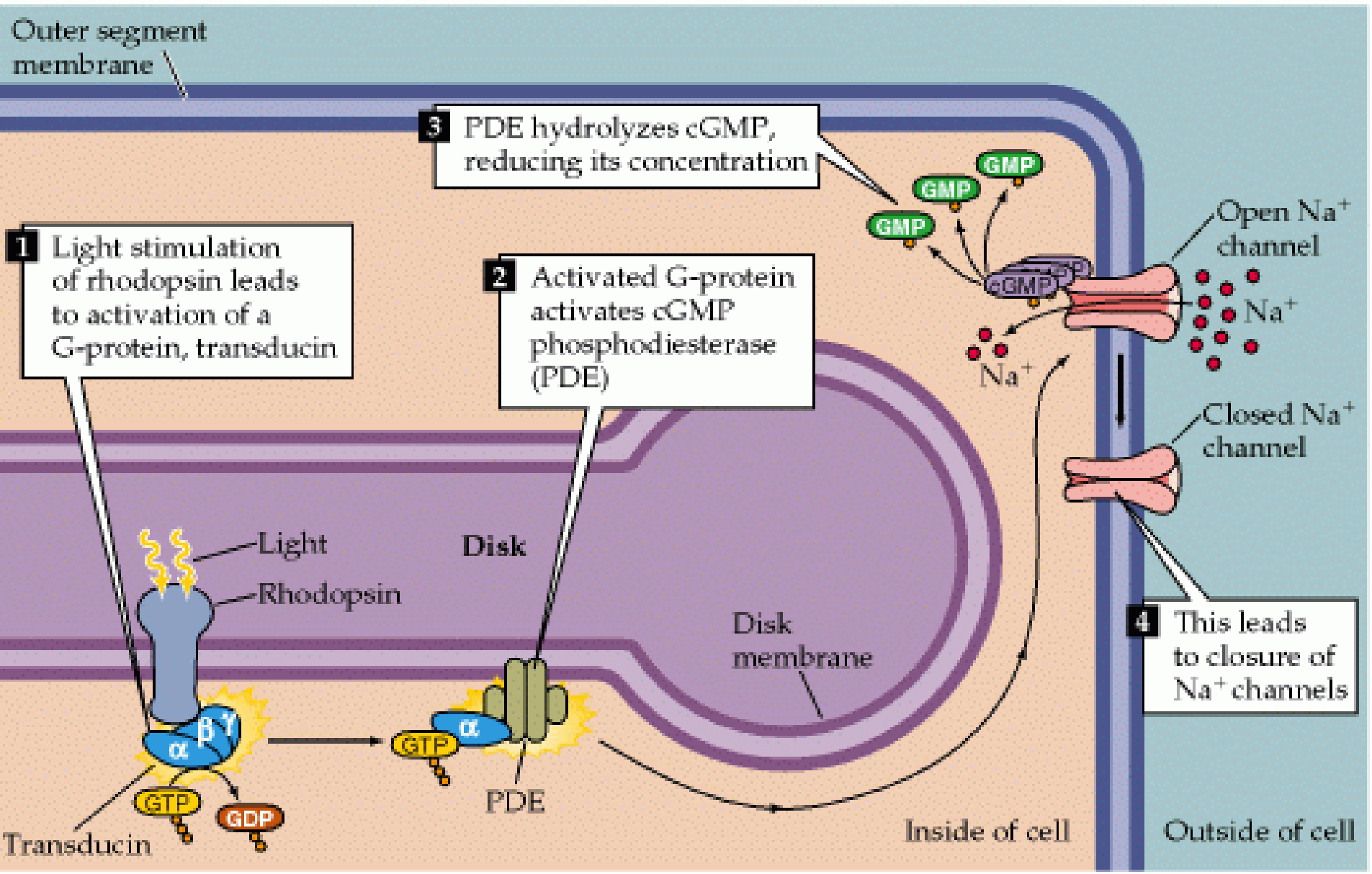




cGMP-gated channels



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

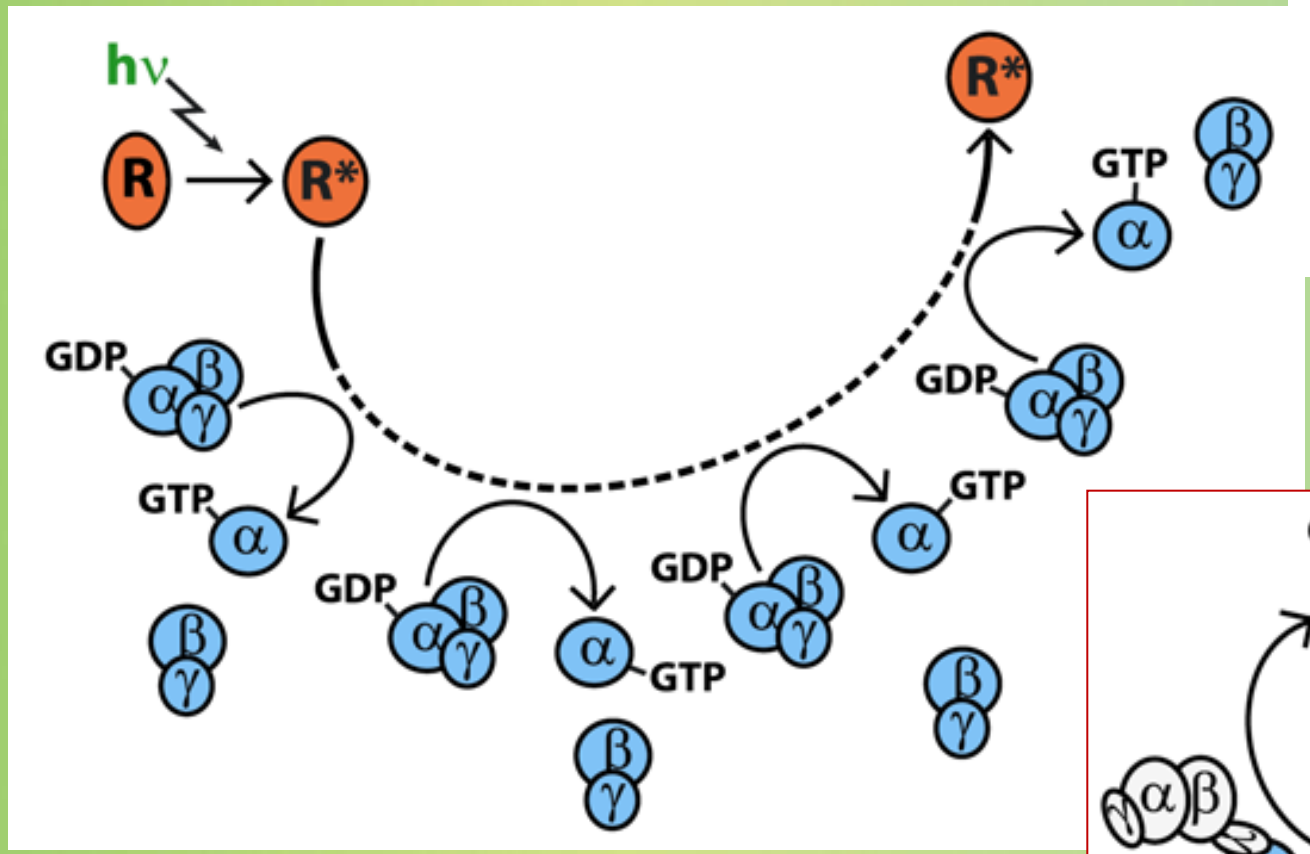

Outer segment membrane
3 PDE hydrolyzes cGMP, reducing its concentration
GMP
GMP
GMP
1 Light stimulation of rhodopsin leads to activation of a G-protein, transducin
2 Activated G-protein activates cGMP phosphodiesterase (PDE)
cGMP
Open Na+ channel
Na+
Na+
Closed Na+ channel
Light
Rhodopsin
Disk
Disk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

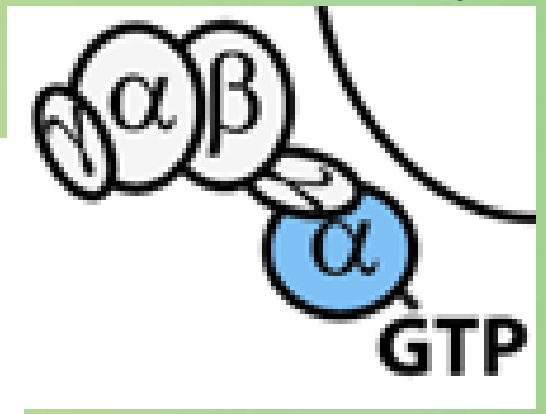
Animation movie

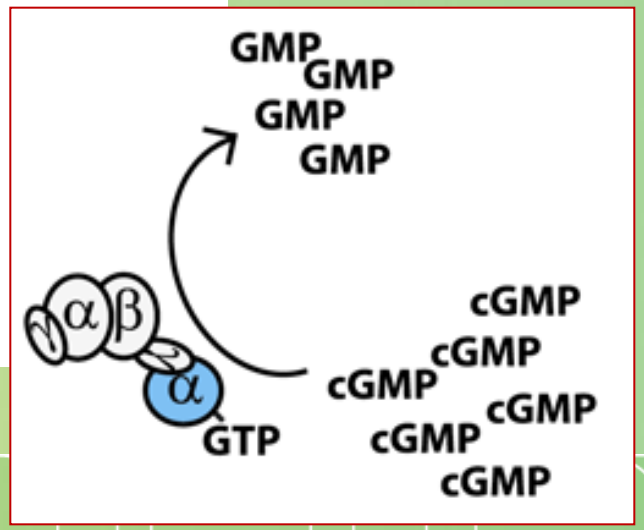
http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/webvision/photomv3-movie1.mov

Signal amplification

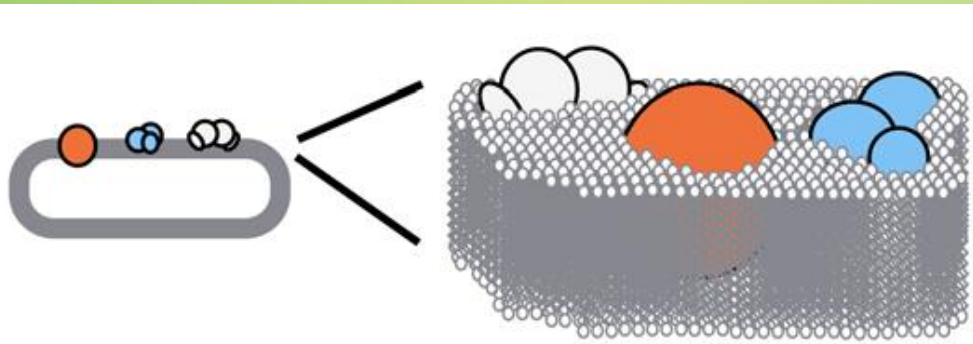
Rhodopsin (1) → Transducin (500)
Transducin (1) → PDE
PDE (1) → cGMP (10^3)

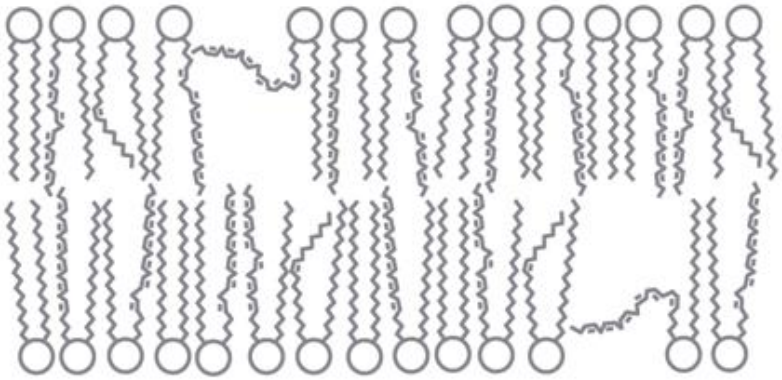






Facilitation of transduction





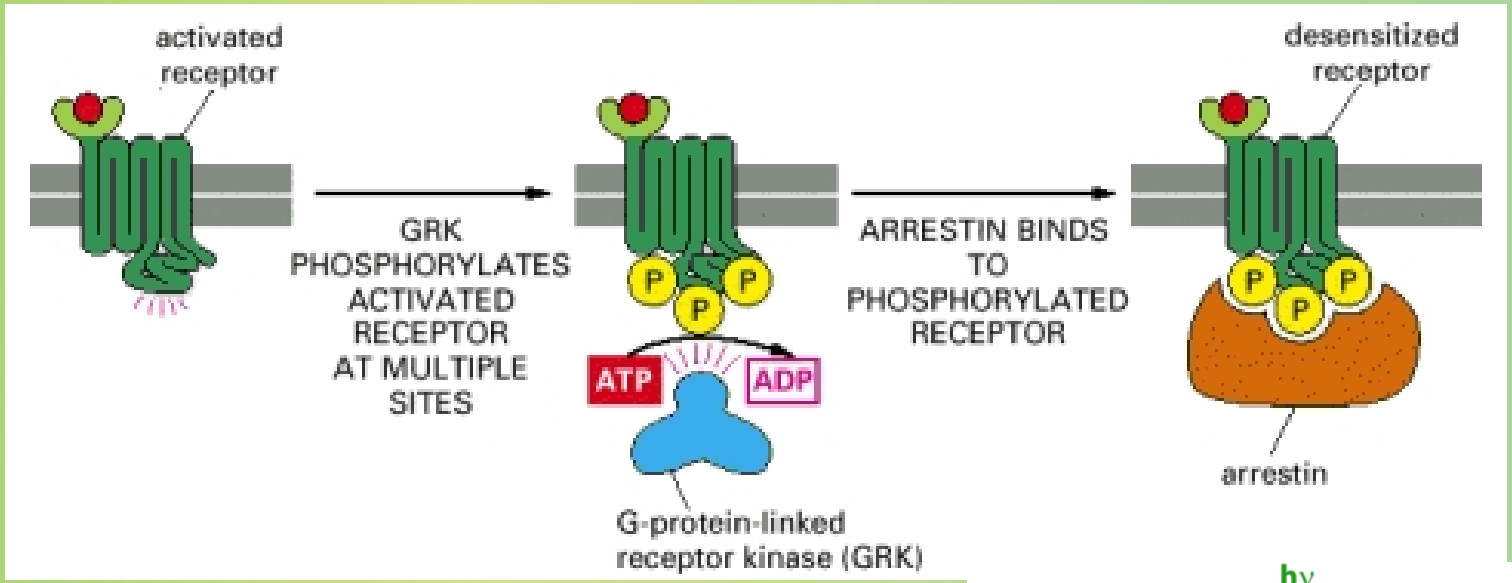
1. 2-dimensional surface
2. low in cholesterol and high content of unsaturated fatty acids
3. Cooperativity of binding: The binding of one cGMP enhances additional binding and channel opening (n = ~3)
4. since multiple cGMP molecules are required to open the channel, it will close when only one or two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

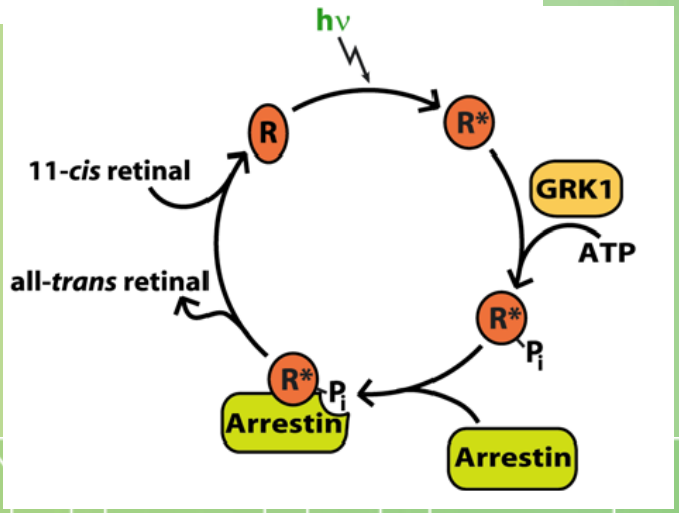
Signal termination

Mechanism I

Arrestin binding

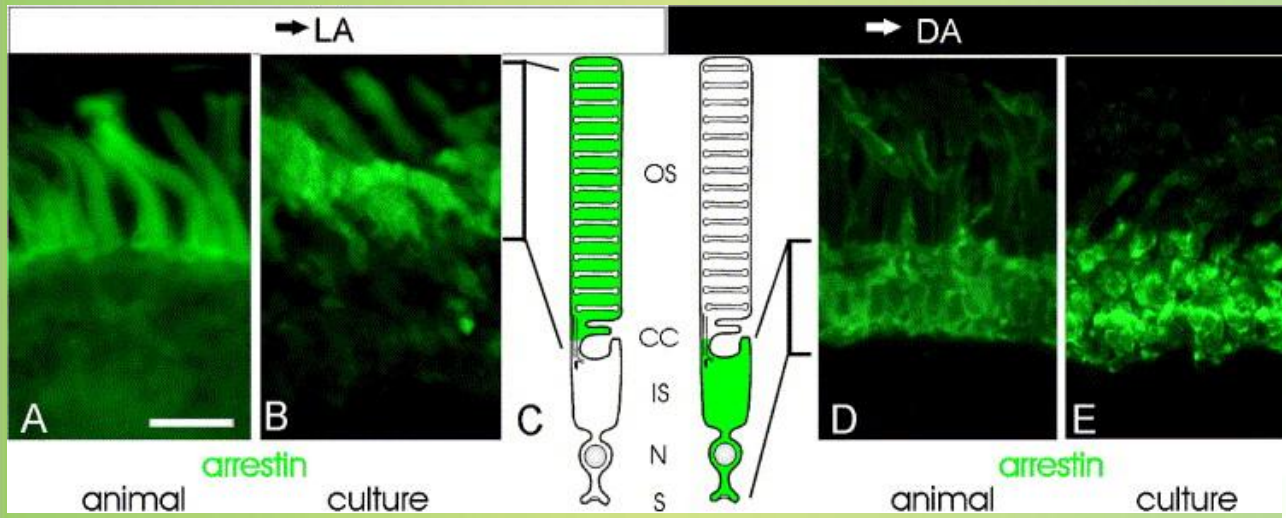


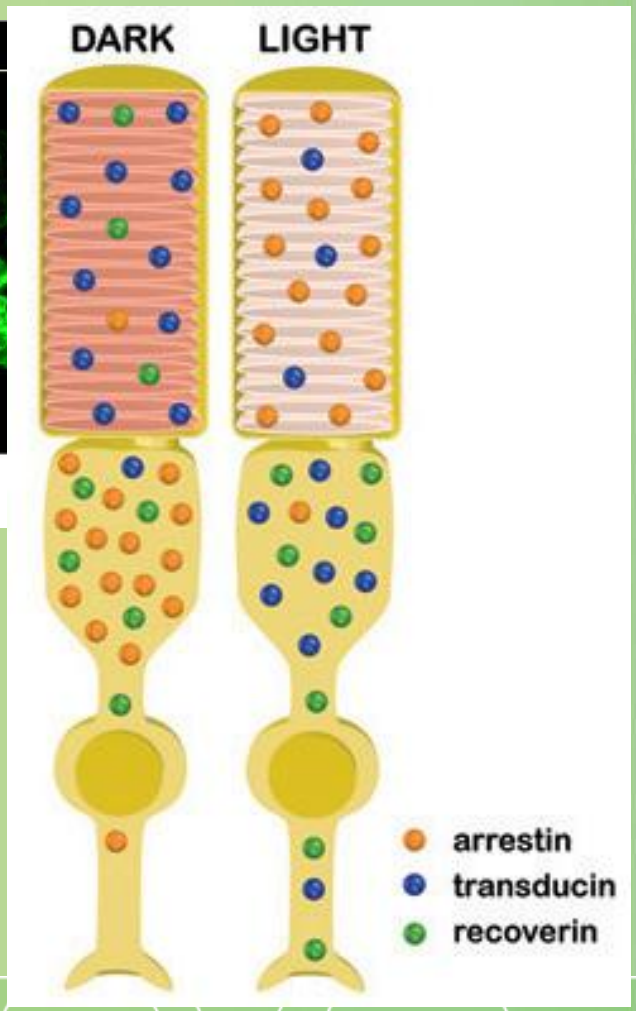
- **Rhodopsin kinase (GRK1) phosphorylates the C-terminus of R*.**
- **Phosphorylation of R* decreases transducin activation and facilitates binding to arrestin, which completely quenches its activity, and releases of the all *trans-retinal* regenerating rhodopsin.**



Mechanism II

Arrestin/transducin distribution

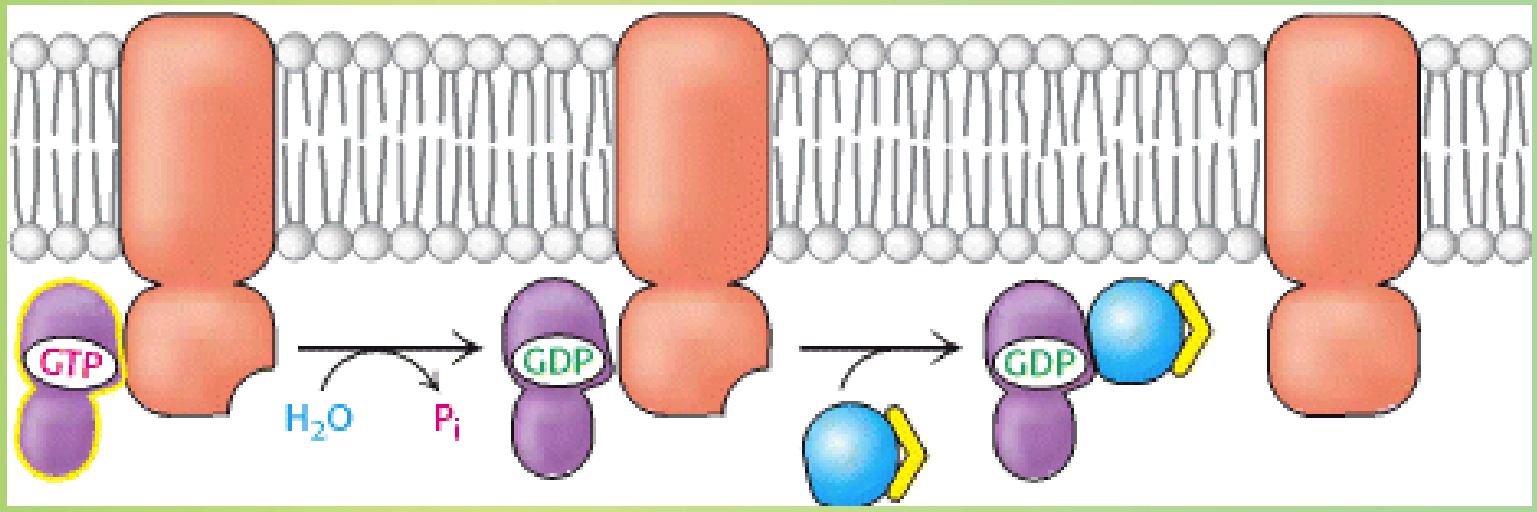




- In dark, the outer segment contains high levels of transducin and low levels of arrestin.
- In light, it is the opposite.

Mechanism III

Intrinsic GTPase activity of G protein

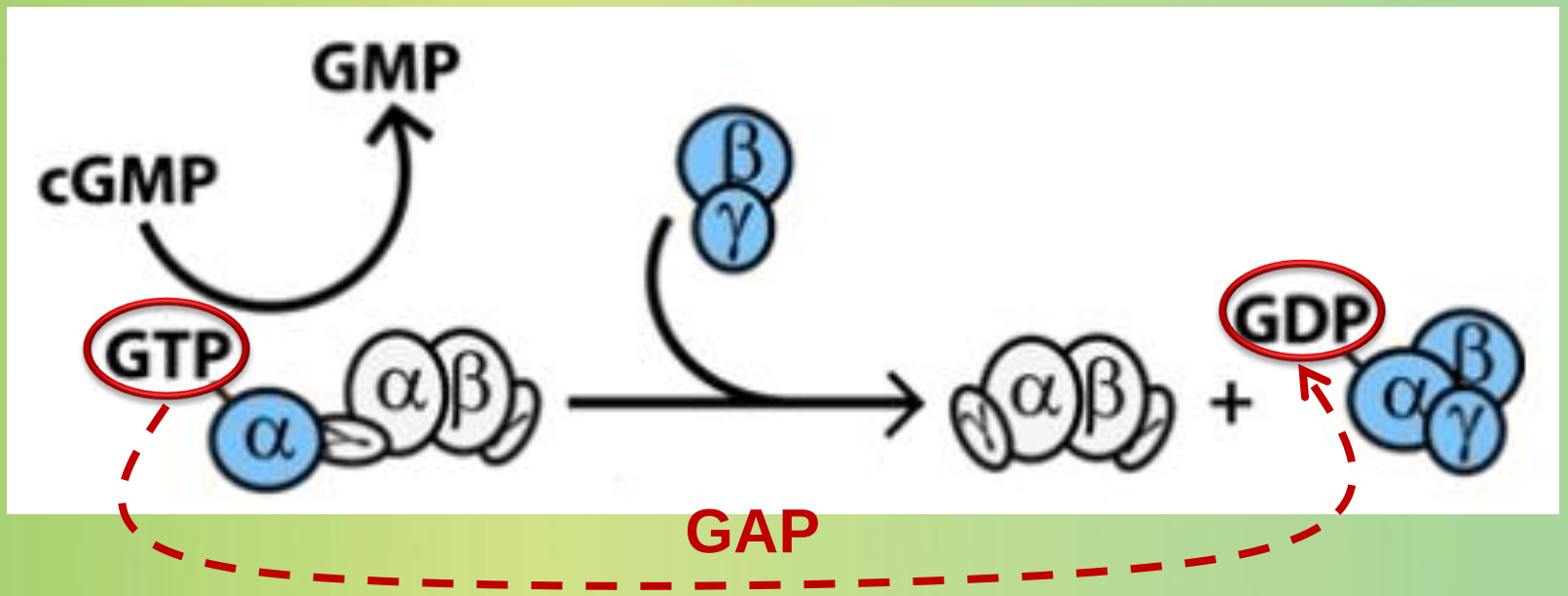


- Transducin has an intrinsic GTPase activity that hydrolyzes GTP to GDP.
- Upon hydrolysis of GTP to GDP, transducin α subunit releases the PDE γ subunit that re-inhibits the catalytic subunit.
- Transducin α-GDP eventually combines with transducin βγ

Mechanism IV

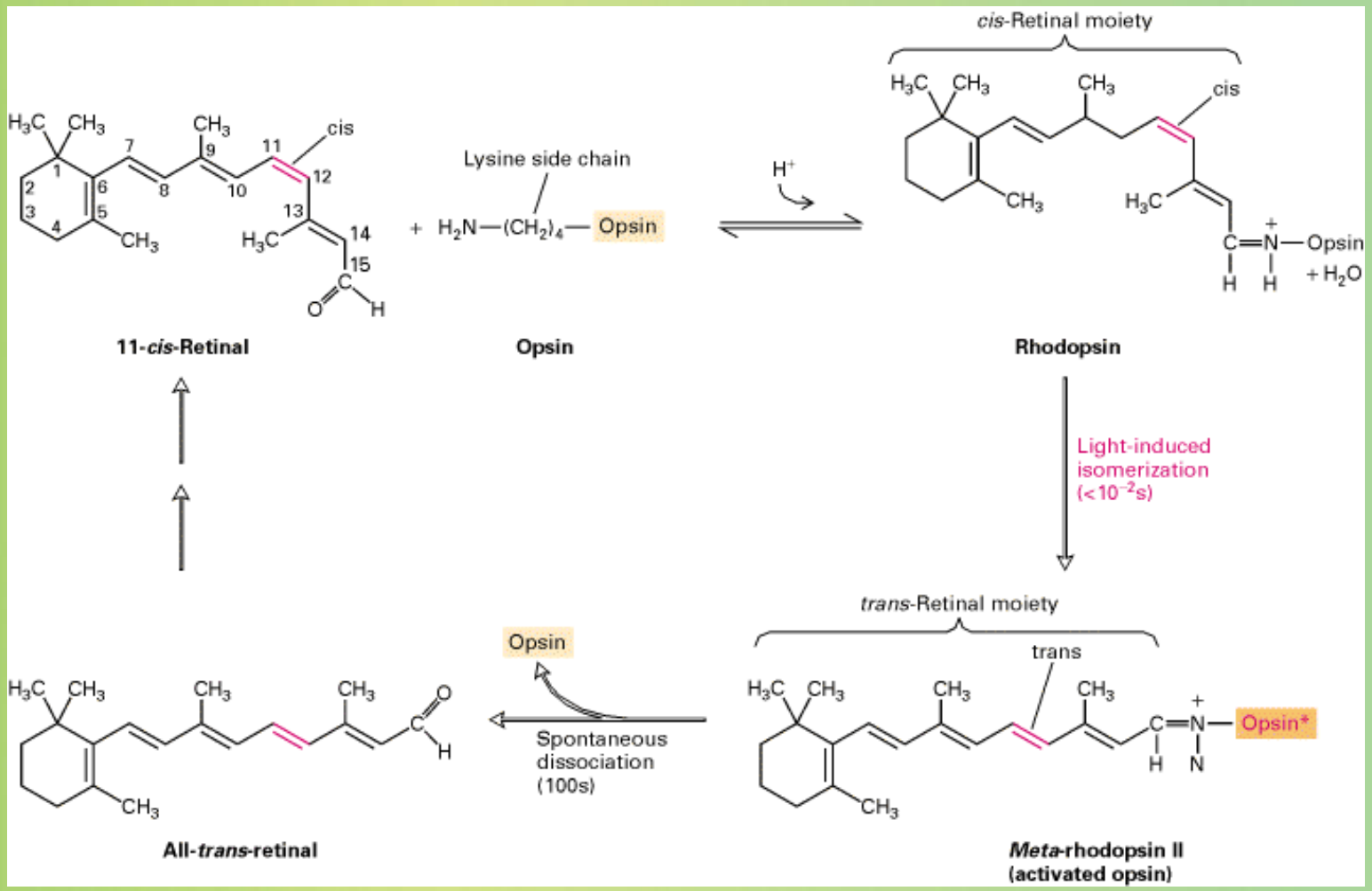
Facilitation of GTPase activity of G protein

- **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.**

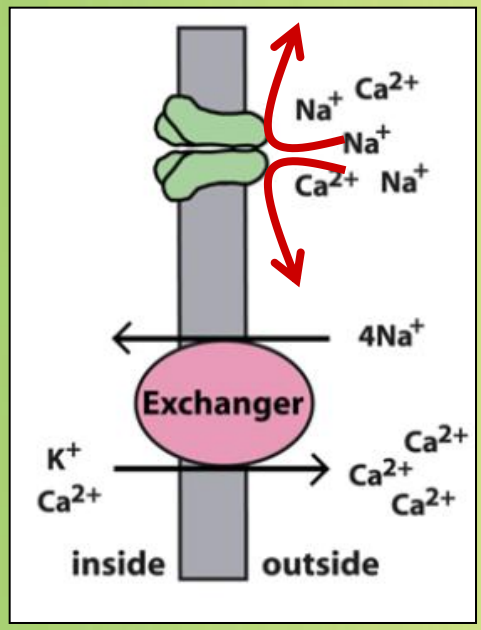


Mechanism V

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

Recovery

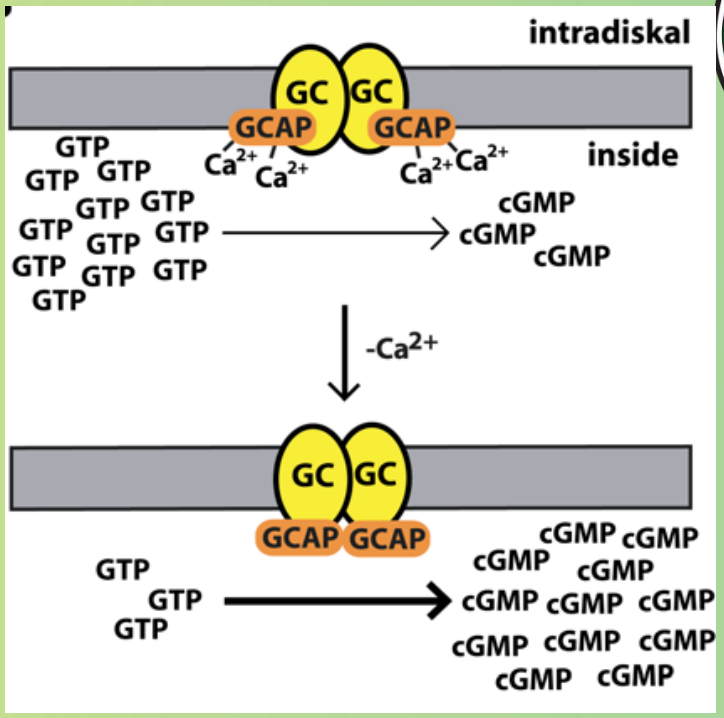
500 nM

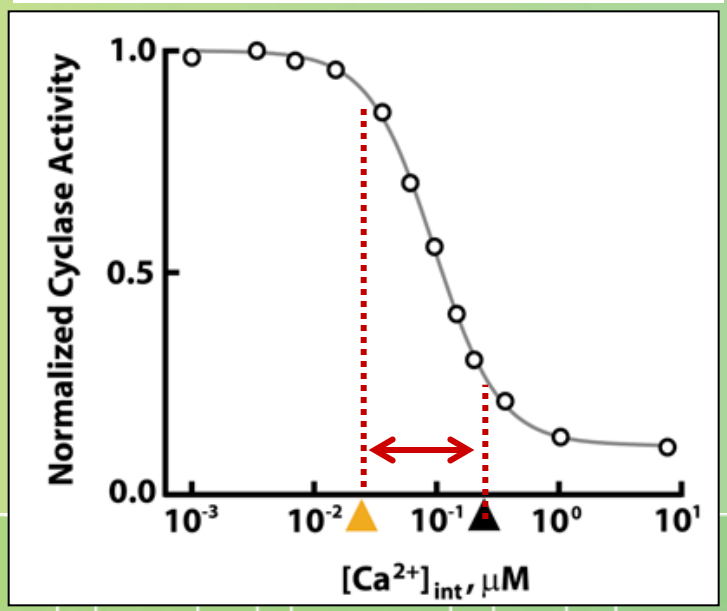
50 nM

Mechanism VI

Guanylate cyclase

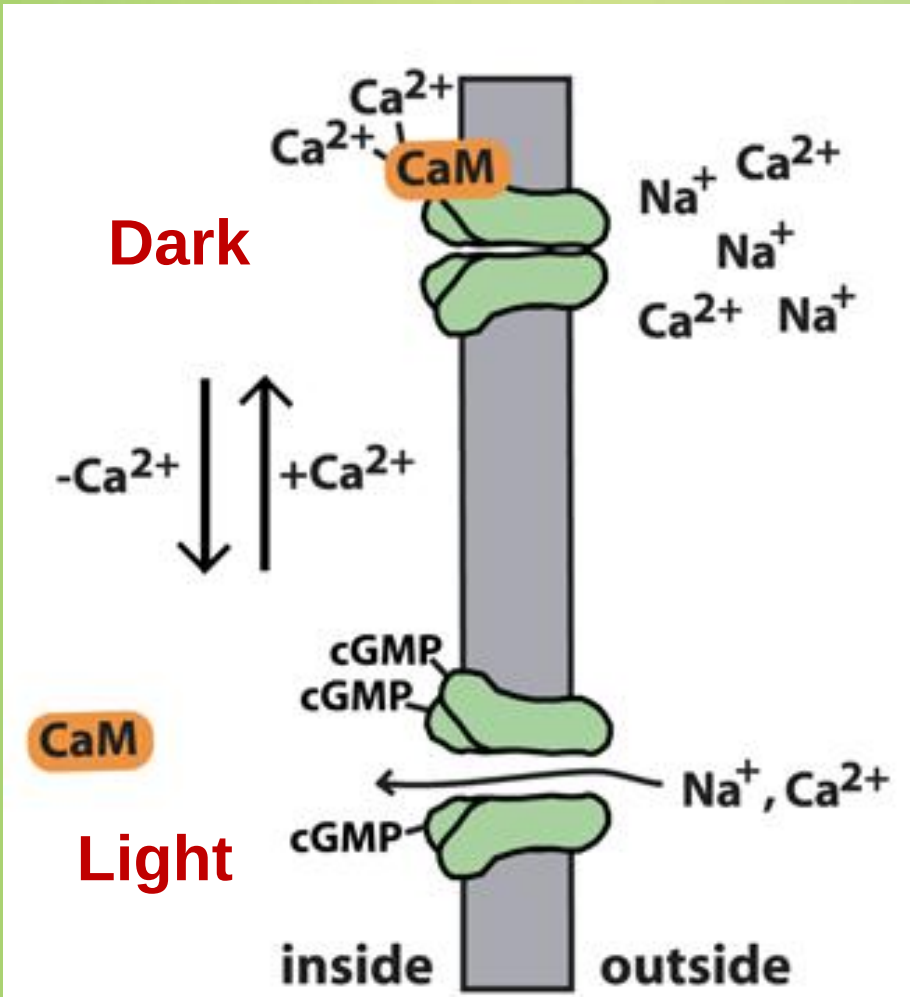
- In the dark, guanylate cyclase-activating proteins (GCAPs) bind Ca^{2+} blocking their activation of guanylate cyclase.
- A decrease in intracellular [Ca^{2+}] causes Ca^{2+} to dissociate from GCAPs leading to full activation of guanylate cyclase subunits, and an increase in the rate of cGMP synthesis.





Mechanism VII

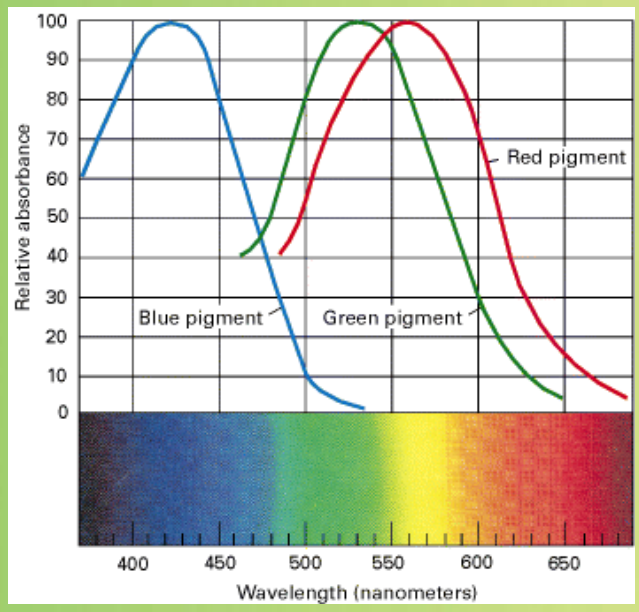
Ca-calmodulin

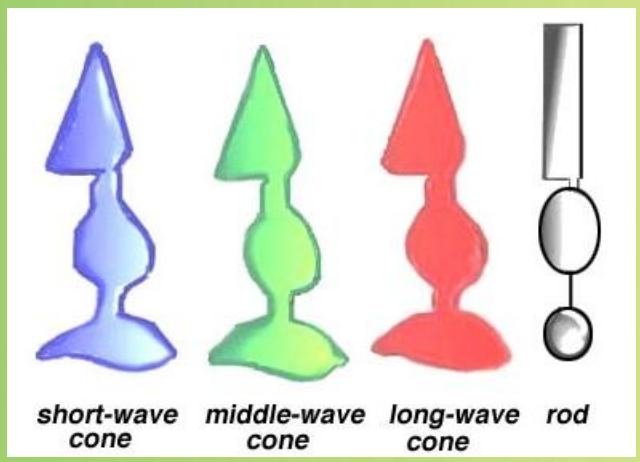


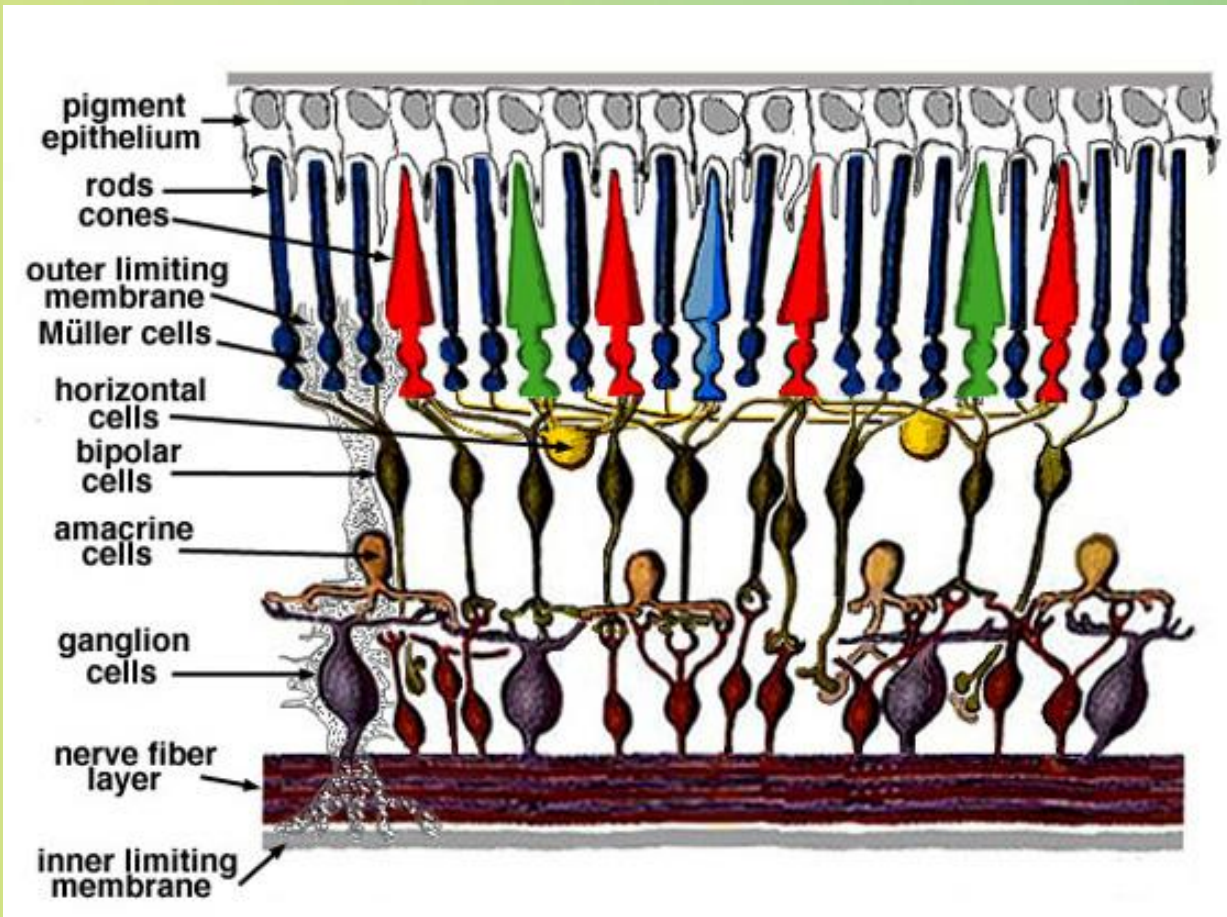
- In the dark, Ca^{2+}-Calmodulin (CaM) binds the channel and shuts it down.
- During visual transduction, the decrease in intracellular [Ca^{2+}] causes CaM to be released, and the channel reopens at lower levels of cGMP.

Color vision

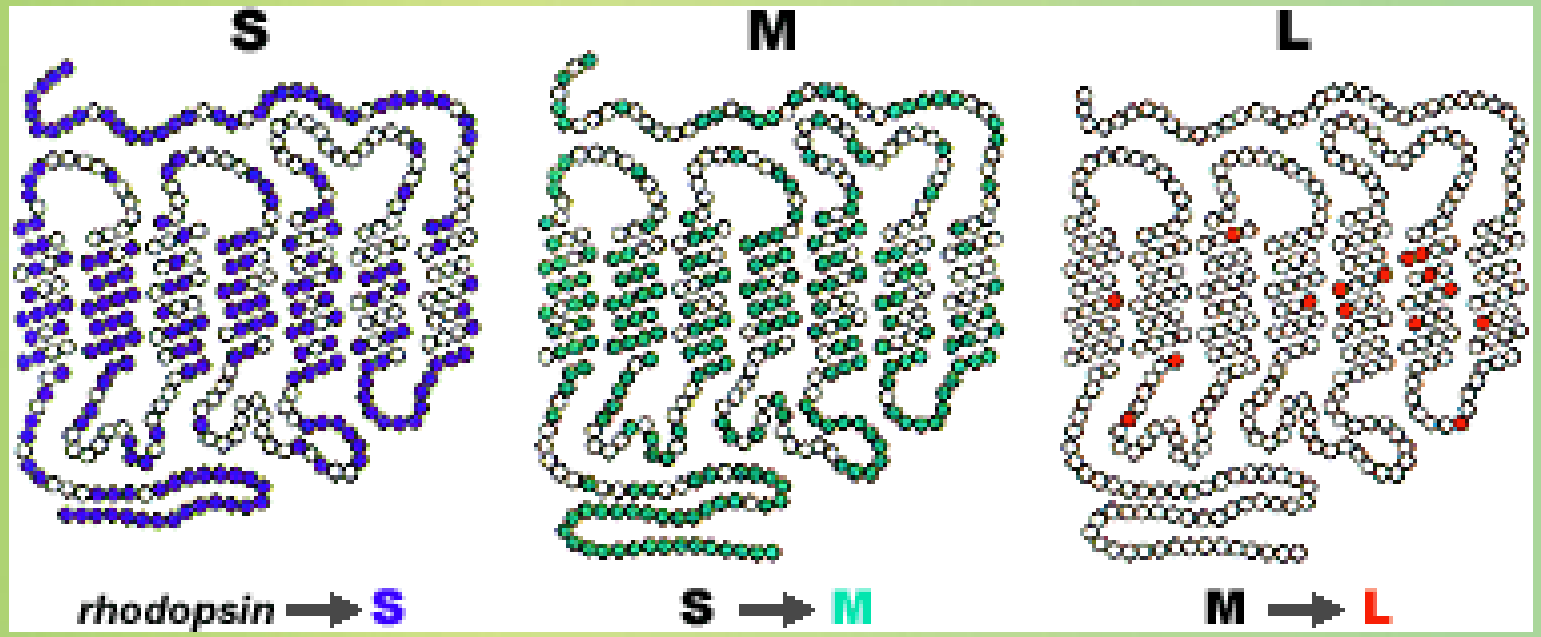
Cone photoreceptor proteins





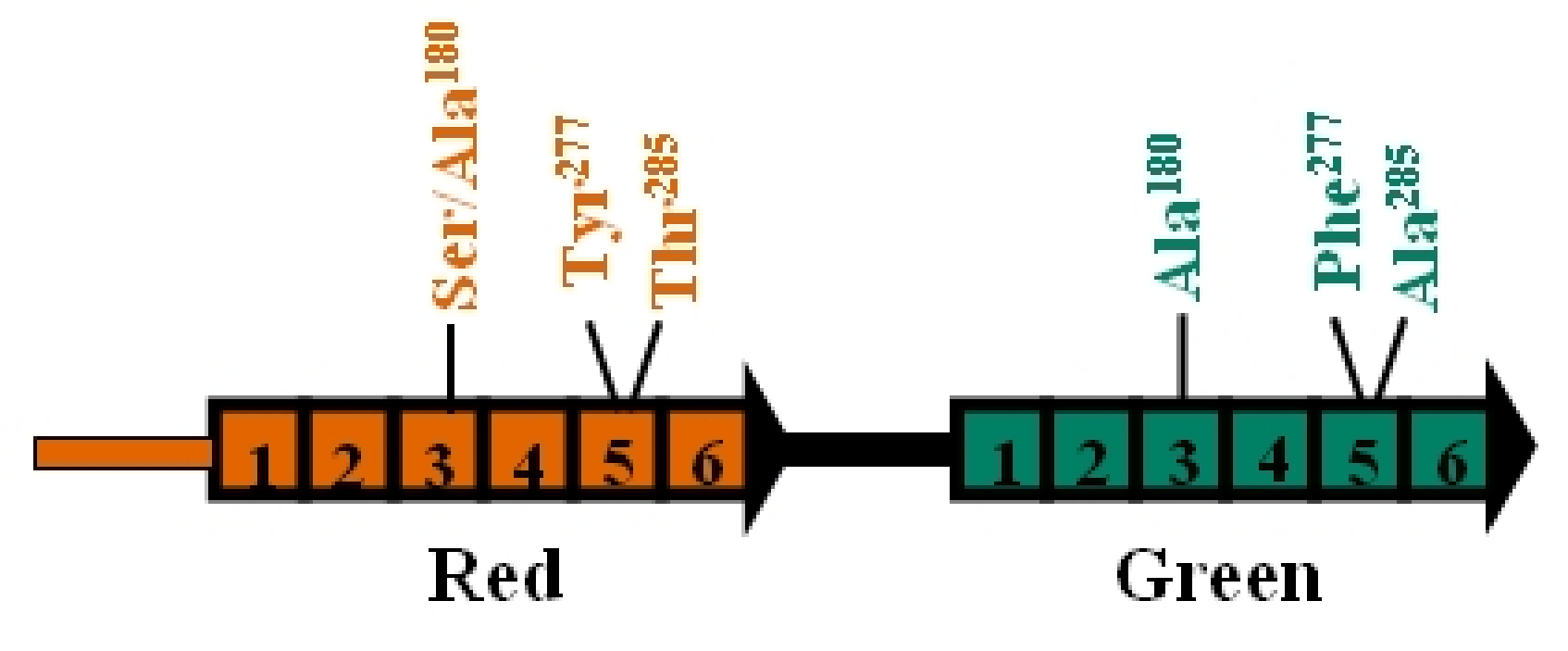


How different are they?



- 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.

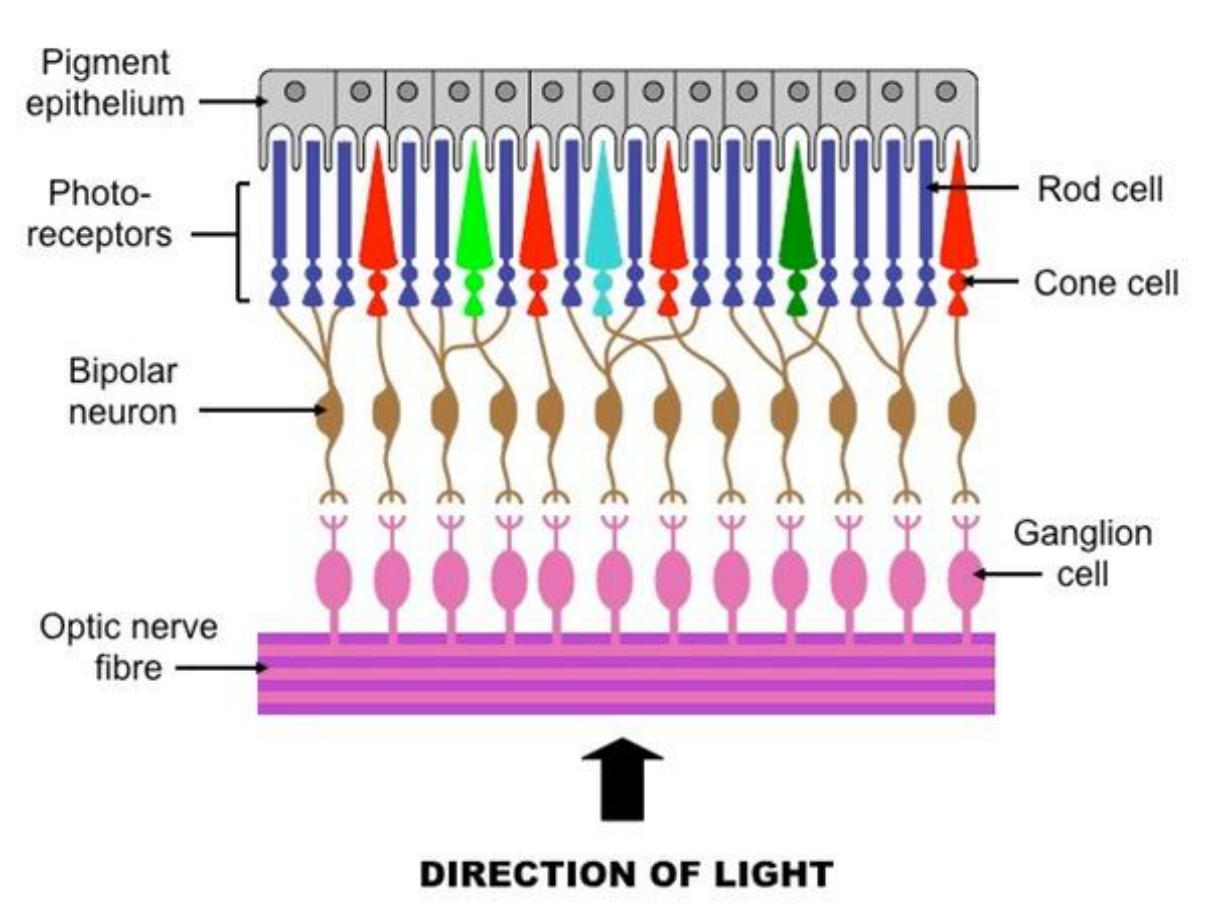
Three important aa residues



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**



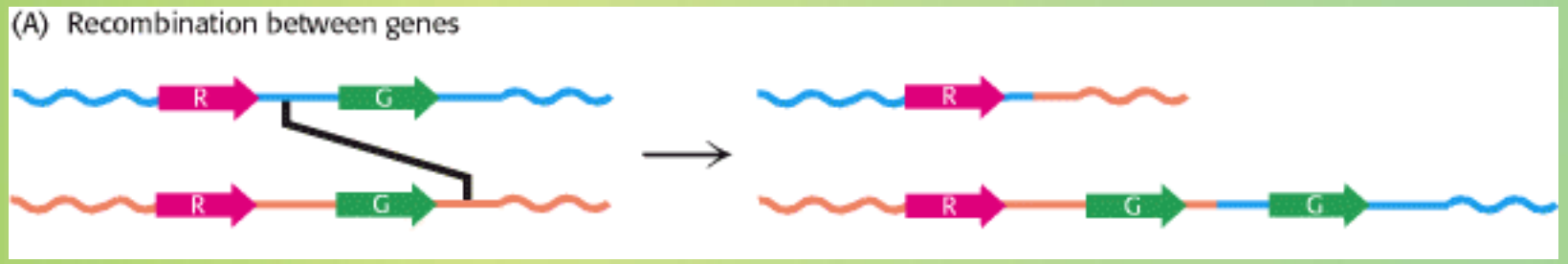
Color blindness

Chromosomal locations

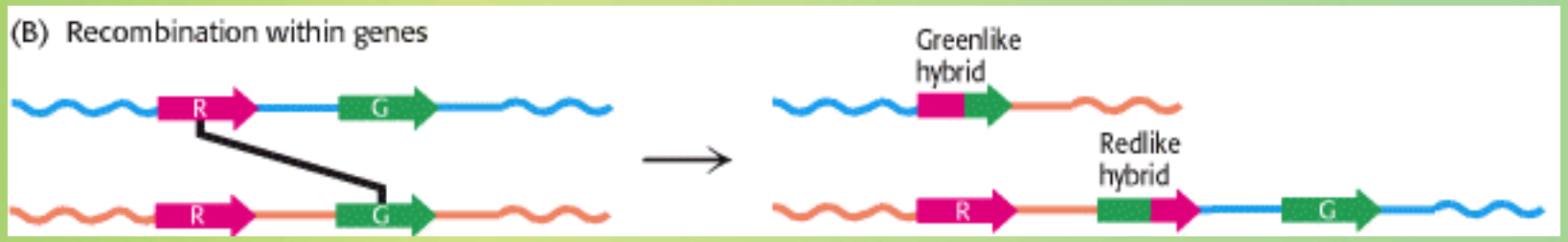
- The "blue" opsin gene: chromosome 7
- The "red" and "green" opsin genes: X chromosome
- The X chromosome normally carries a cluster of from 2 to 9 opsin genes.
- Multiple copies of these genes are fine.

Red-green homologous recombination

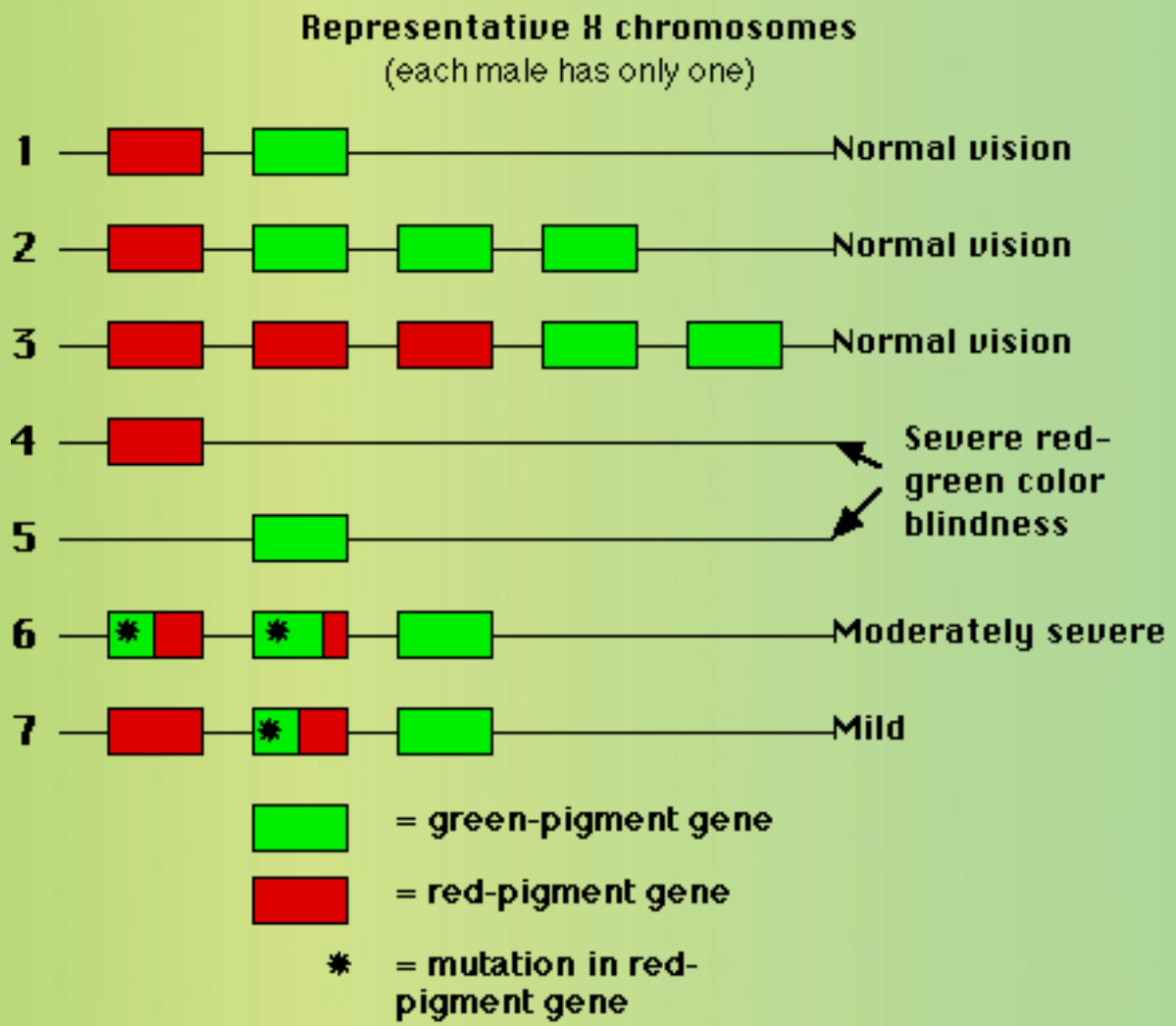
- **Between transcribed regions of the gene (inter-genic)**



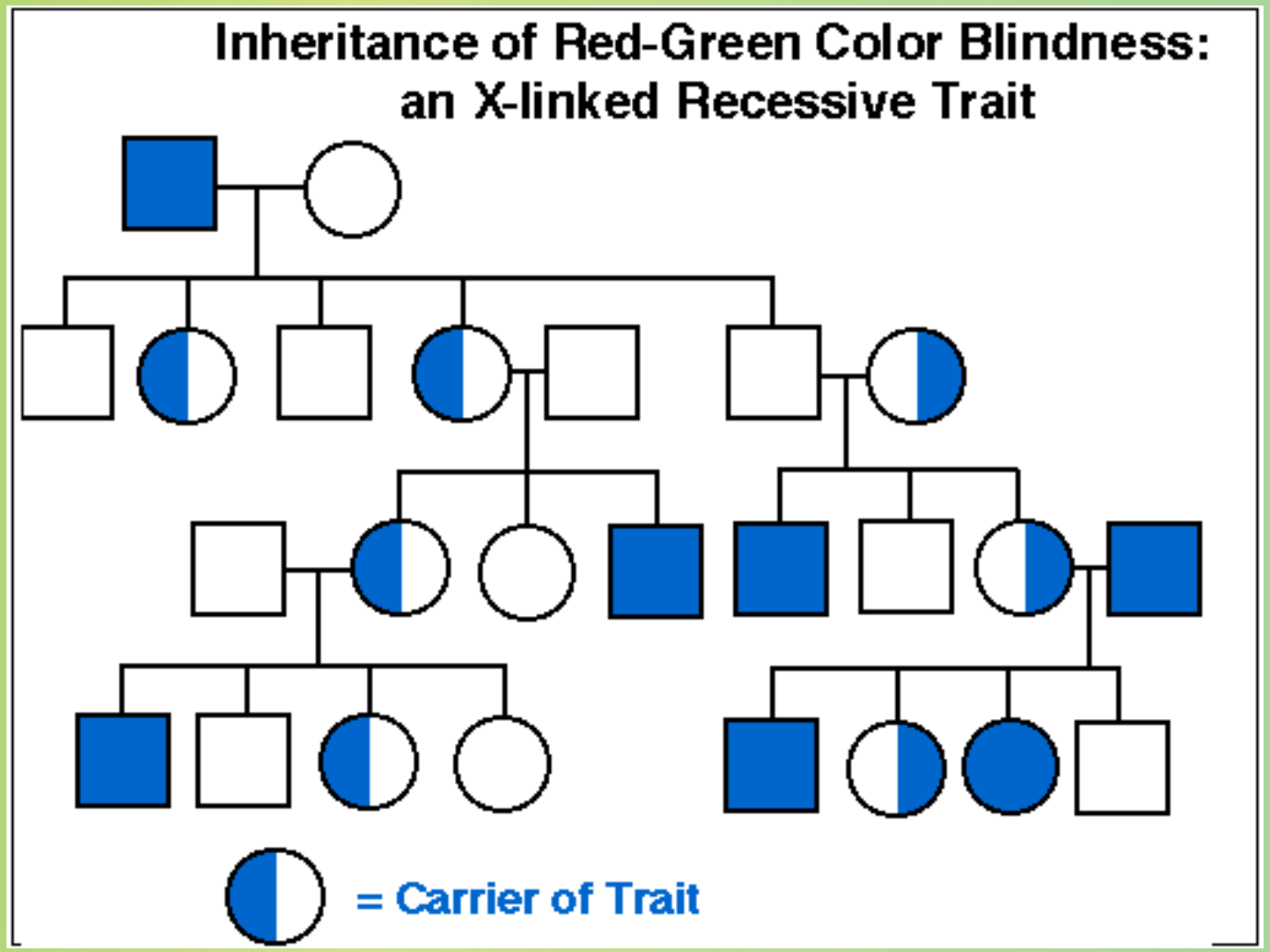
- **Within transcribed regions of the gene (intra-genic)**



Genetic probabilities



Pedigree


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

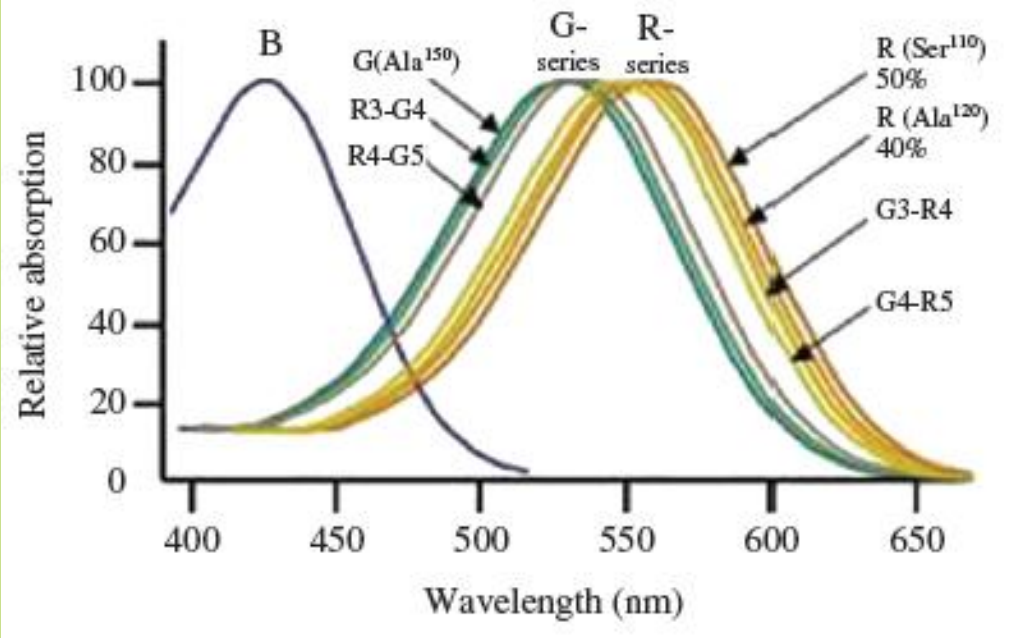
Examples

Red blindness

Green blindness

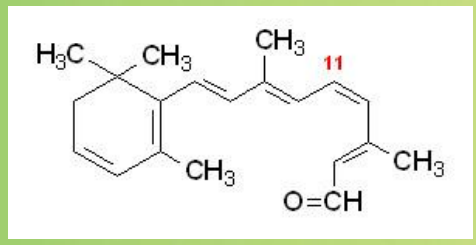
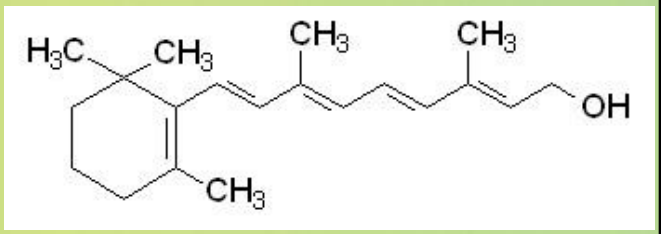
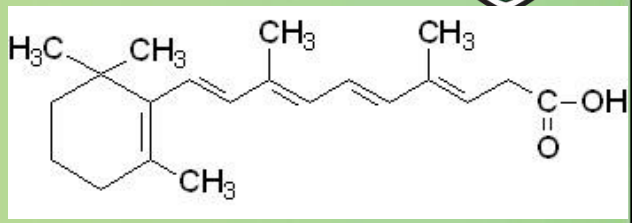
Single nucleotide polymorphism

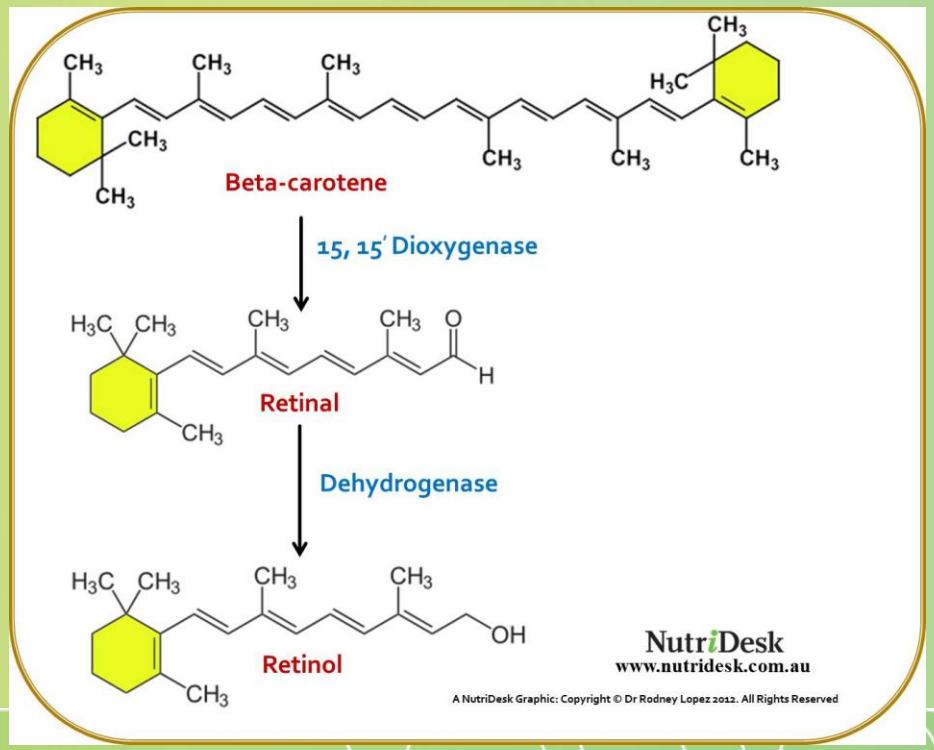
Location	180
AA change	Serine → Alanine
Wavelength	560 nm → 530 nm



Metabolism of vitamin A

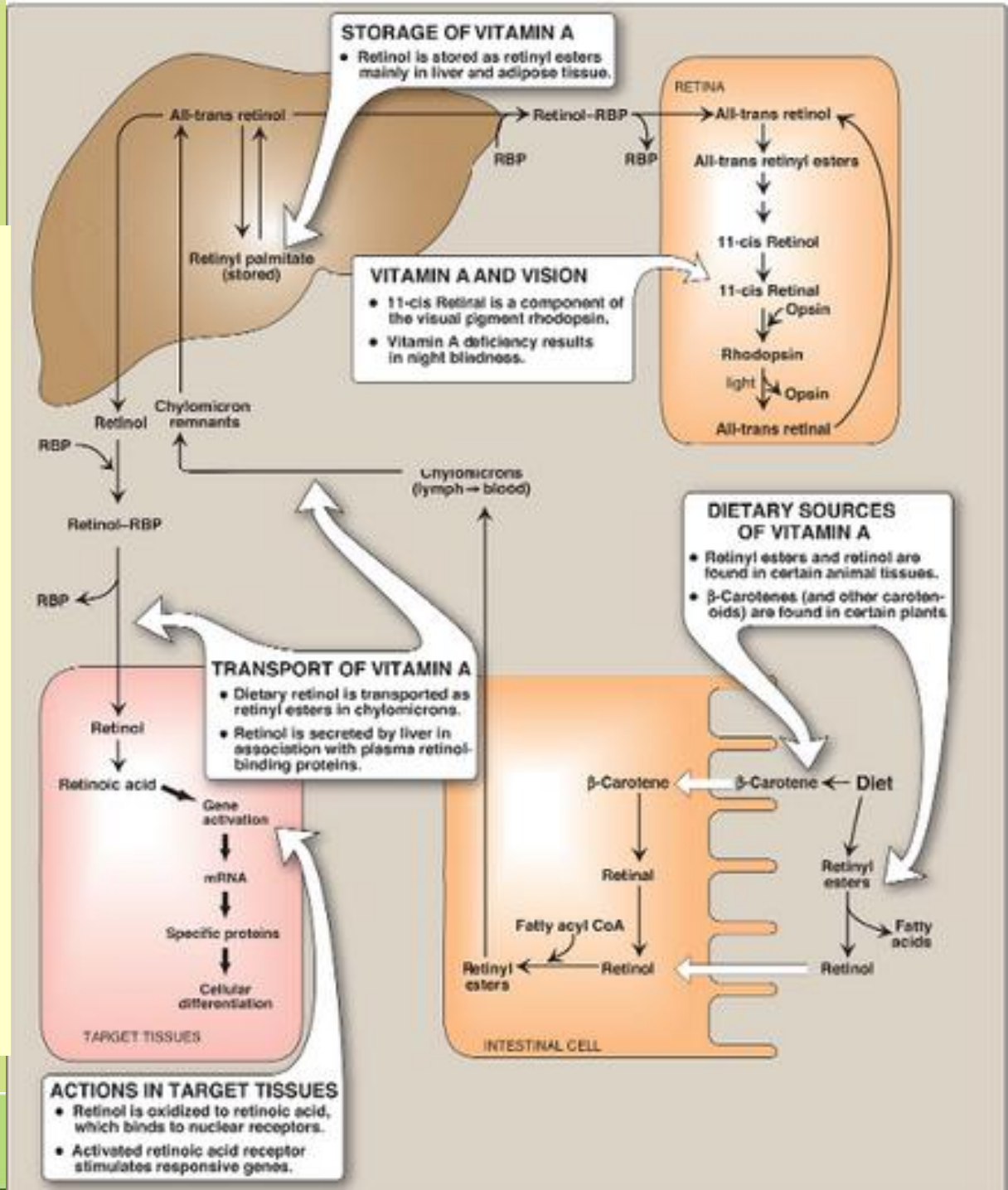
Source of vitamin A

		
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 where it is converted into retinyl esters. REs are packaged into chylomicrons and secreted into the lymphatic circulation. The lipoprotein lipase converts chylomicrons 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 or released and transported by retinol-binding proteins to target cells where it is catabolized into retinal, retinoic acid (RA), or other metabolites.



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)