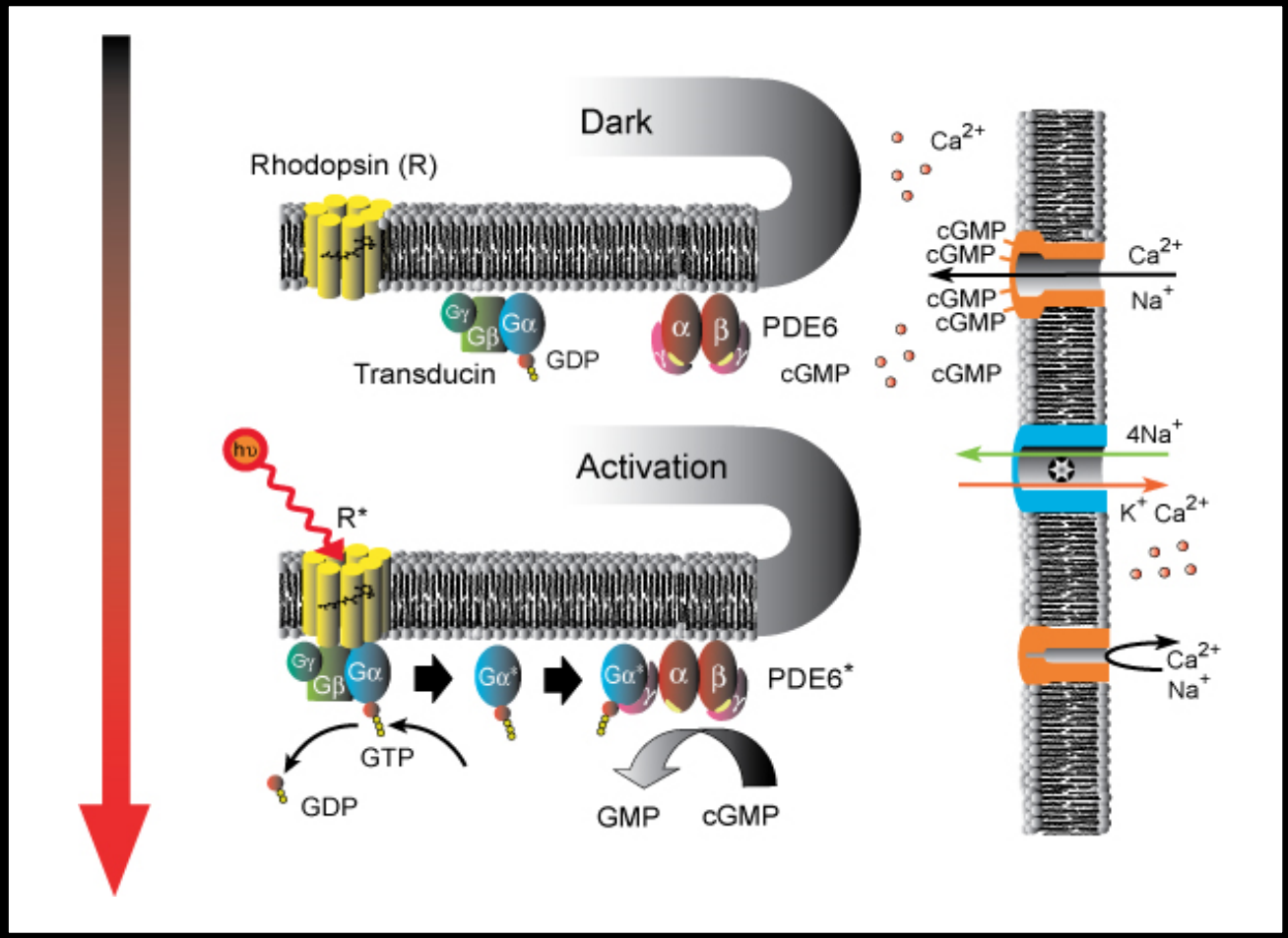
Visual pigments and phototransduction



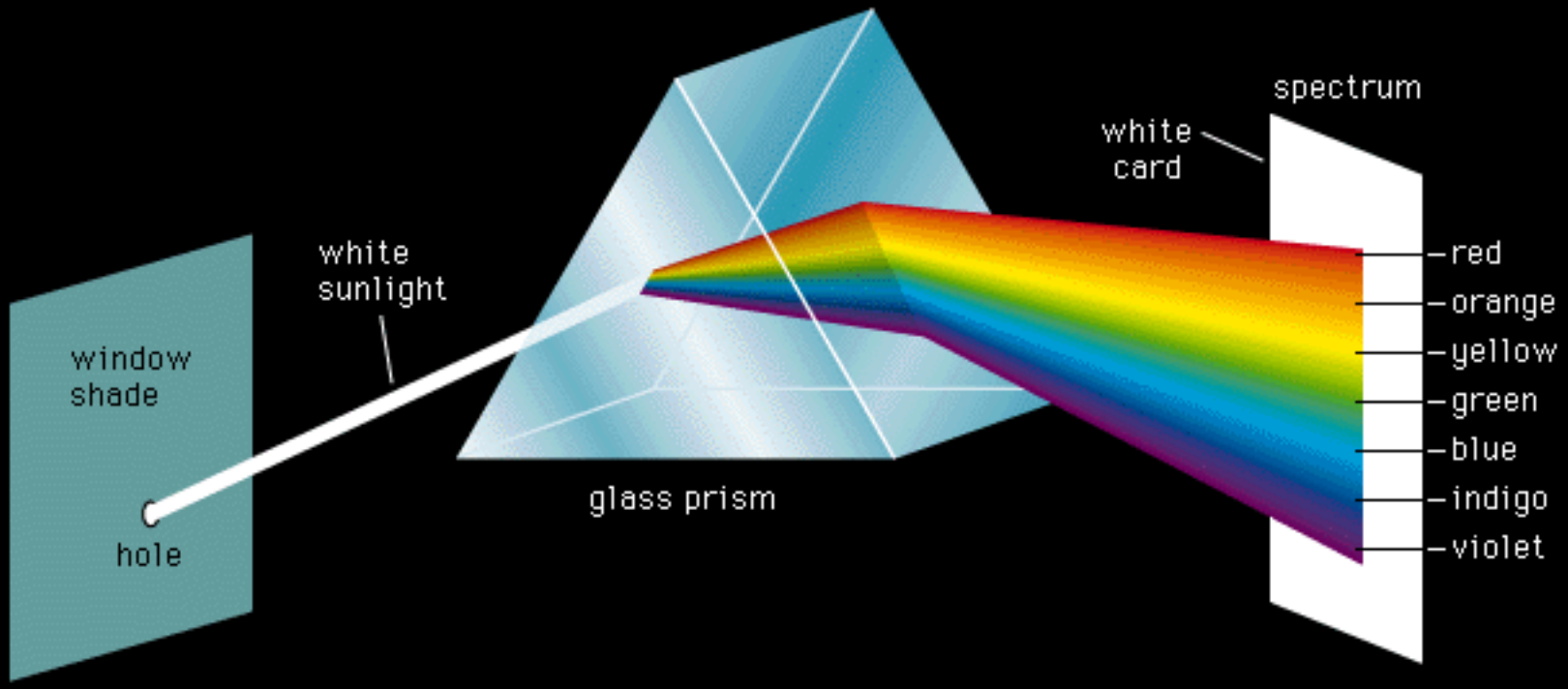
Revision Course in Basic Sciences for FRCOphth. Part 1

Andrew Stockman

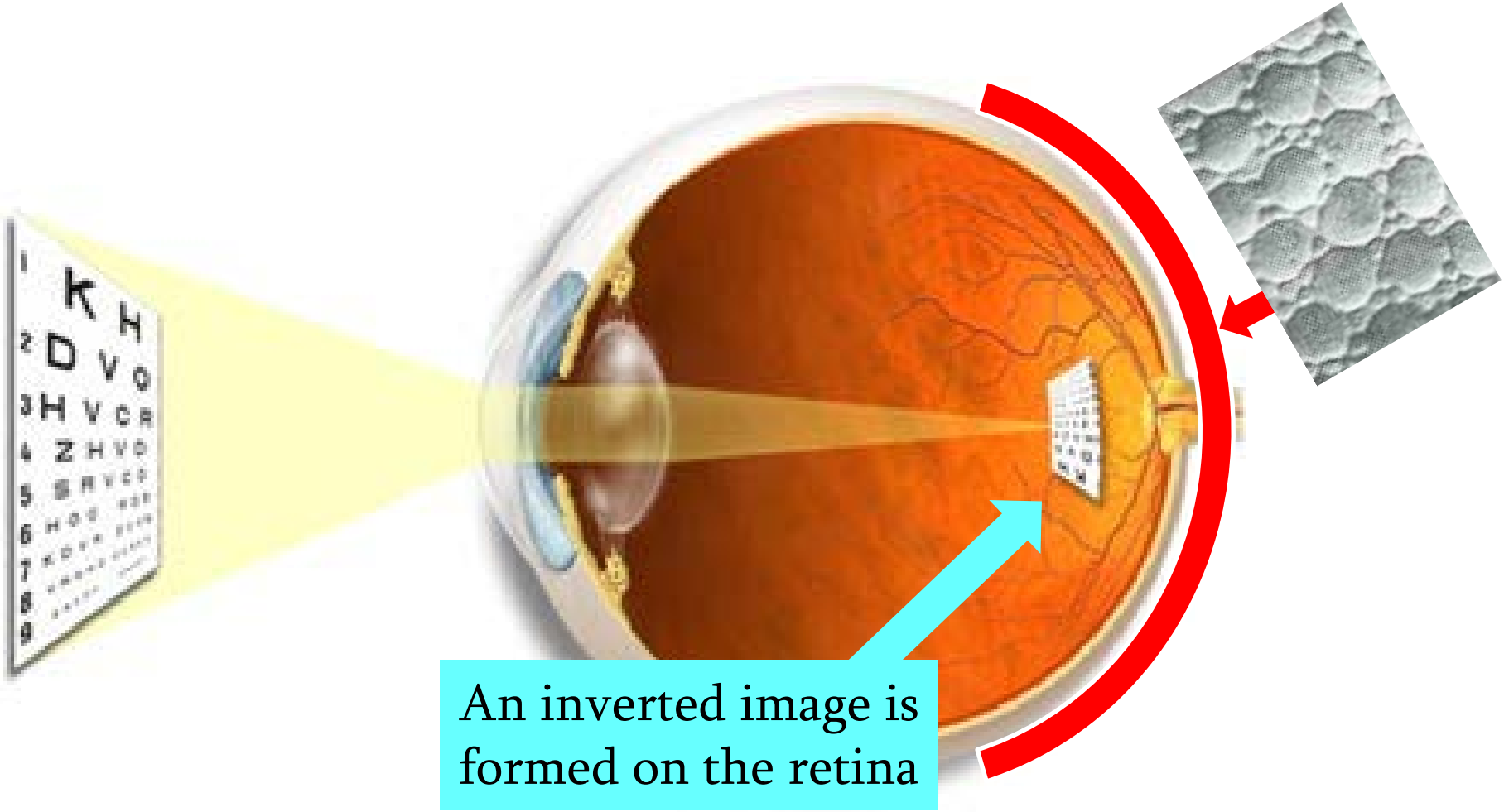
Background

Light

400 - 700 nm is important for vision



The retina is carpeted with light-sensitive rods and cones



An inverted image is formed on the retina

Rods and cones

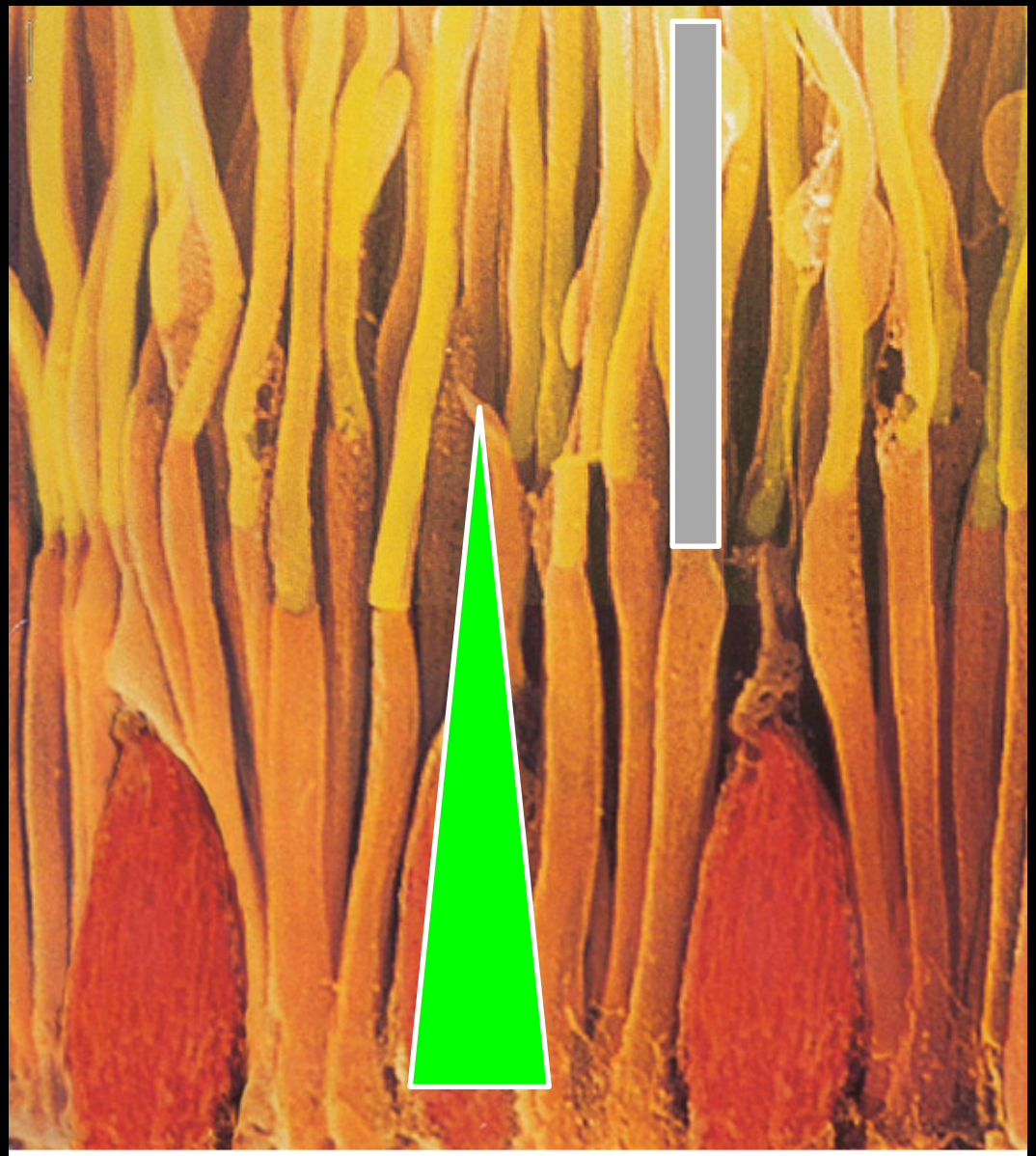
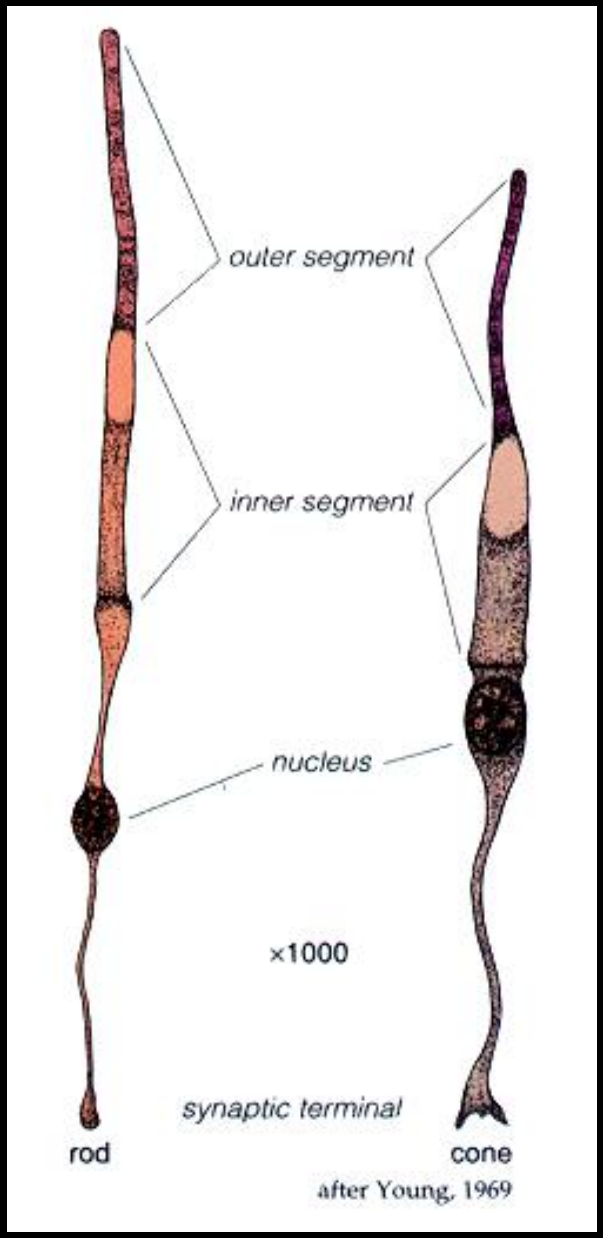


Fig1b. Scanning electron micrograph of the rods and cones of the primate retina. Image adapted from one by Ralph C. Eagle/Photo Researchers, Inc.

Webvision



Human photoreceptors

- Rods
  - Achromatic night vision
  - 1 type
- Cones
  - Daytime, achromatic *and* chromatic vision
  - 3 types

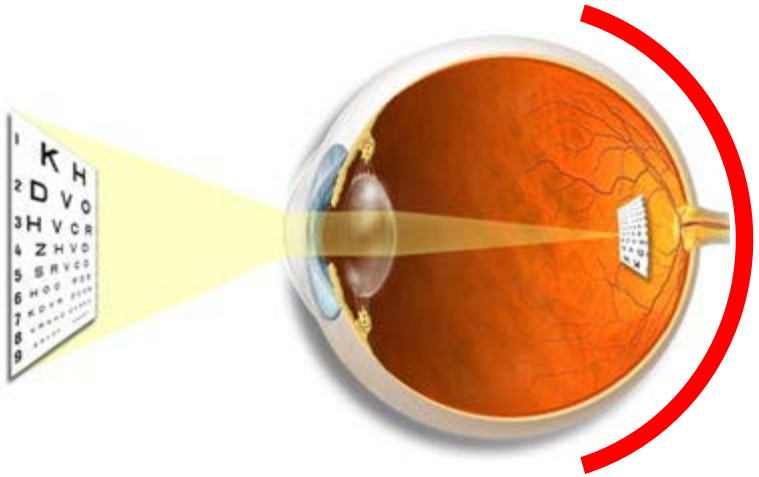
Rod

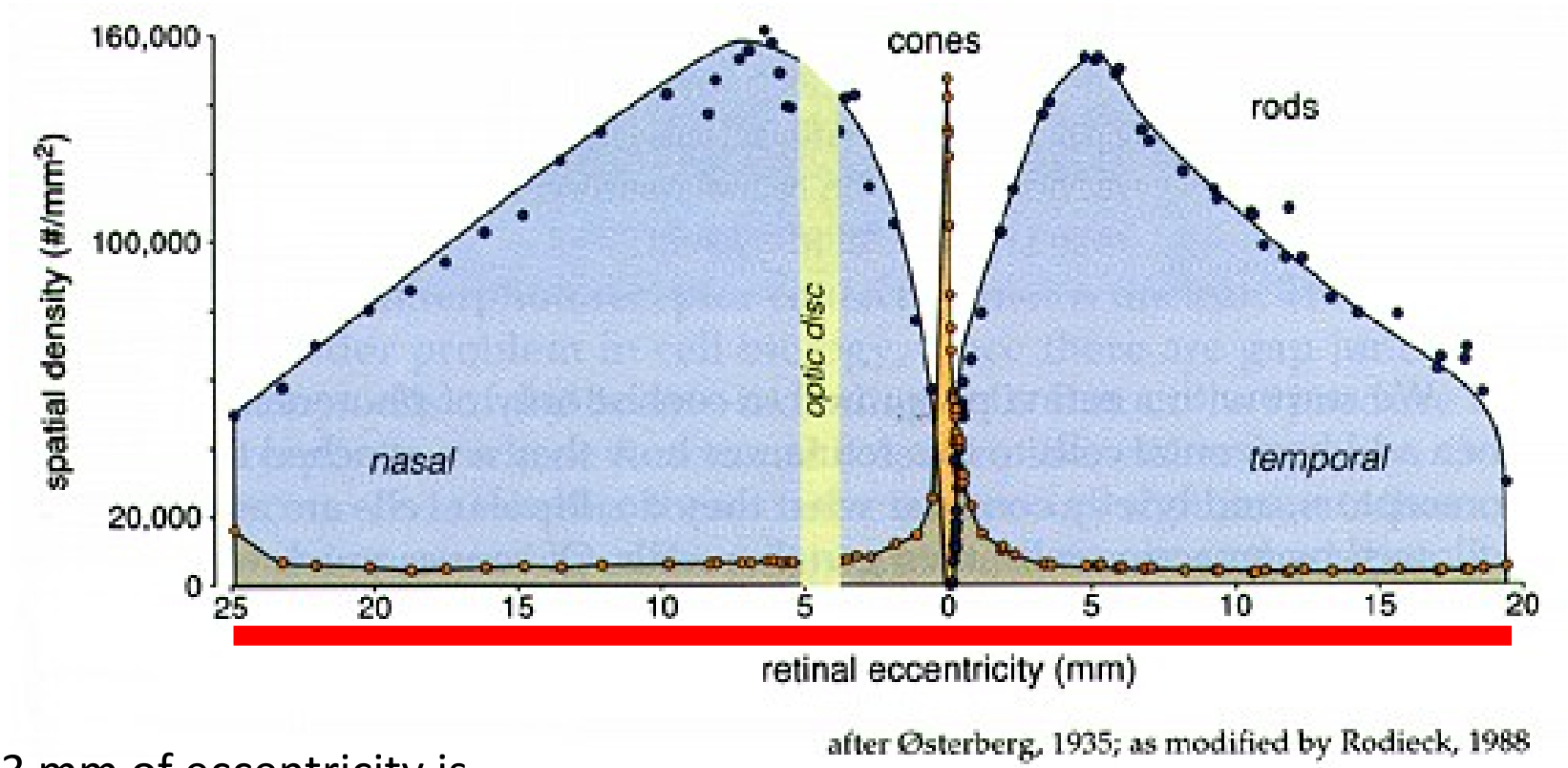
Long-wavelength-sensitive (L) or "red" cone

Middle-wavelength-sensitive (M) or "green" cone

Short-wavelength-sensitive (S) or "blue" cone

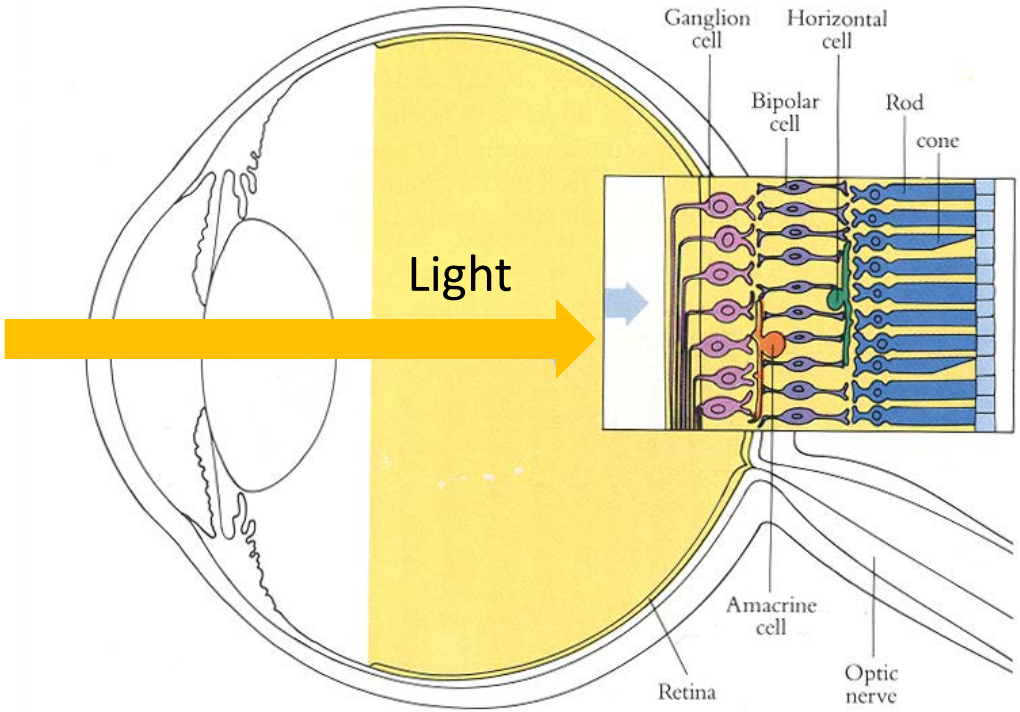
Rod and cone distribution

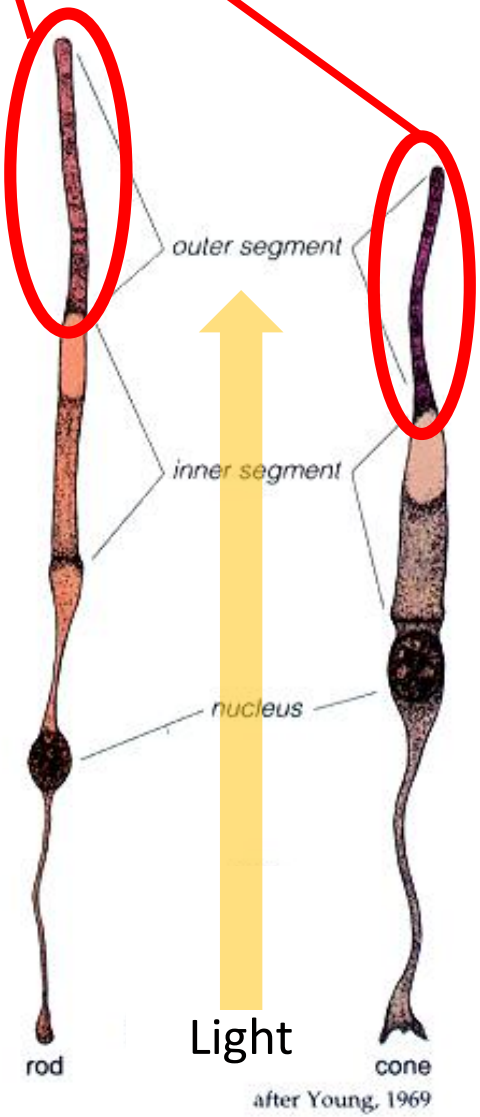




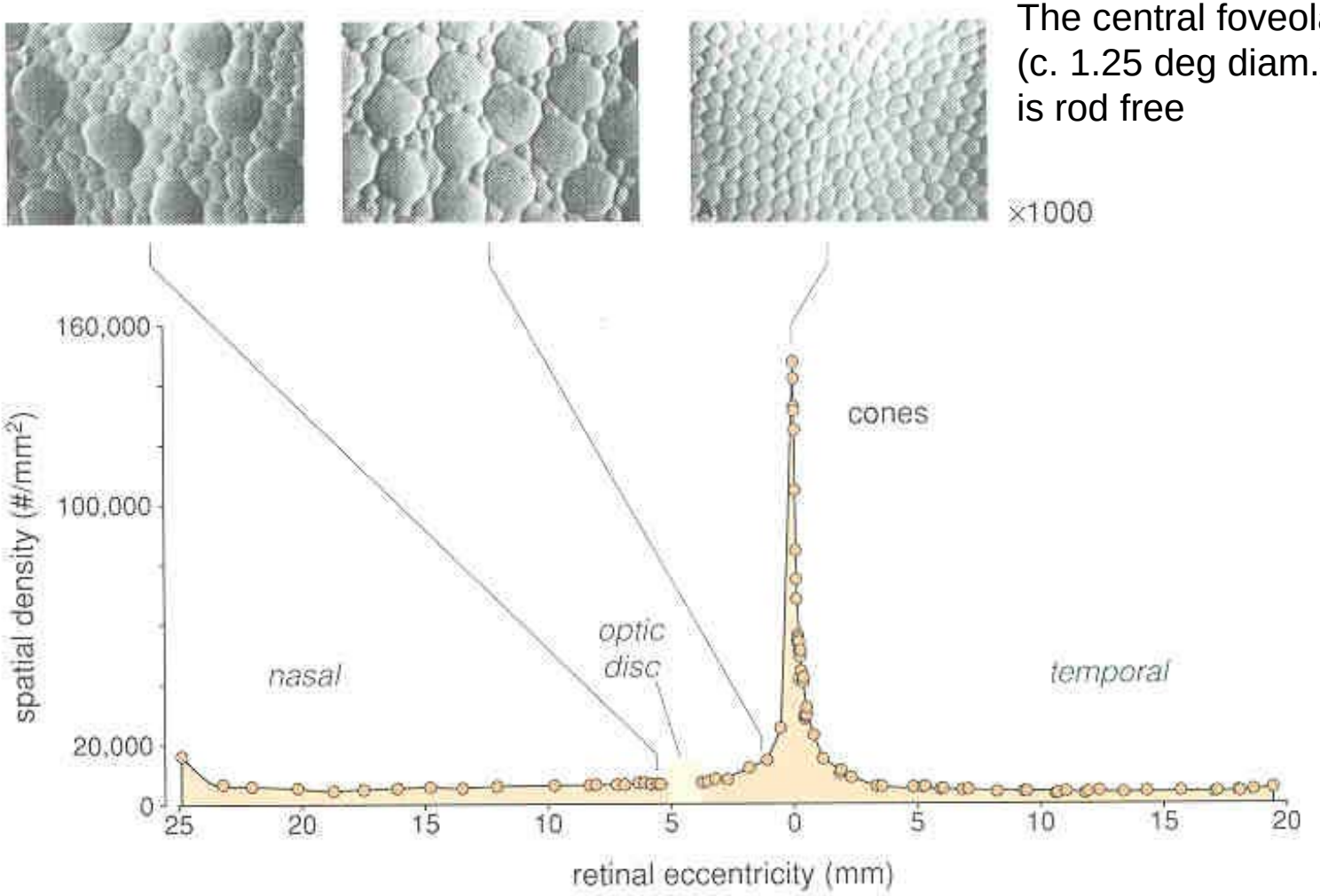
0.3 mm of eccentricity is about 1 deg of visual angle

The light-sensitive photopigment lies inside the rod and cone outer segments.





Human photoreceptor mosaics



The central foveola (c. 1.25 deg diam.) is rod free

0.3 mm of eccentricity is about 1 deg of visual angle

after Østerberg, 1935; as modified by Rodieck 1988; micrographs from Curcio et al., 1990

Rods and cones

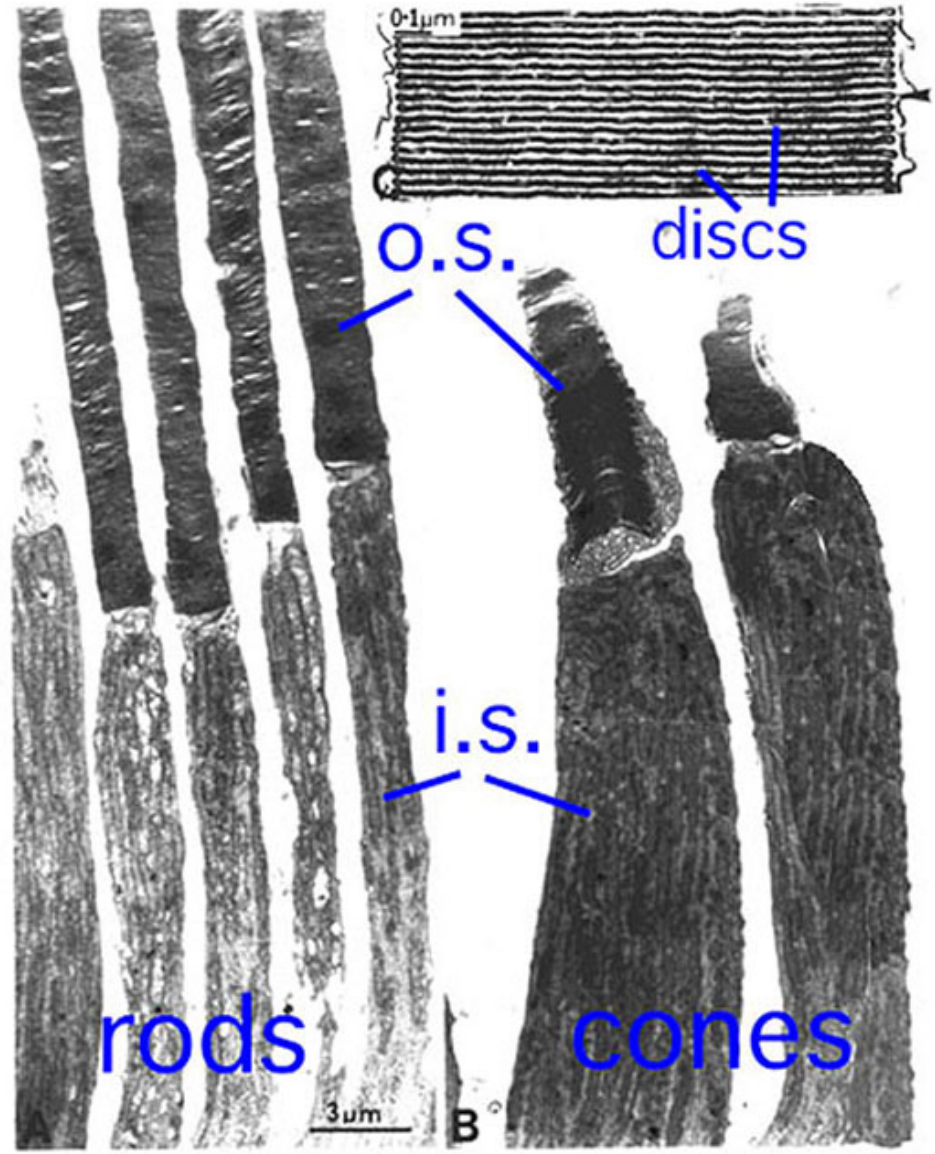
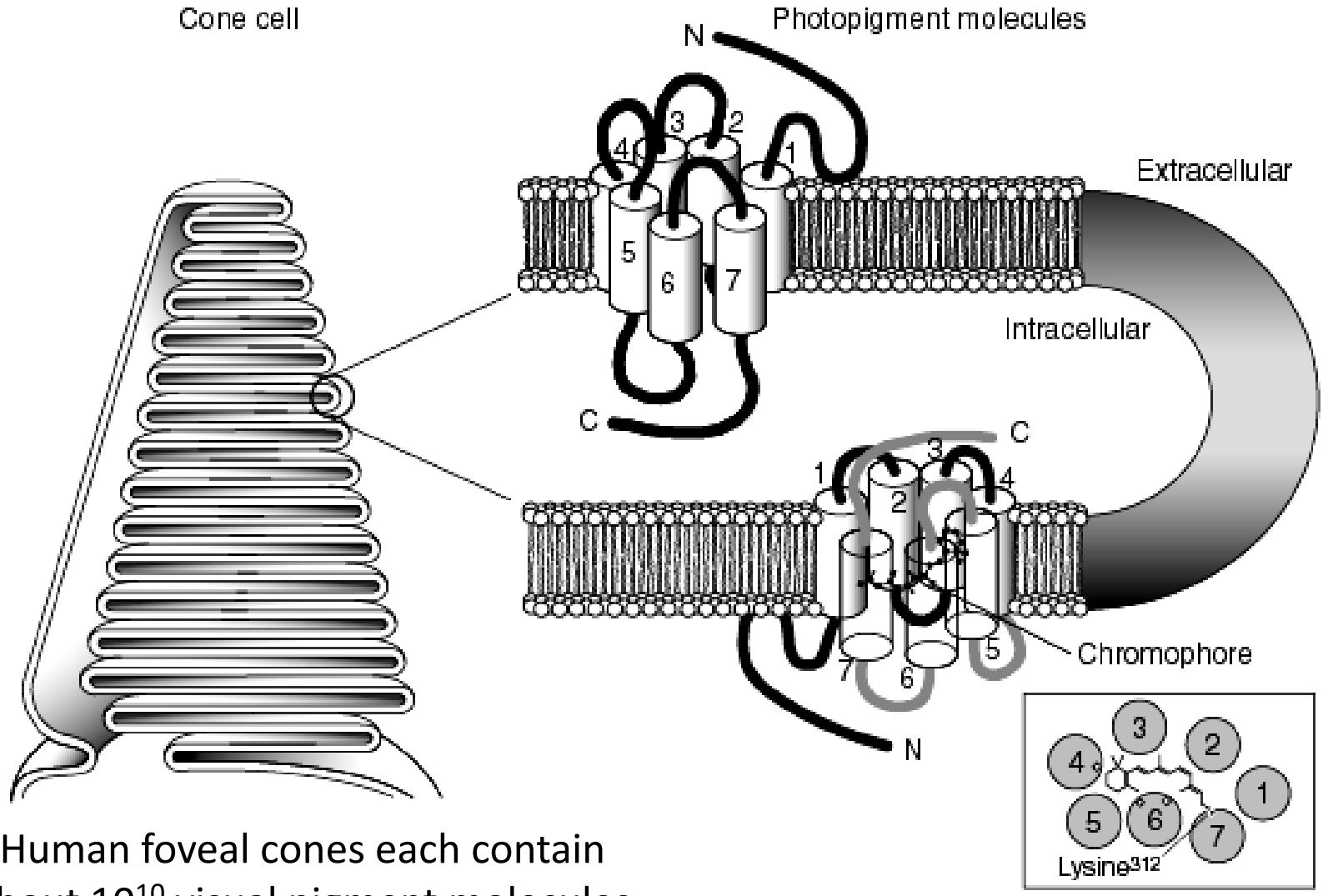


Fig 2. Low magnification EM image of monkey rods and cones with an enlargement of the outer segment discs.

Webvision

Arrangement of visual pigment molecules in a cone

The molecule consists of protein, opsin, forming 7 transmembrane α-helices, surrounding the chromophore, retinal, the aldehyde of Vitamin A



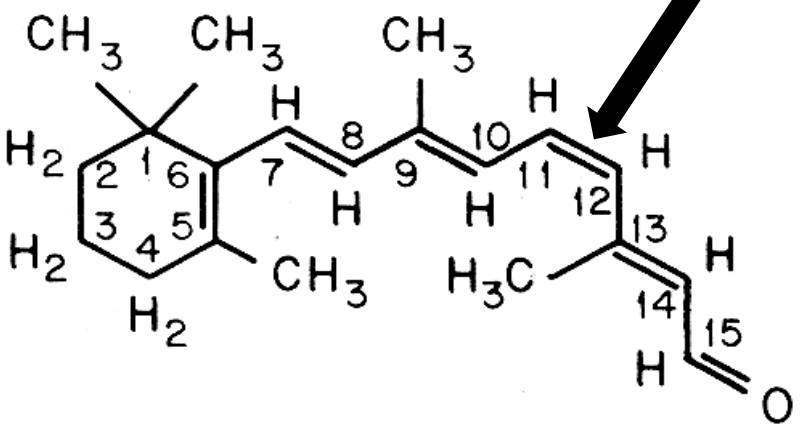
Human foveal cones each contain about 10^{10} visual pigment molecules

Chromophore

(*chromo-* colour, + *-phore,* producer)
Light-catching portion of any molecule

11-*cis* retinal

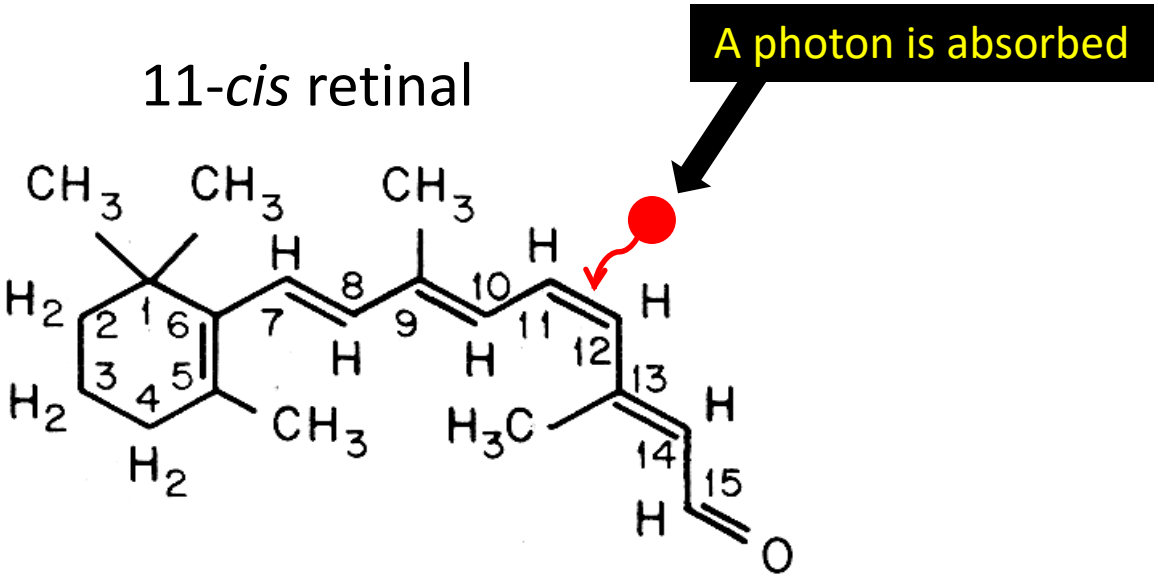
The molecule is twisted at the 11th carbon.



Chromophore

(*chromo-* colour, + *-phore,* producer)
Light-catching portion of any molecule

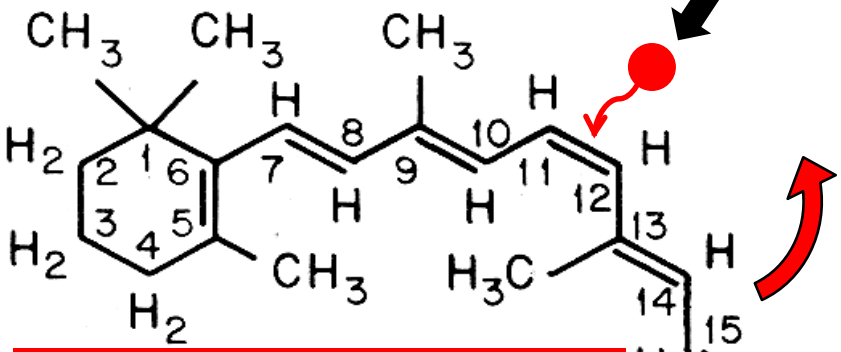
11-*cis* retinal



Chromophore

(*chromo-* colour, + *-phore,* producer)
Light-catching portion of any molecule

11-*cis* retinal



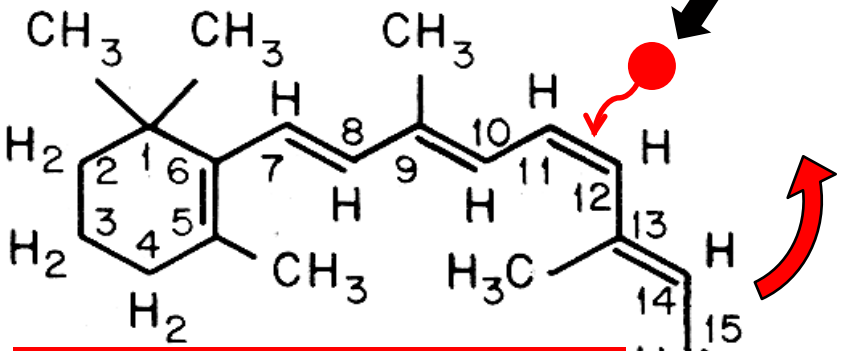
the energy of which initiates a conformational change to…

Chromophore

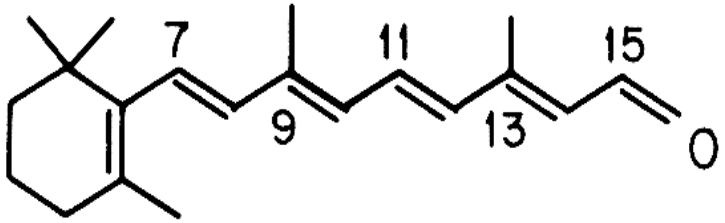
(*chromo-* colour, + *-phore,* producer)
Light-catching portion of any molecule

A photon is absorbed

11-*cis* retinal

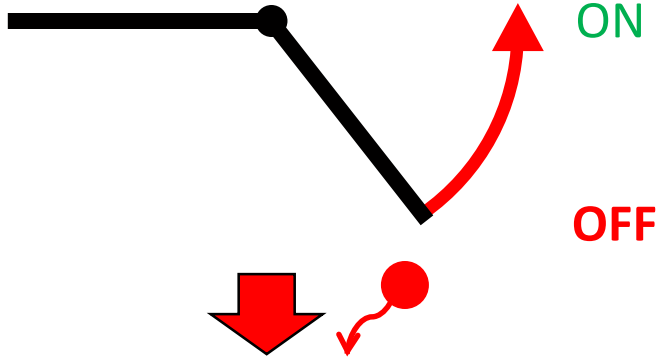


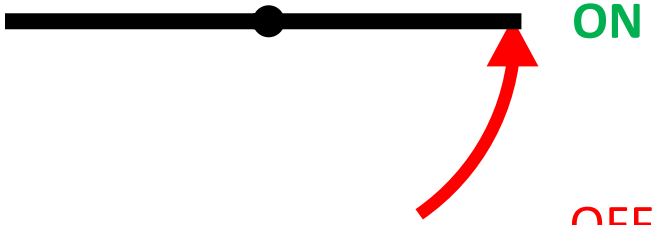
the energy of which initiates a conformational change to…



all-*trans* retinal

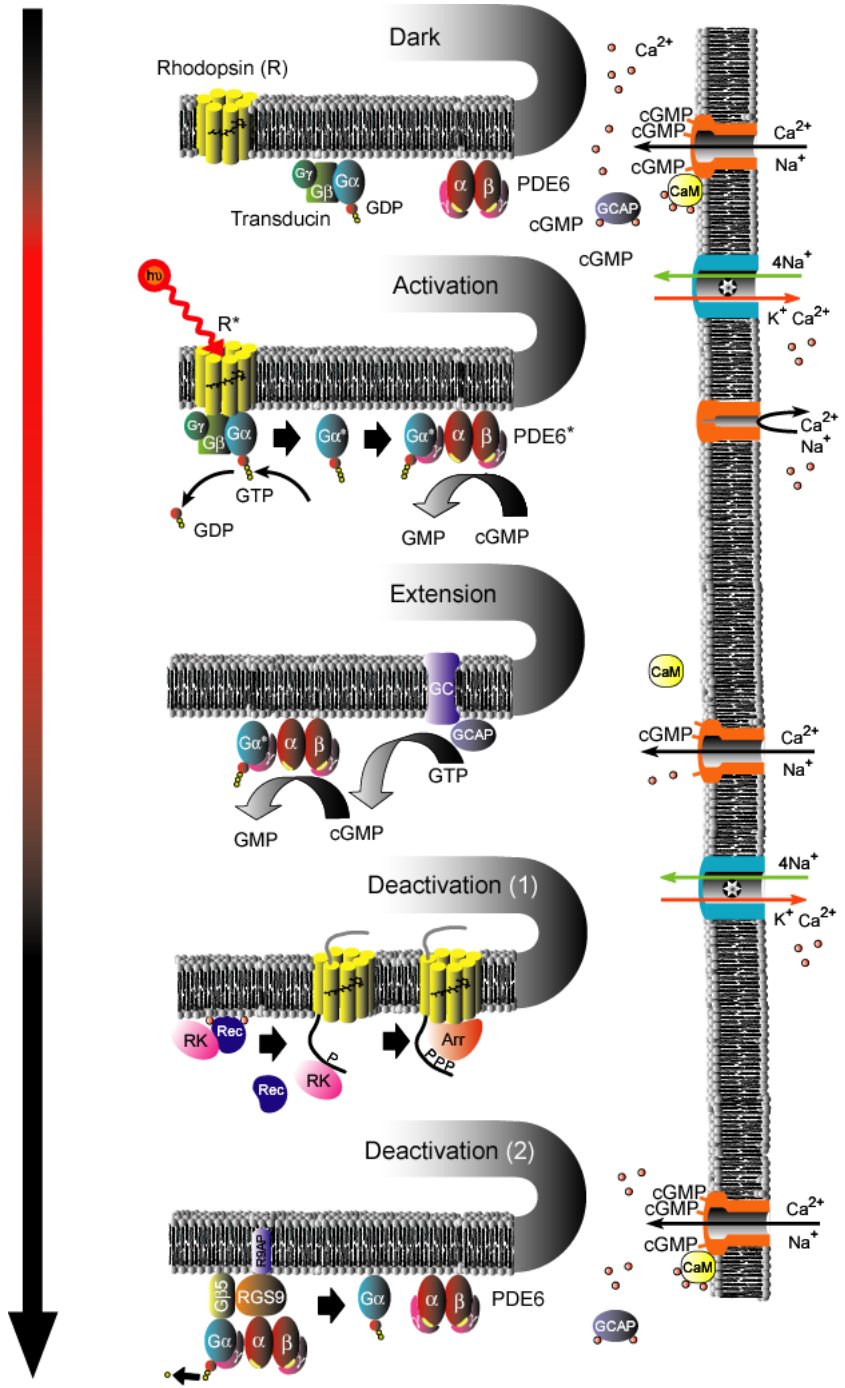
Think of the molecule as a photo-sensitive switch!





Phototransduction

Energy of absorbed photon is converted (transduced) to an electrical neural signal, the receptor potential.

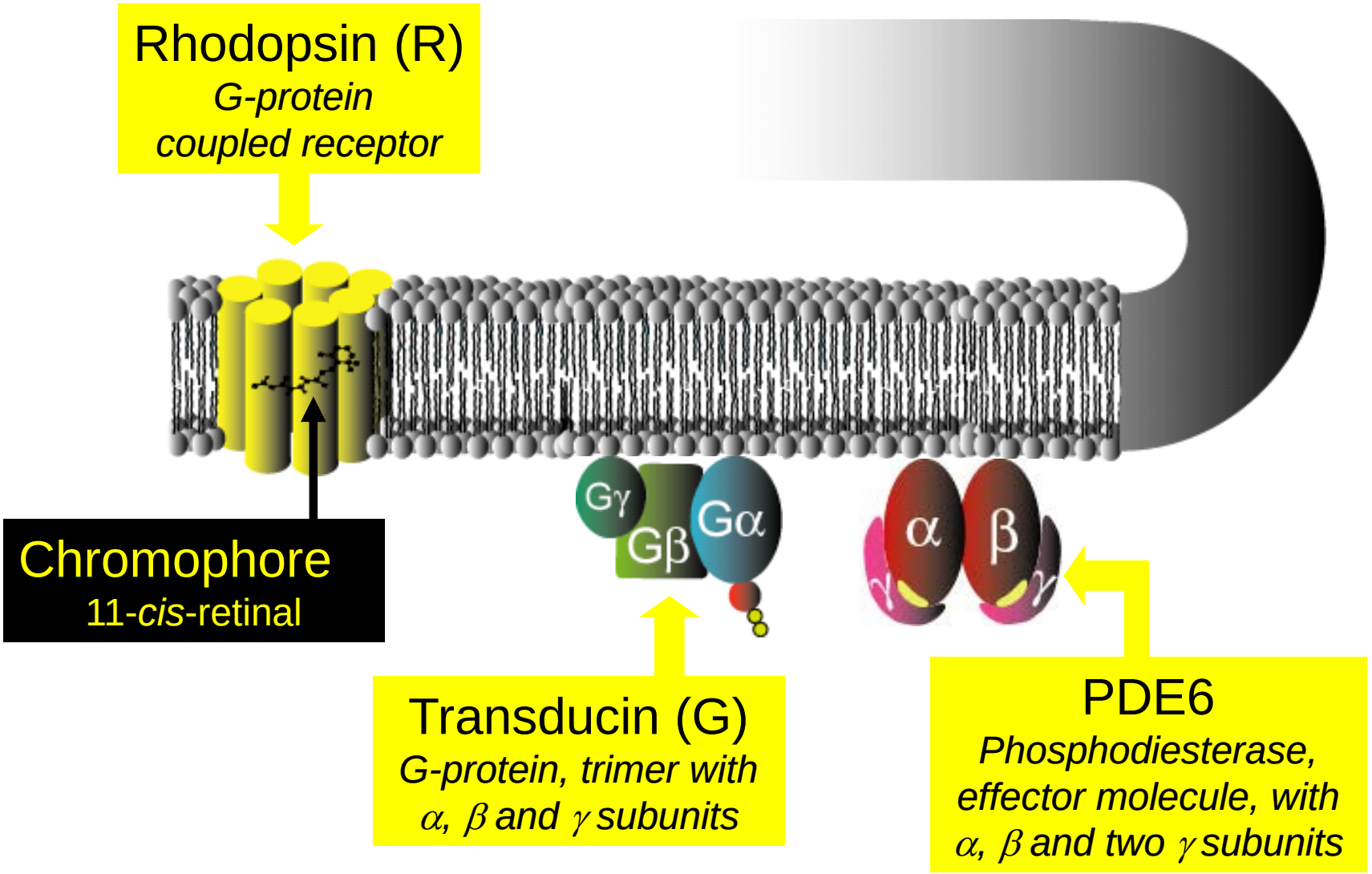


Phototransduction

- Activation
- Range extension
- Deactivation

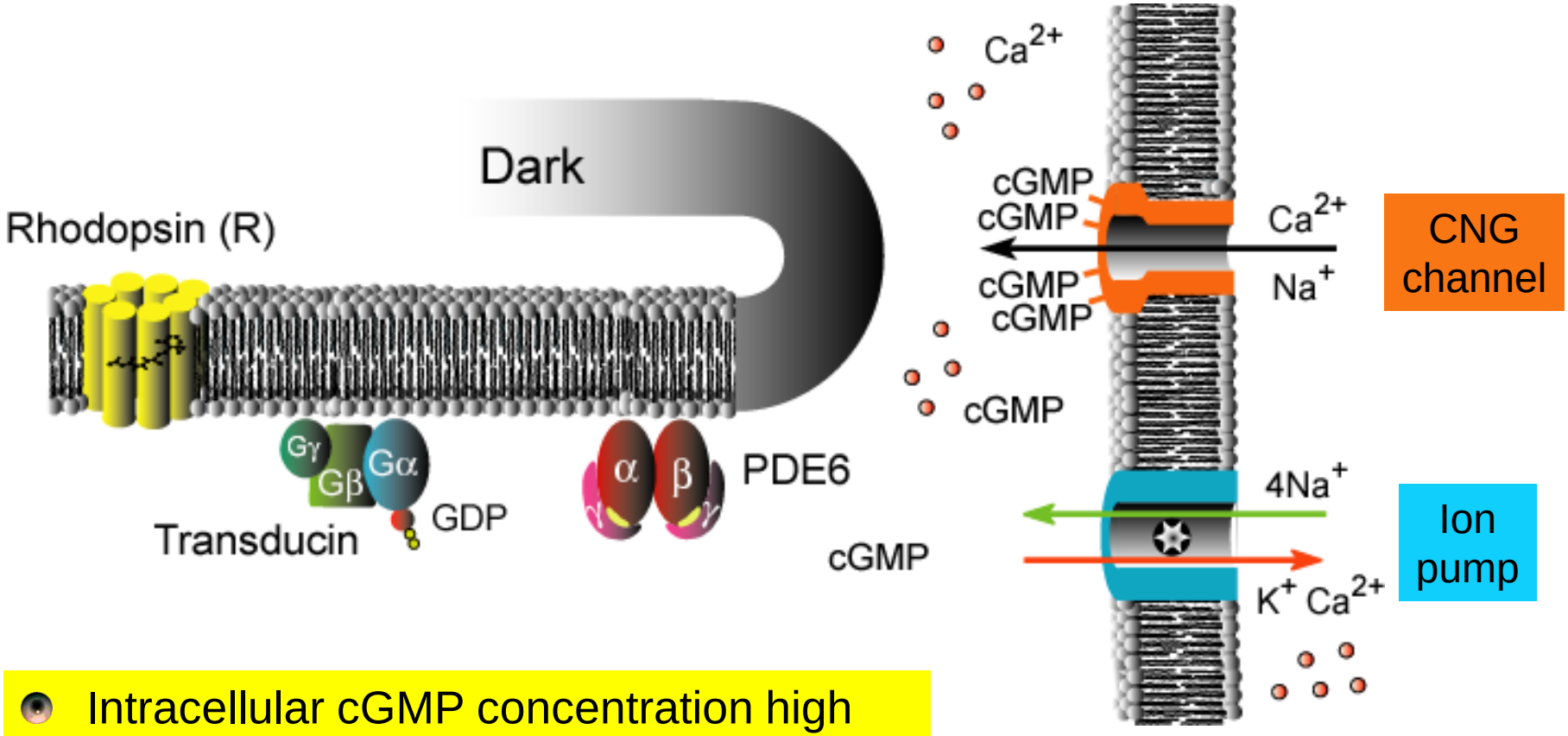
Inspired by:
Pugh, Nikonov, & Lamb (1999). *Current Opinion on Neurobiology, 9*, 410-418.
Burns & Arshavsky (2005). *Neuron, 48*, 387-401.

Main molecular players in the cascade…



In the Dark…

In the Dark

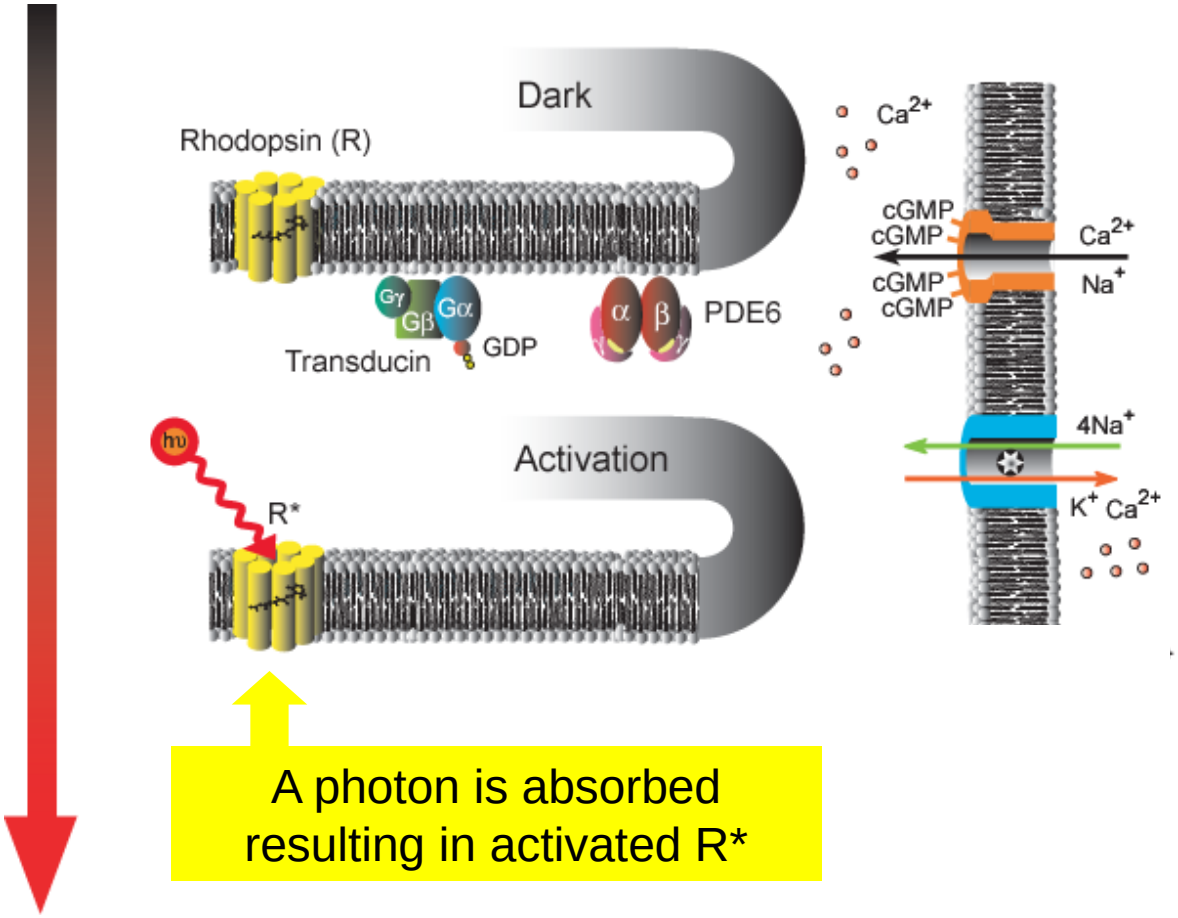


- Intracellular cGMP concentration high
- CNG channels open

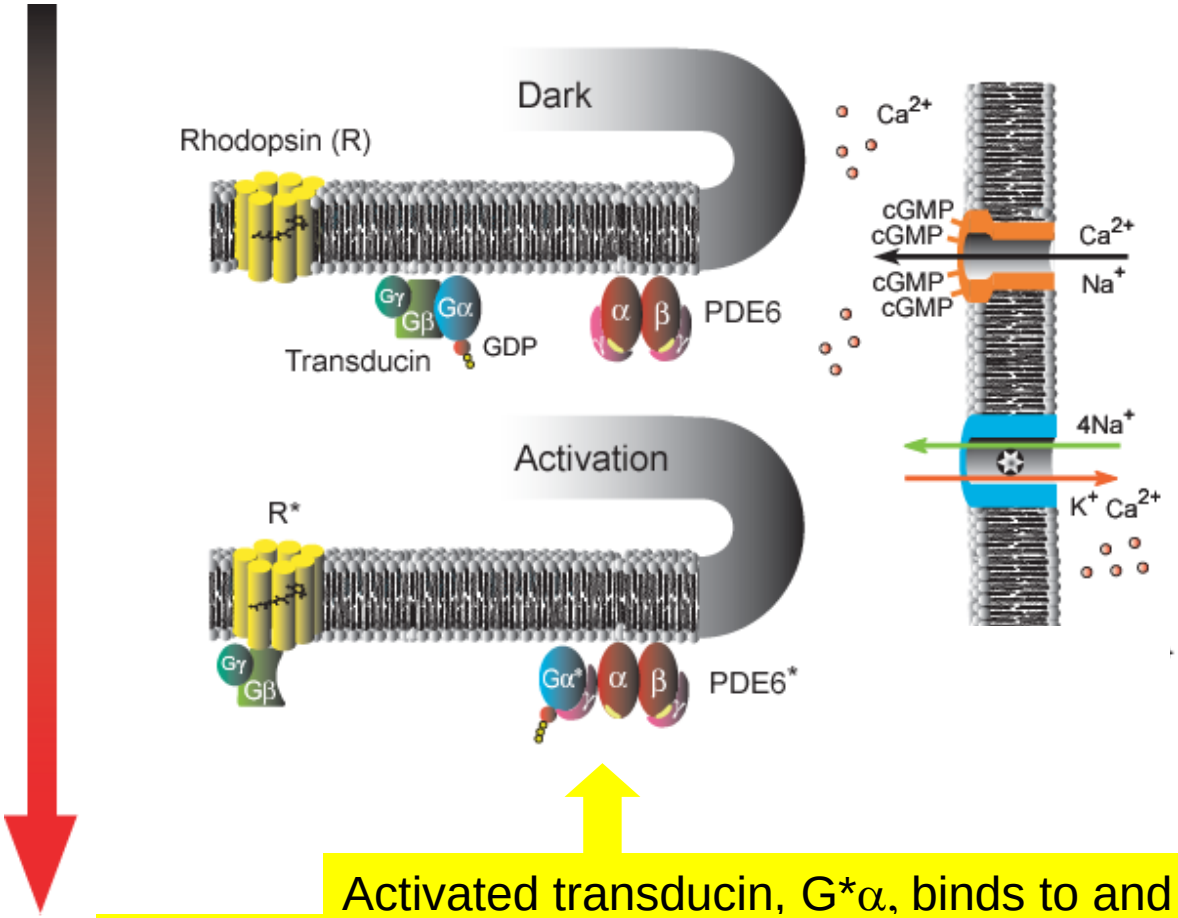
CNG = Cyclic Nucleotide Gated channel

Activation steps

Activation steps



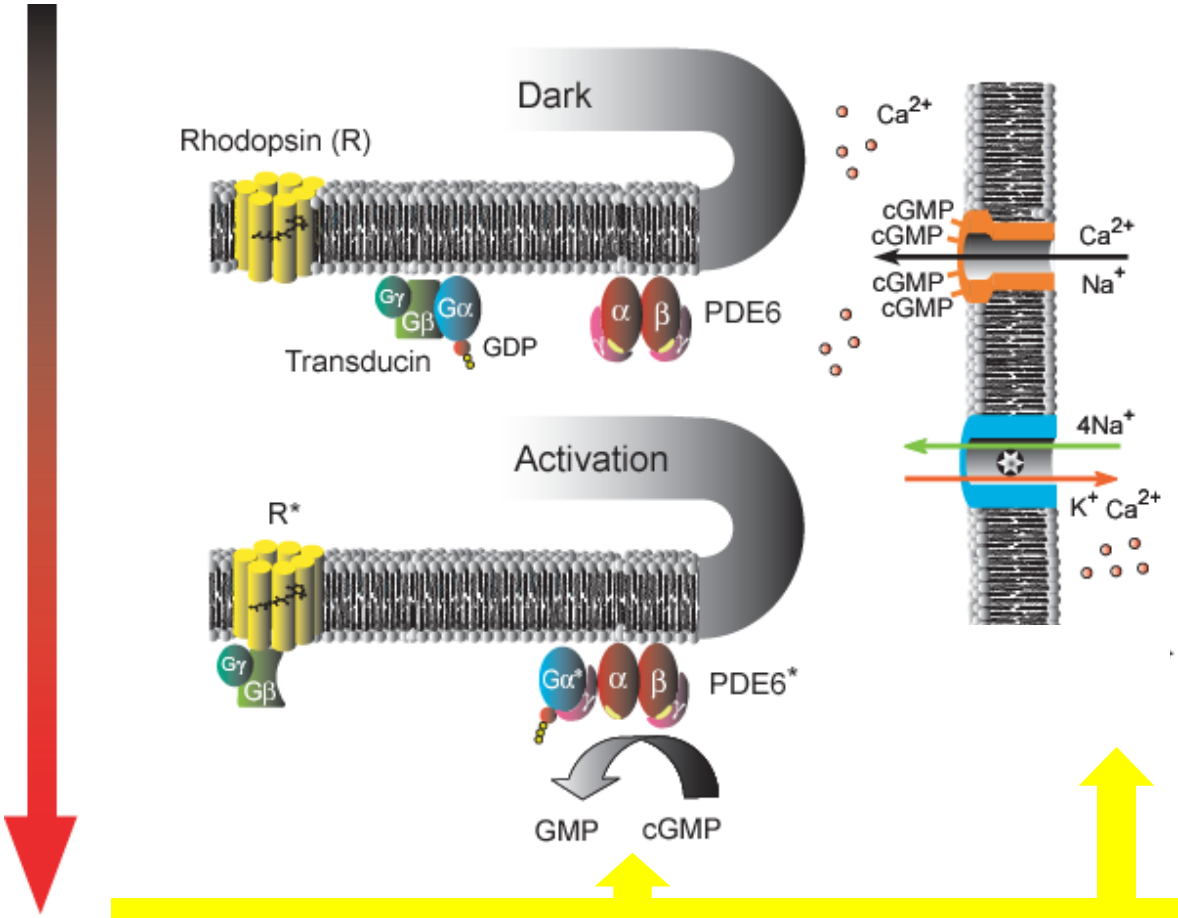
Activation steps



Activated transducin, G*α, binds to and activates

R* catalyses the exchange of GDP for GTP on the G-protein, producing the activated subunit G*α, which dissociates

Activation steps



Ion pump continues to reduce [Ca^{2+}] in the cell

The drop in cGMP leads to closure of the CNG channels, which blocks the entry of Na^+ and Ca^{2+} ions into the outer segment, causing the outer segment to hyperpolarize.

How many photons are needed for us to detect light (when fully dark-adapted)?

When fully dark-adapted, we can detect as few as 7-10 photons.

How is this possible?

Amplification

The absorption of a single photon is sufficient to change the membrane conductance. How?

A single R* catalyses the activation of c. 500 transducin molecules. Each G*α can stimulate one PDE6*, which in turn can break down 10^3 molecules of cGMP per second. Thus, a single R* can cause the hydrolysis of $>10^5$ molecules of cGMP per second!

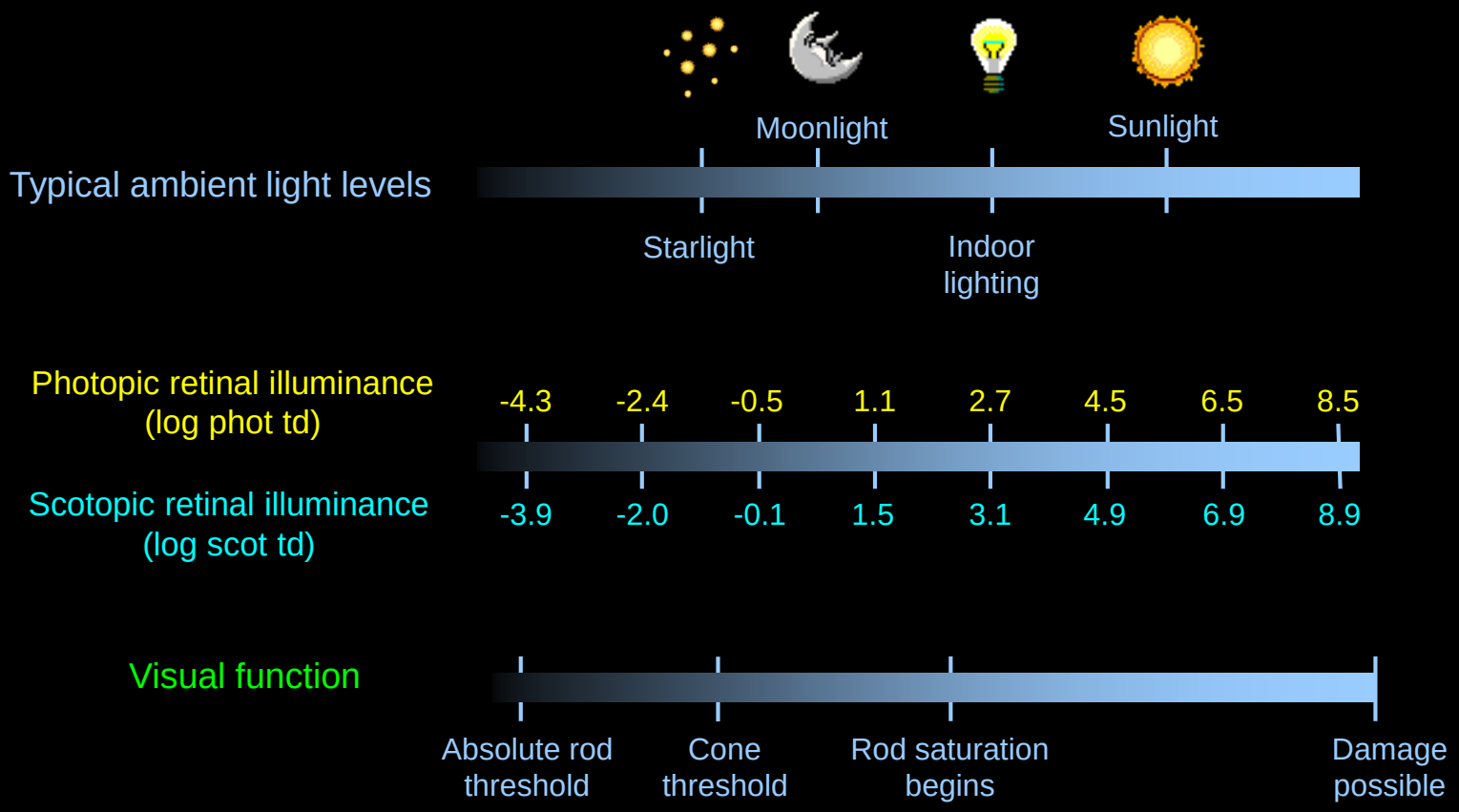
Amplification is beneficial at low light levels, but what negative effect will it have at high light levels?

An important function of the photoreceptor and the transduction cascade is:

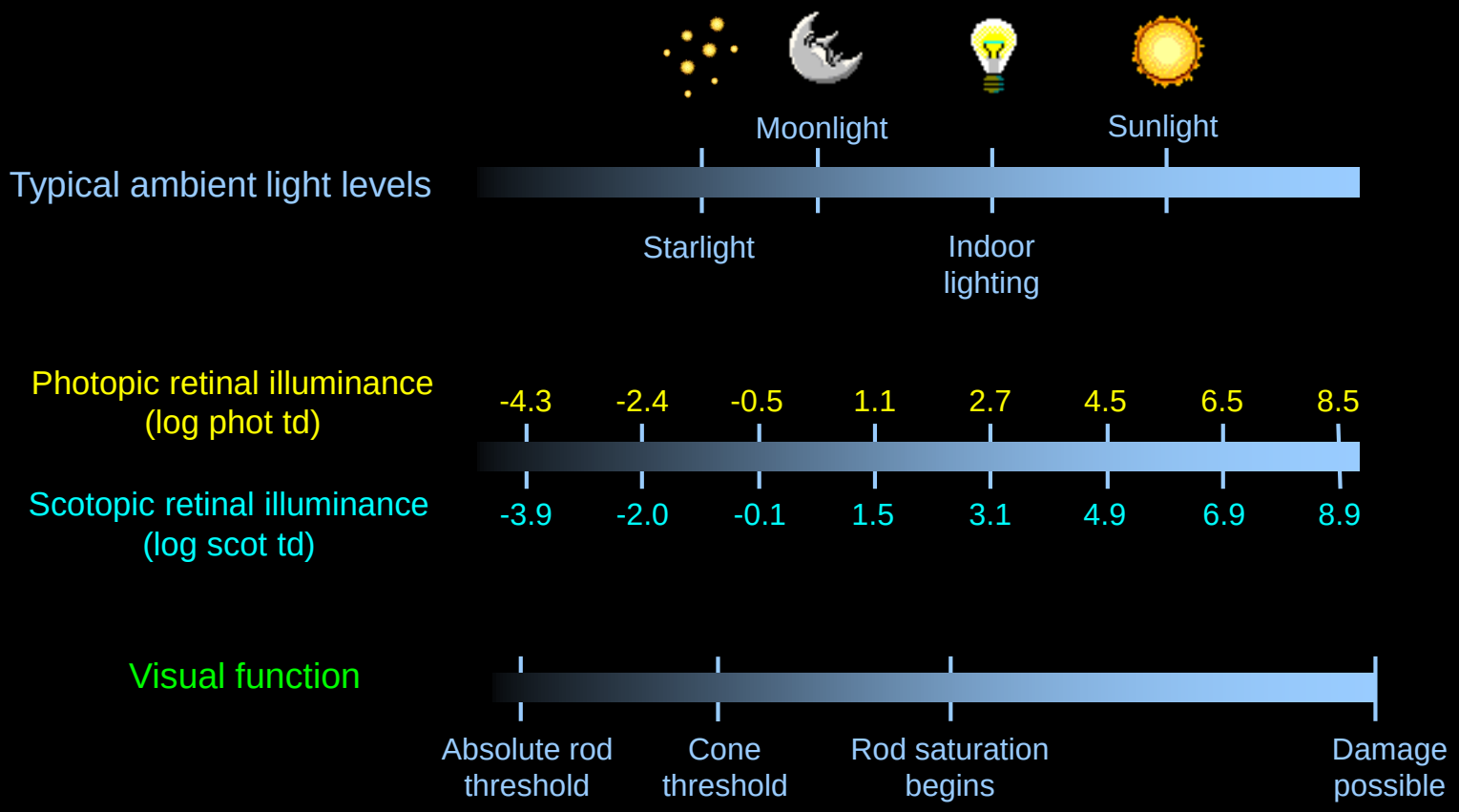
Range extension and light adaptation

Why is light adaptation or sensitivity regulation important?

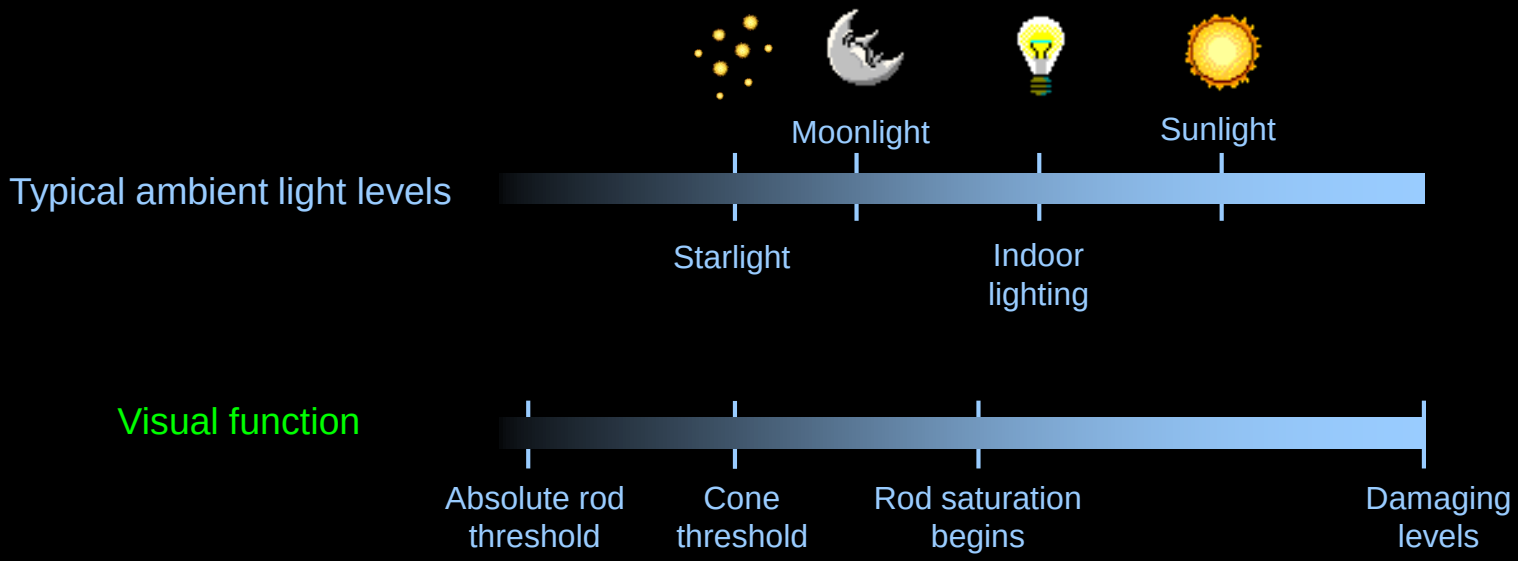
- The visual system must maintain itself within a useful operating range over the roughly 10^{12} change in illumination: from absolute rod threshold to levels at which photoreceptor damage can occur.



- It must do so despite the fact that that a typical postreceptoral neuron can operate over a range of only c. 10^3.

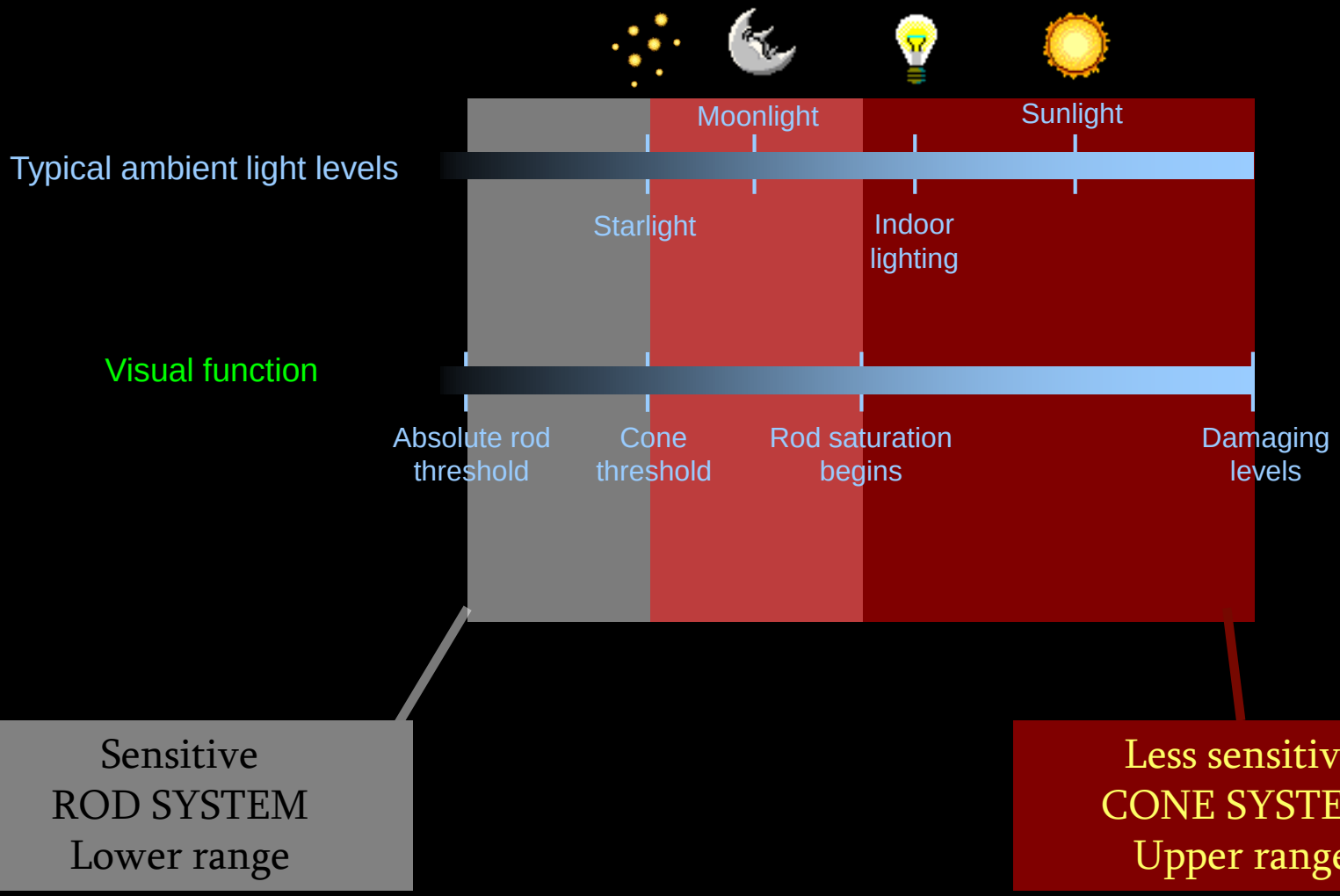


It achieves it in part by having two types of photoreceptor:

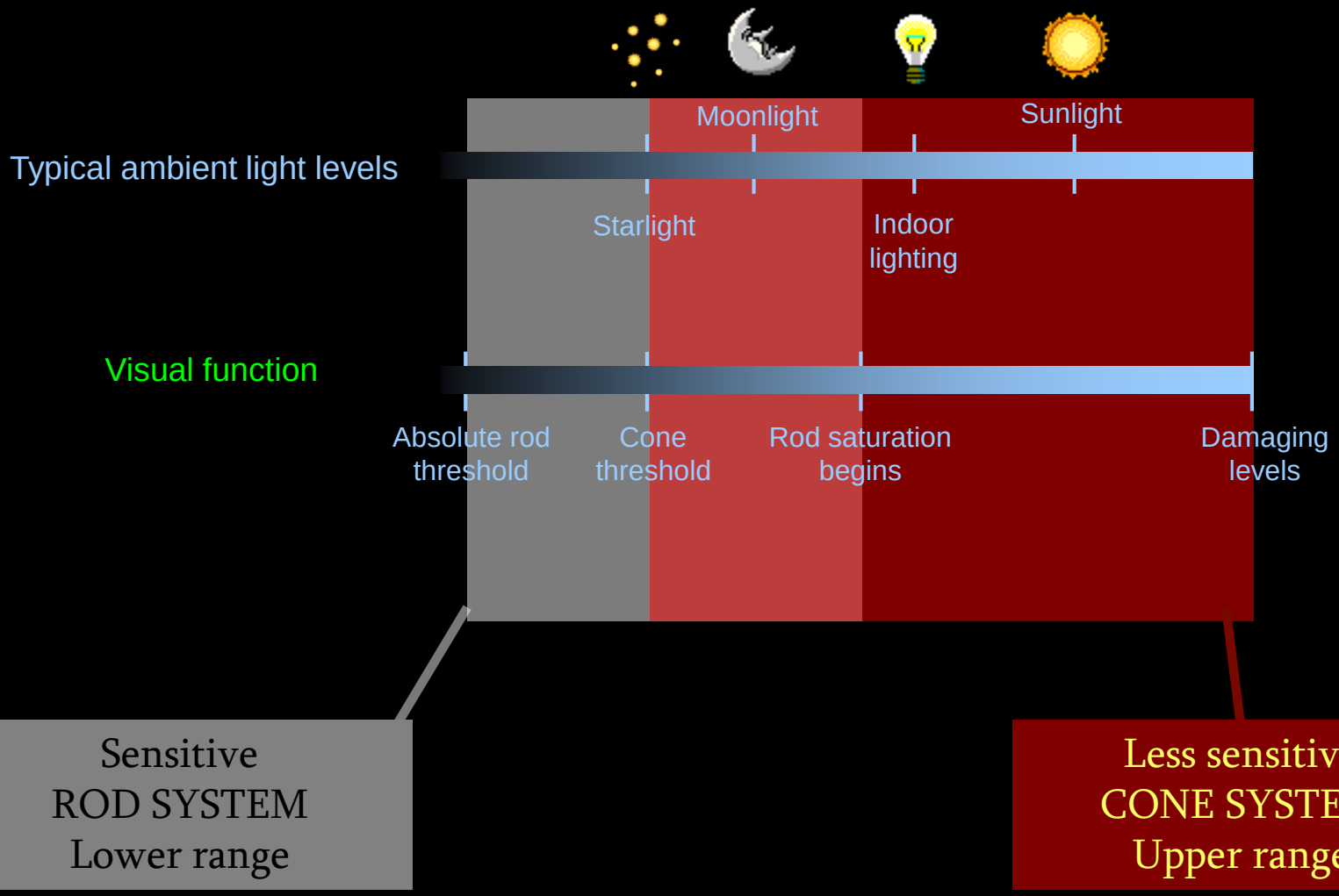


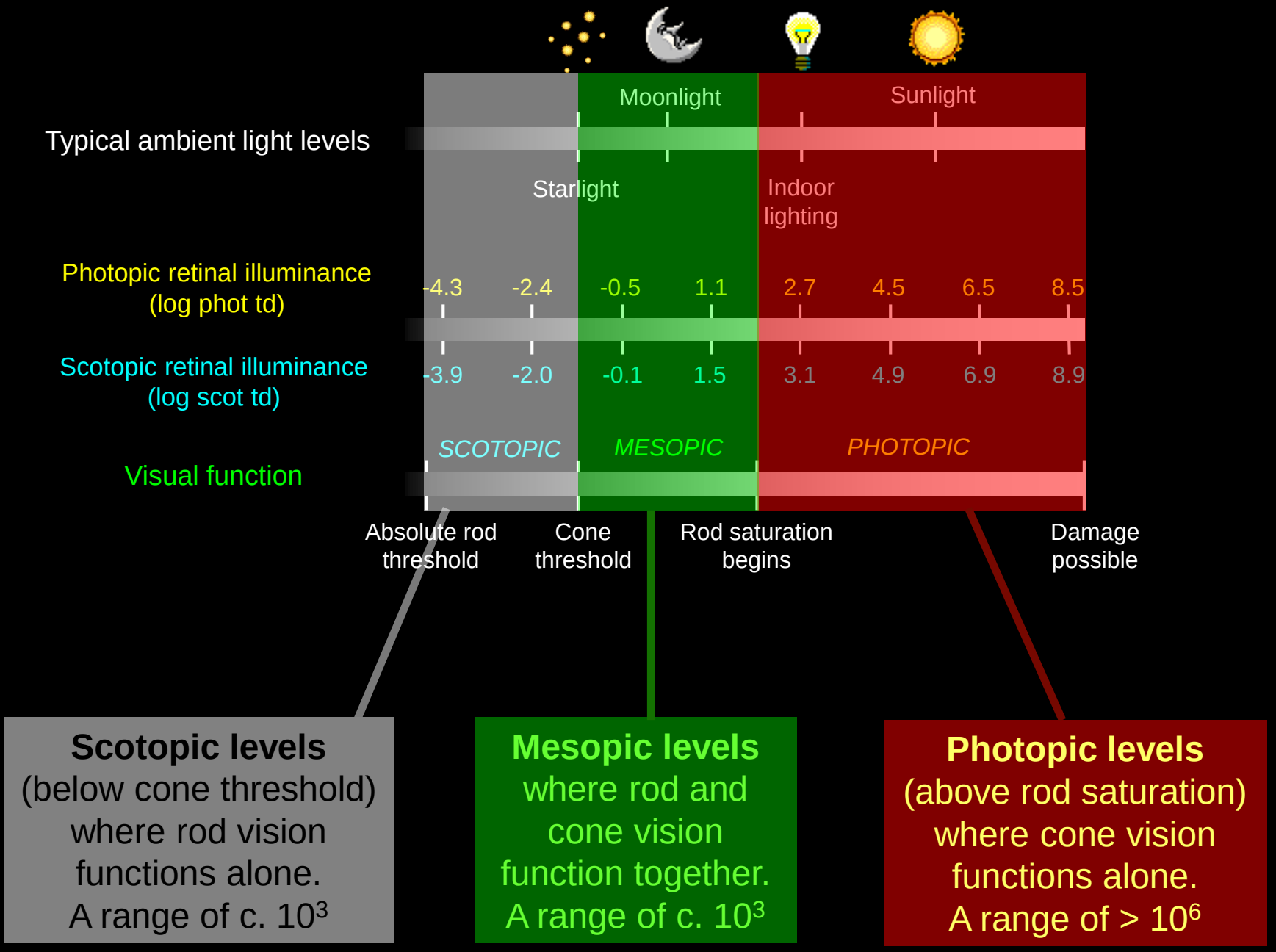
Rods that are optimized for low light levels

Cones that are optimized for higher light levels



Even with two systems, each of them must still operate over an enormous range.




Typical ambient light levels
Photopic retinal illuminance (log phot td)
Scotopic retinal illuminance (log scot td)
Visual function
Moonlight
Sunlight
Starlight
Indoor lighting
-4.3
-2.4
-0.5
1.1
2.7
4.5
6.5
8.5
-3.9
-2.0
-0.1
1.5
3.1
4.9
6.9
8.9
SCOTOPIC
MESOPIC
PHOTOPIC
Absolute rod threshold
Cone threshold
Rod saturation begins
Damage possible
Scotopic levels (below cone threshold) where rod vision functions alone. A range of c. 10³
Mesopic levels where rod and cone vision function together. A range of c. 10³
Photopic levels (above rod saturation) where cone vision functions alone. A range of > 10⁶

Adaptation and sensitivity…

System must ADAPT to changes in light level

Ideally, the system should be very sensitive at low light levels, so that it can detect a few photons, but then much, much less sensitive at high light levels.

How is this achieved within the transduction cascade?

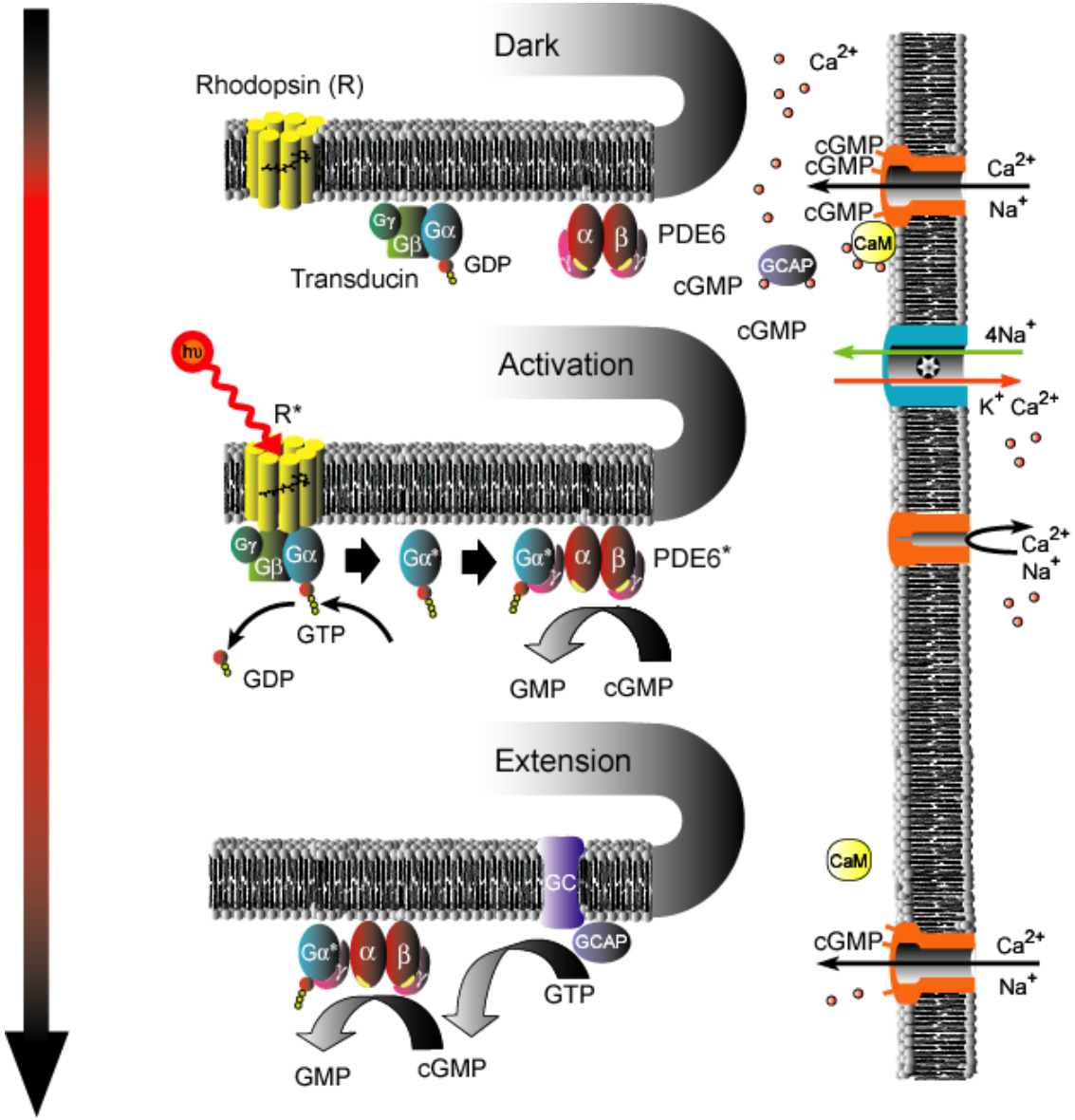
Amplification

At low light levels the sensitivity is very high.

A single R* catalyses the activation of c. 500 transducin molecules. Each G*α can stimulate one PDE6*, which in turn can break down 10^3 molecules of cGMP per second. Thus, a single R* can cause the hydrolysis of $>10^5$ molecules of cGMP per second!

But as the light level increases, the system will saturate (as you run out of “stuff”).

Range extension (1)

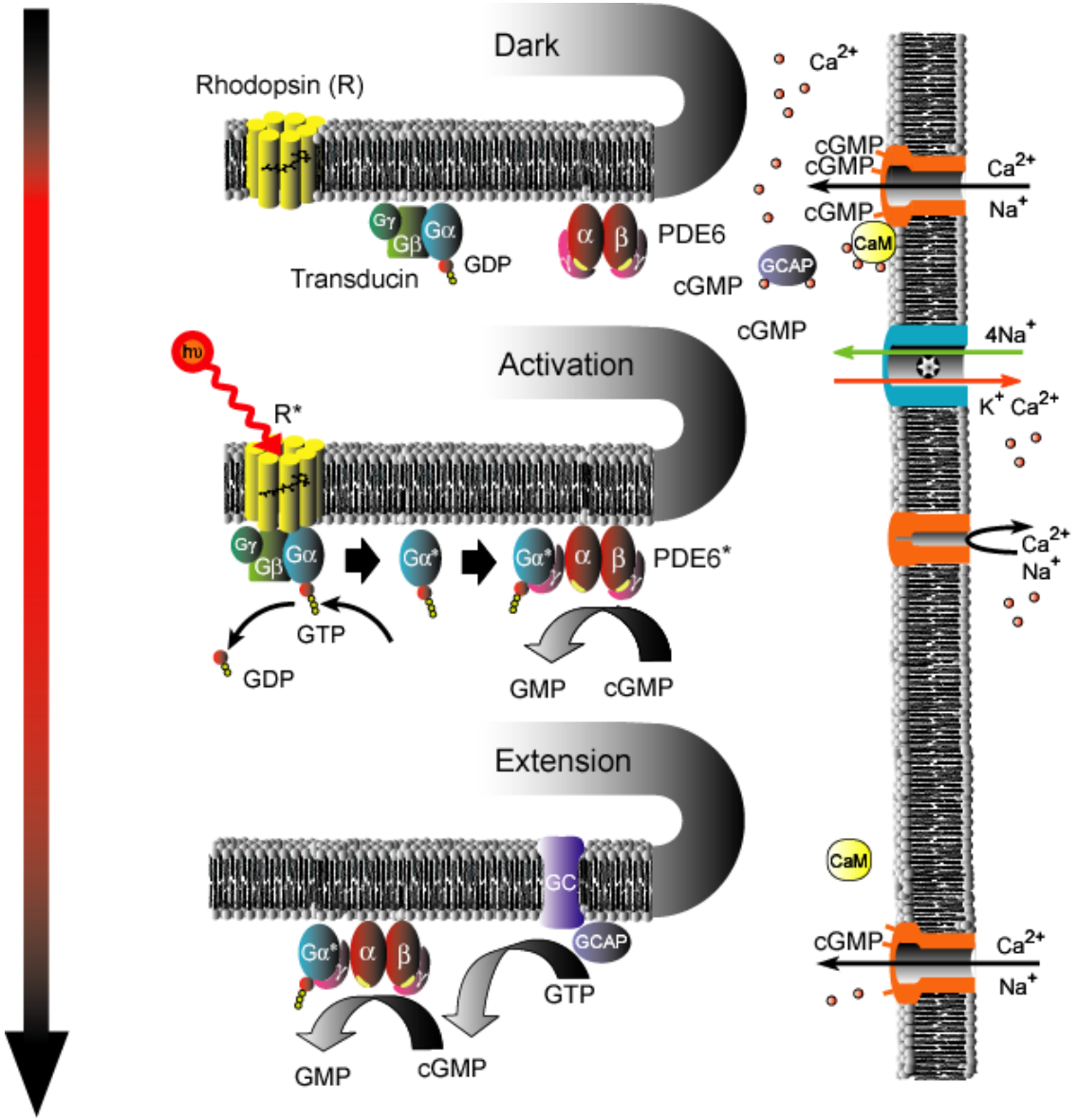


Calmodulin

Reduction in [Ca^{2+}] causes Calmodulin (CaM) to dissociate from the CNG channels raising the affinity of the channels for cGMP

Need less cGMP to open the CNG channels

Range extension (2)



Calmodulin

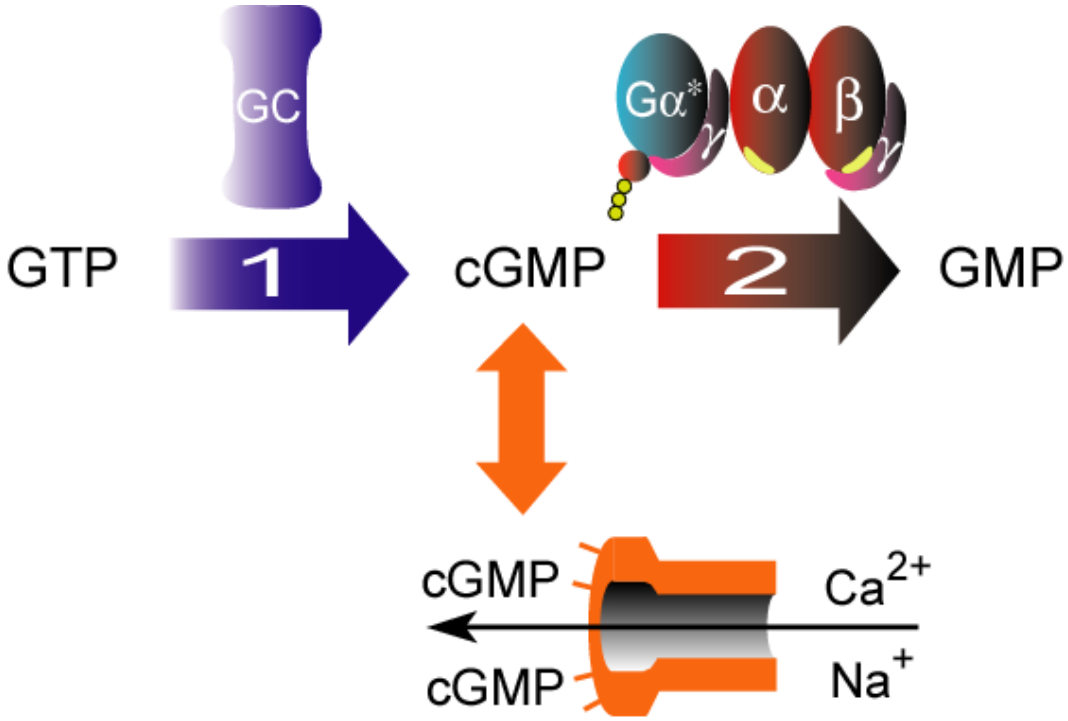
Reduction in [Ca^{2+}] causes Calmodulin (CaM) to dissociate from the CNG channels raising the affinity of the channels for cGMP

GCAP

Reduction in [Ca^{2+}] causes dissociation of Ca^{2+} from GCAP, allowing it to bind to GC increasing the rate of resynthesis of cGMP

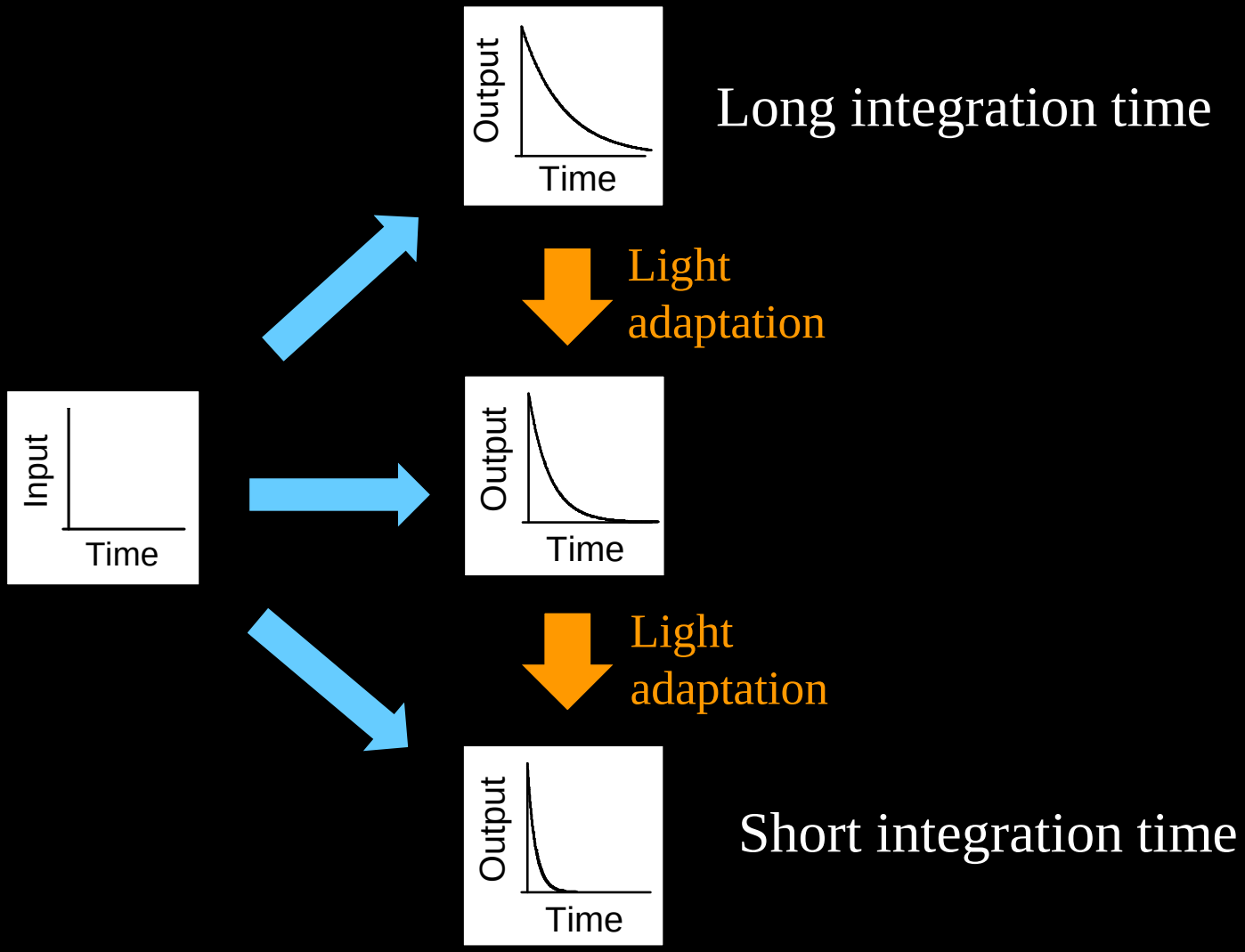
Speeding up the visual response

The increase in concentration of G*α-PDE6* in light speeds up rate of reaction 2 (the removal of cGMP) and thus increases the overall speed of the visual response



How does speeding up the visual response help light adaptation?

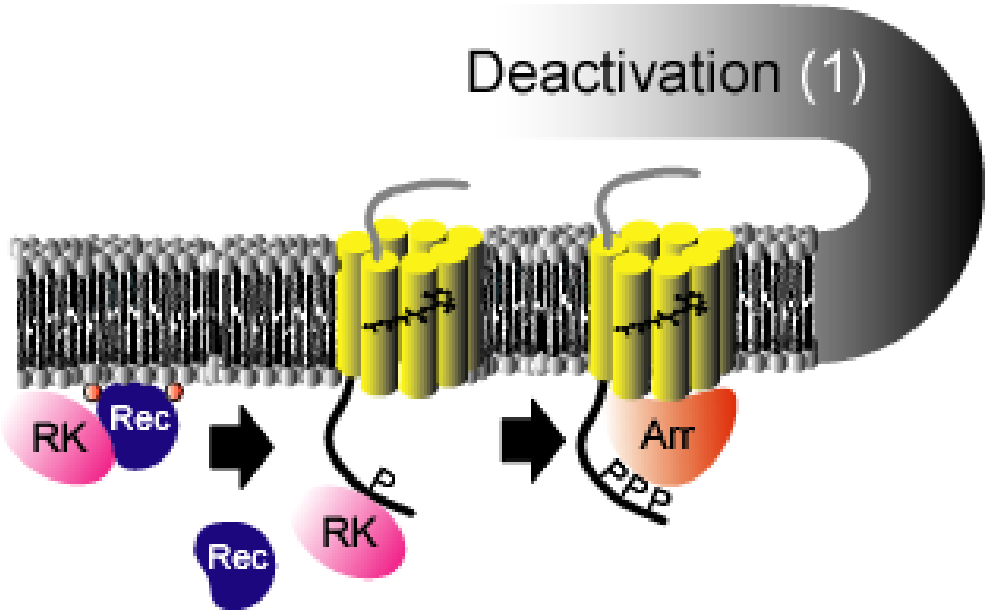
What are the benefits of this type of adaptation?



Deactivation

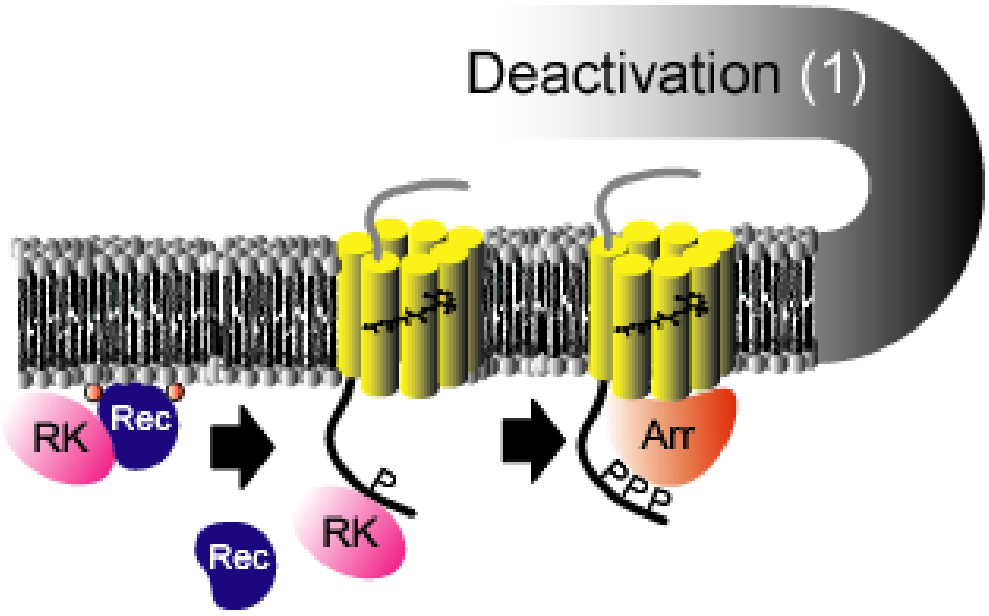
Speeding up deactivation also decreases temporal integration.

Deactivation steps
(turn things off more quickly in the light)



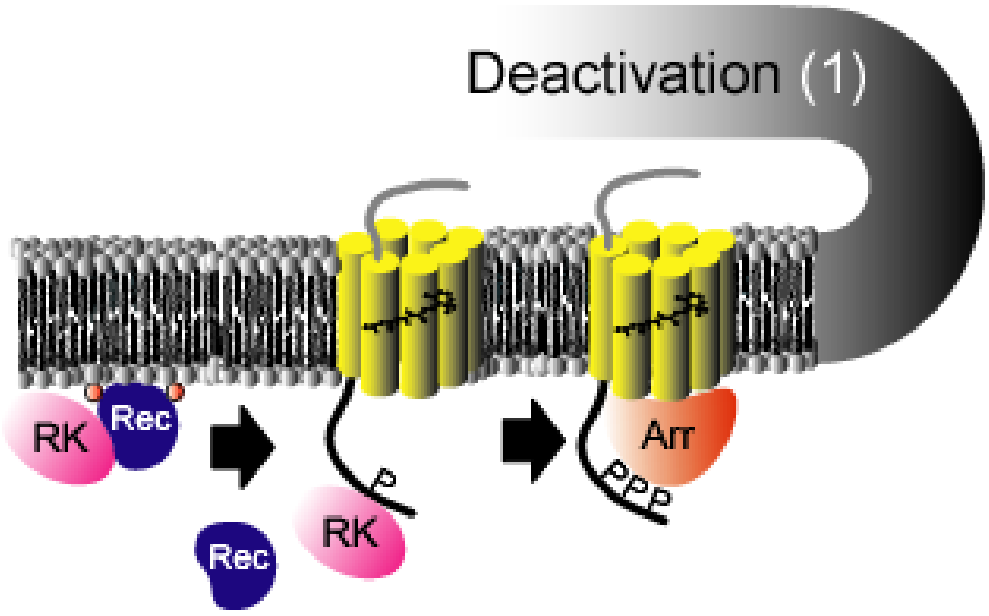
Rec-2Ca^{2+} forms a complex with RK, blocking its activity. When [Ca^{2+}] drops, Ca^{2+} dissociates and Rec goes into solution.

Deactivation steps



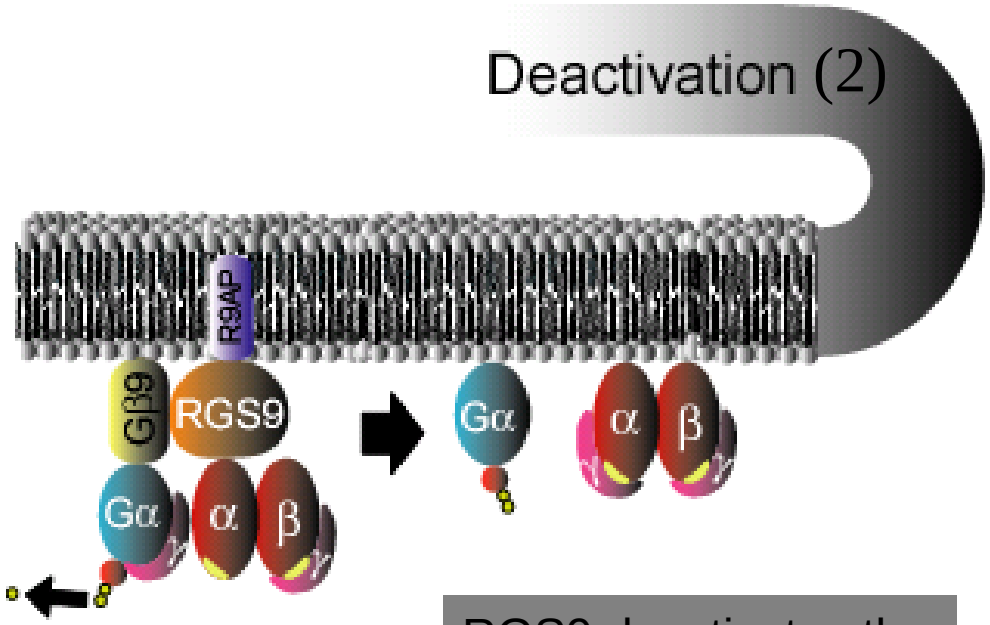
Free RK multiply phosphorylates R*

Deactivation steps



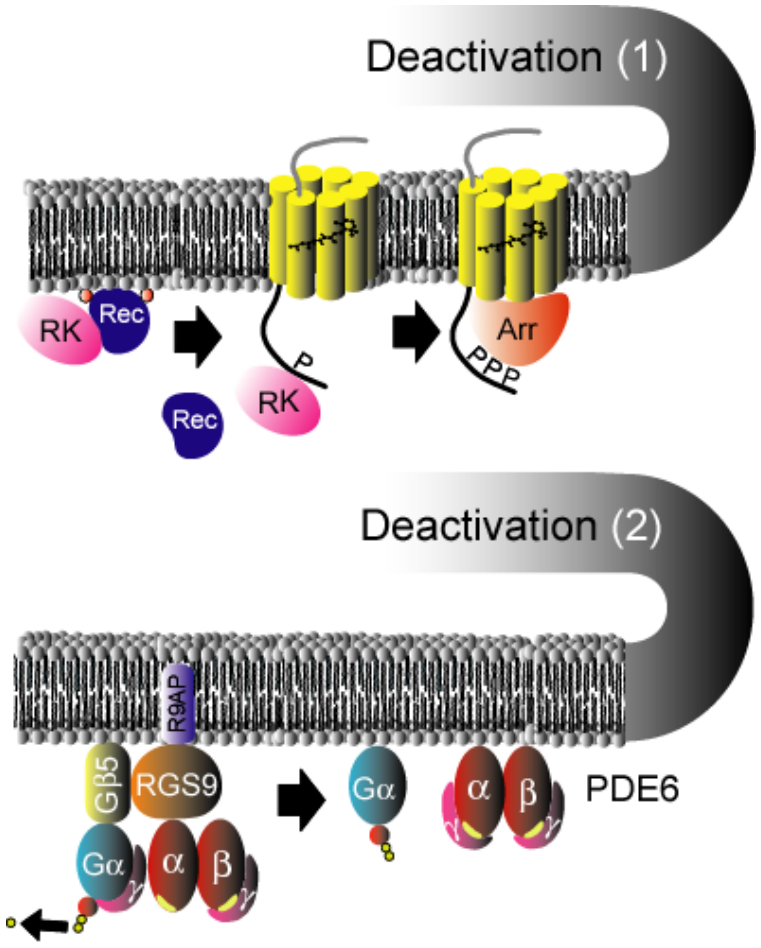
Arrestin (Arr) quenches the phosphorylated R*

Deactivation steps



RGS9 deactivates the activated G*α-PDE6* complex.

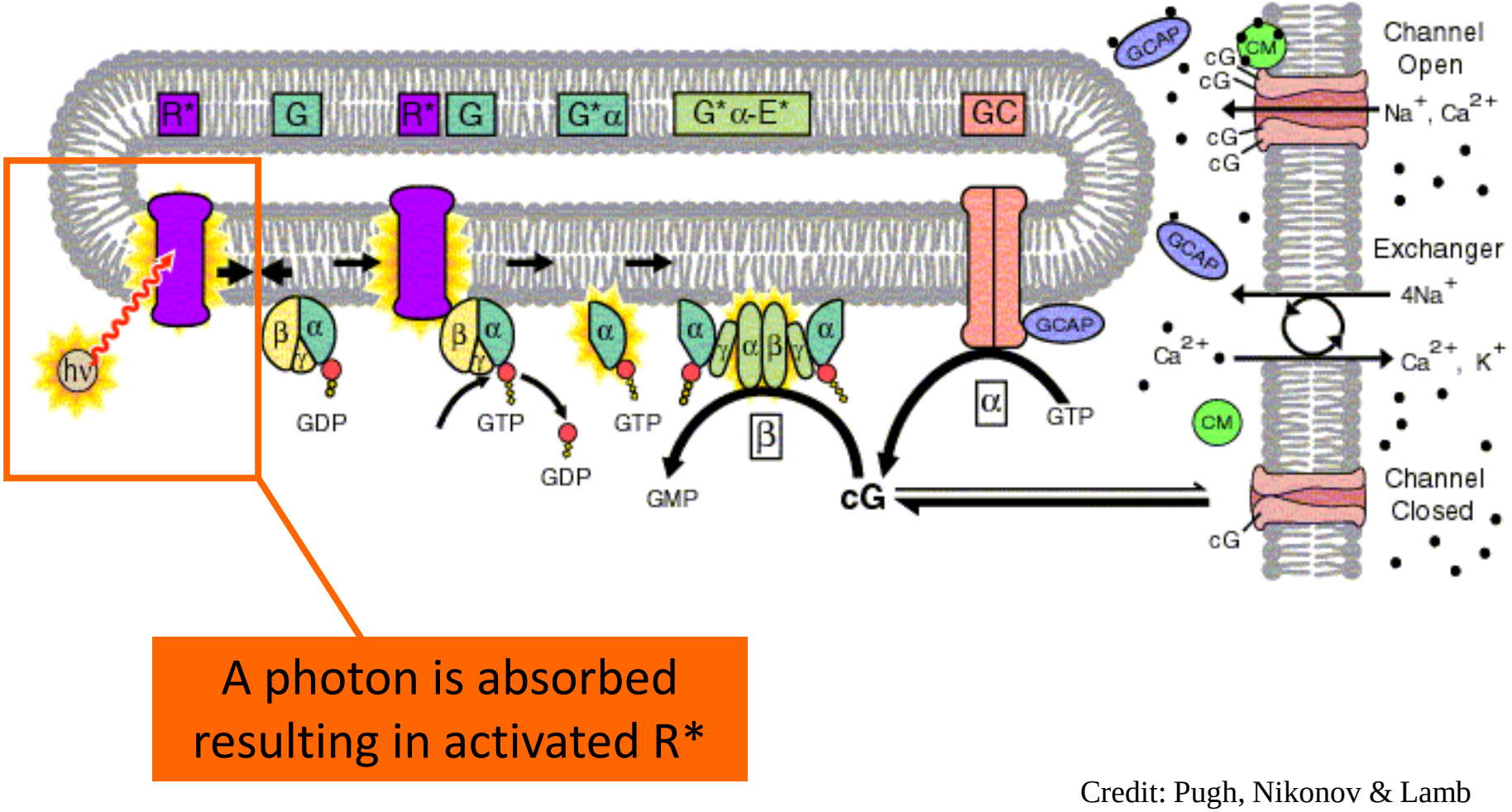
Deactivation steps



Second run through…

Phototransduction cascade activation stages

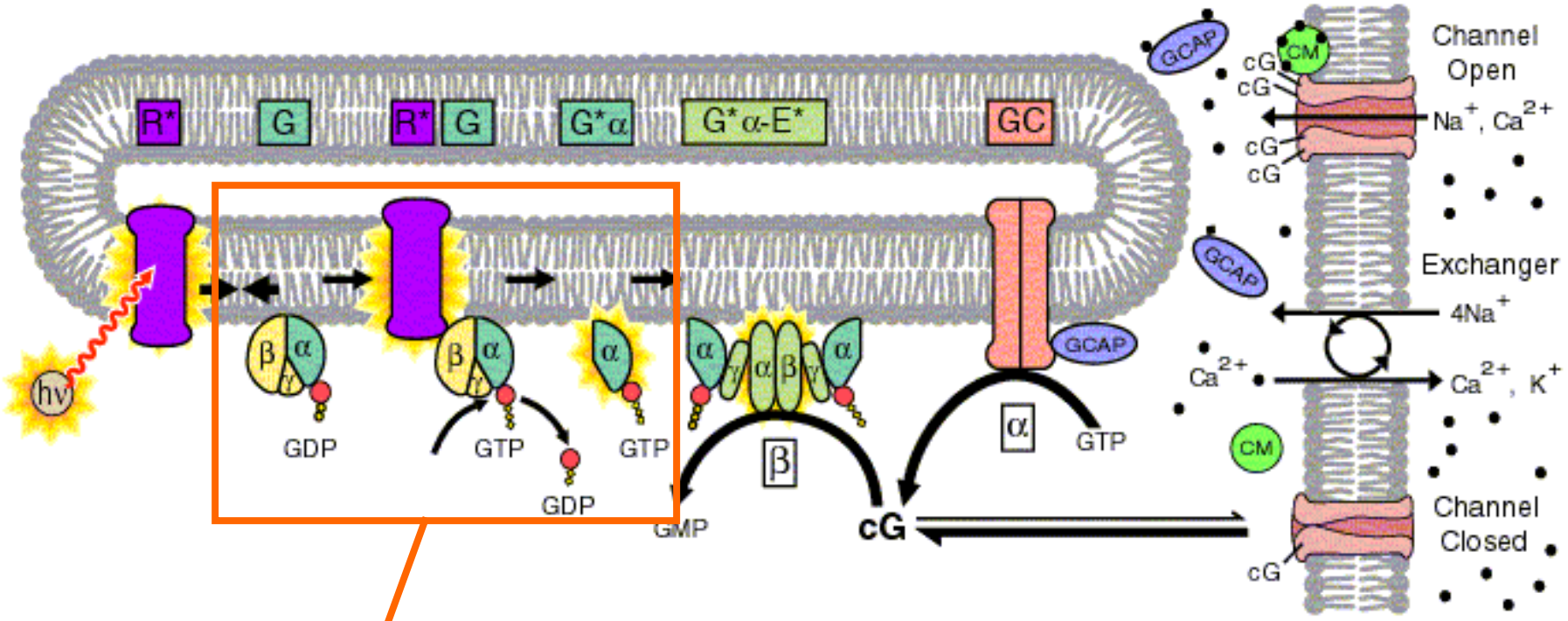
Activation steps of the phototransduction cascade



A photon is absorbed resulting in activated R*

Credit: Pugh, Nikonov & Lamb

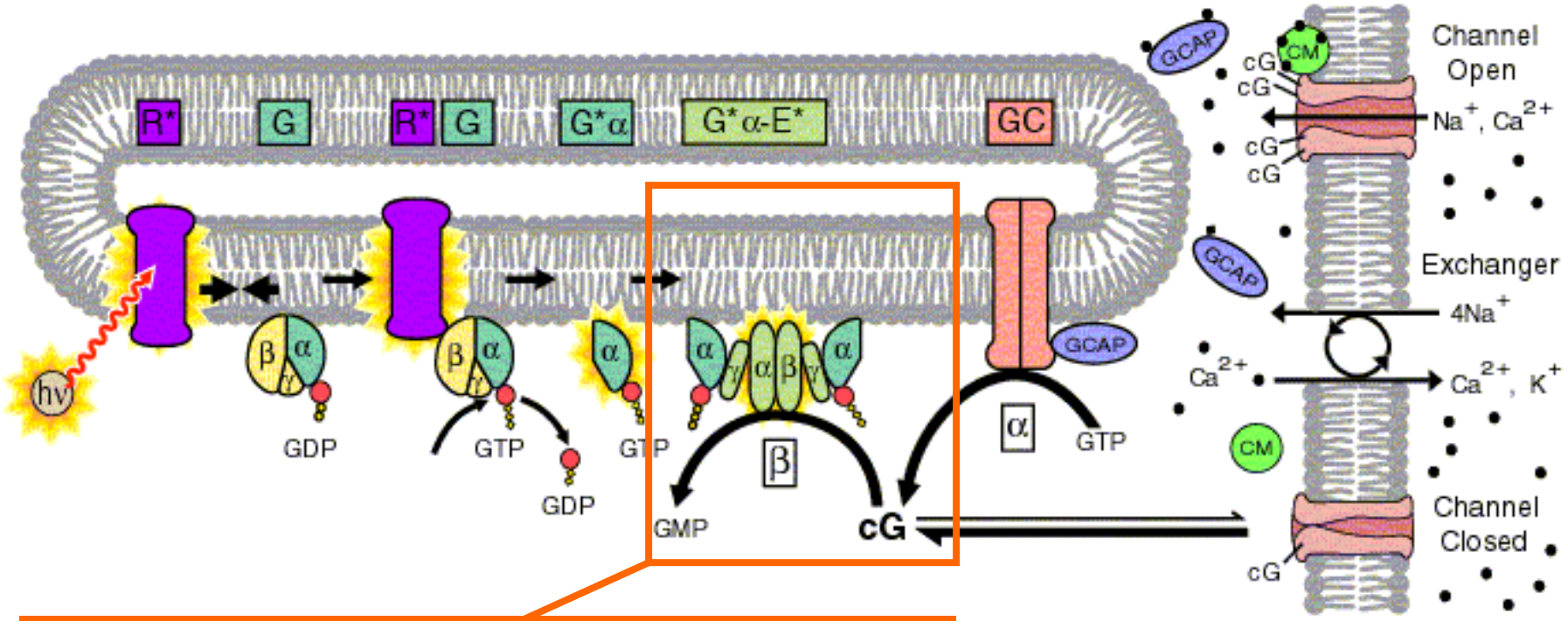
Activation steps of the phototransduction cascade



R* catalyses the exchange of GDP for GTP on the G-protein, producing the activated transducin, subunit G*α (Gα-GTP).

Credit: Pugh, Nikonov & Lamb

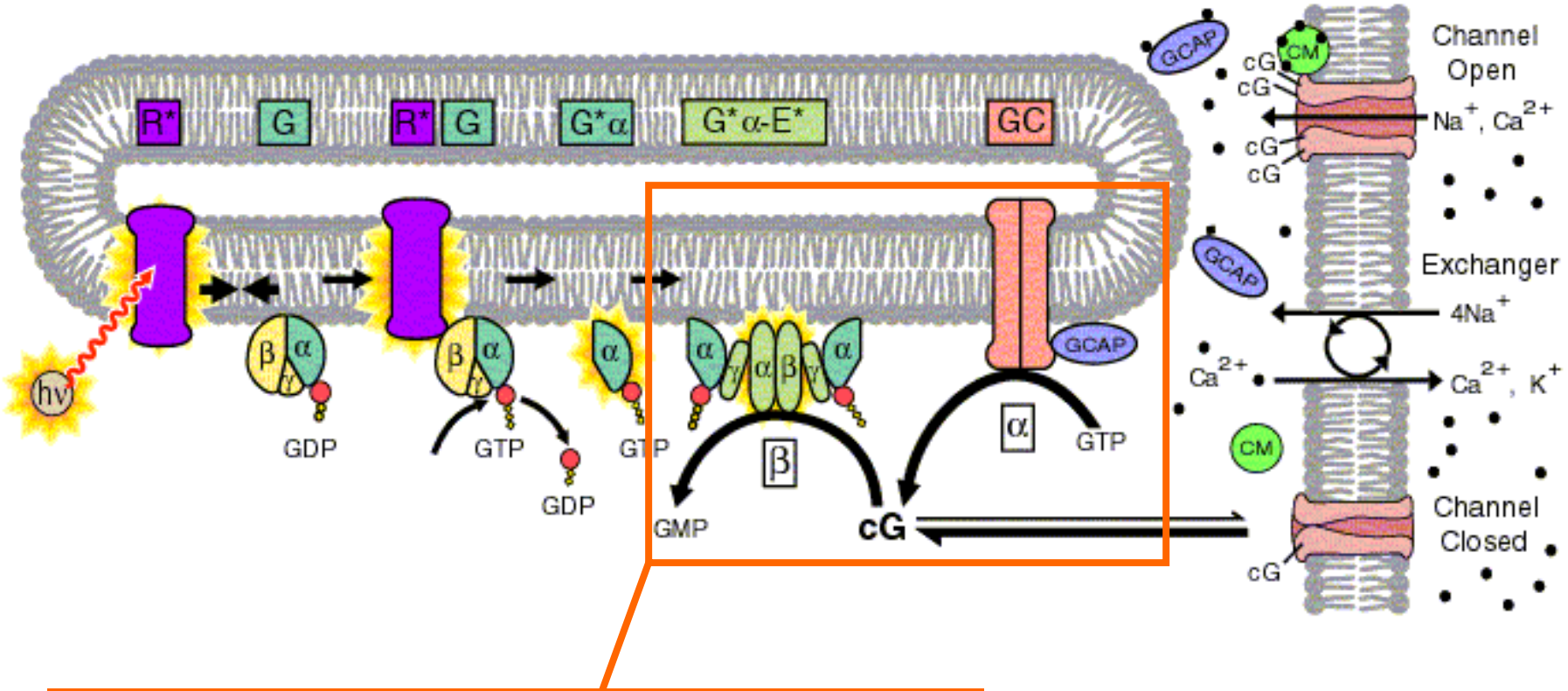
Activation steps of the phototransduction cascade



Activated transducin, G*α, in turn, binds to and activates phospho-diesterase (PDE6) by displacing γ inhibitory subunits to produce PDE6*.

Credit: Pugh, Nikonov & Lamb

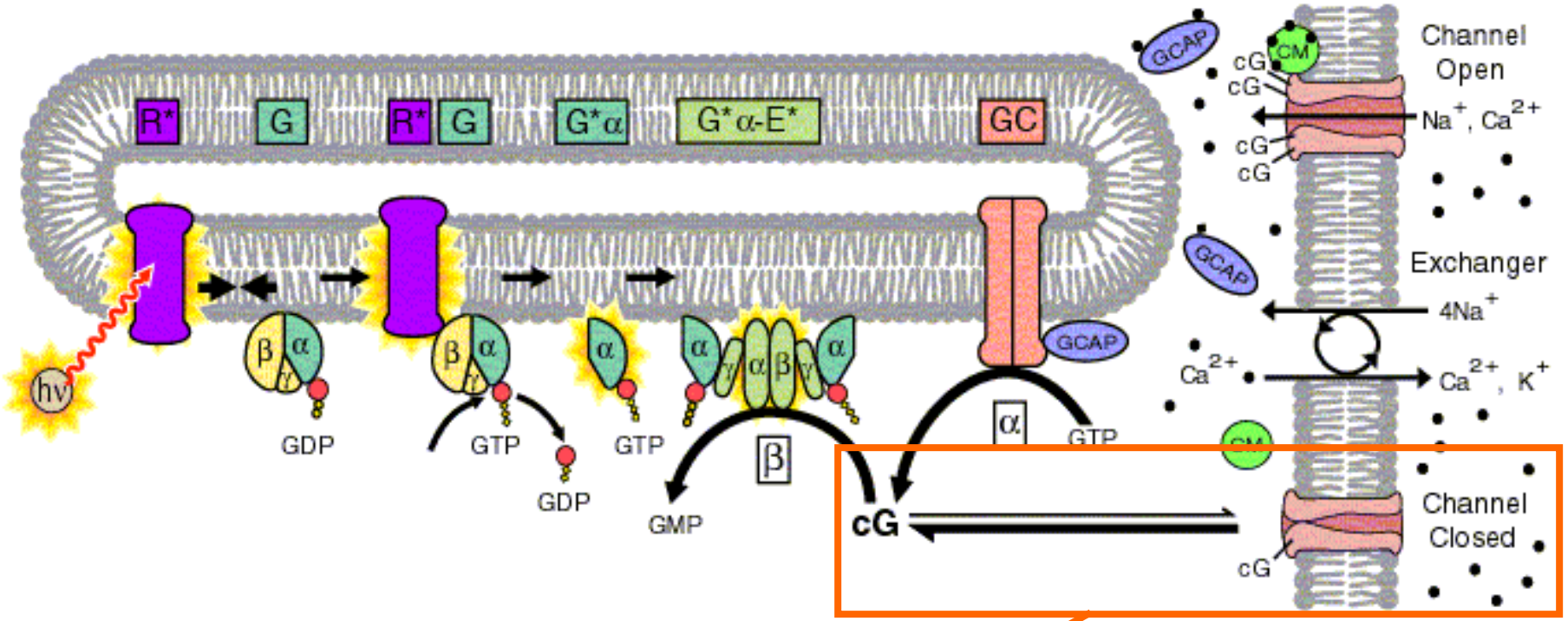
Activation steps of the phototransduction cascade



PDE6* (G*α-E*) activity produces a local drop in cytoplasmic cG (cGMP)

Credit: Pugh, Nikonov & Lamb

Activation steps of the phototransduction cascade

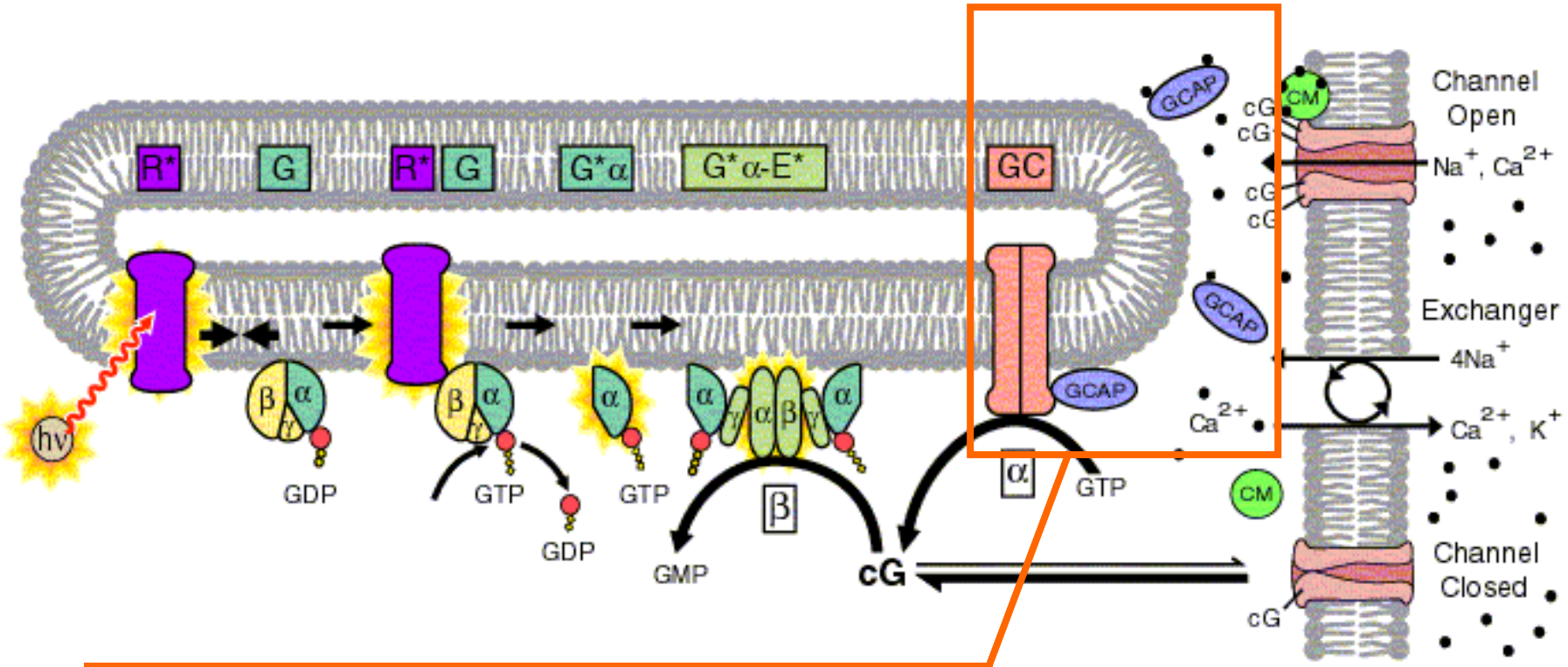


A drop in cGMP leads to closure of cGMP gated channels, blocking the entry of Na^+ and Ca^{2+} into the outer segment. The ion exchanger continues to function lowering [Ca^{2+}] in the outersegment.

Credit: Pugh, Nikonov & Lamb

Phototransduction cascade inactivation steps

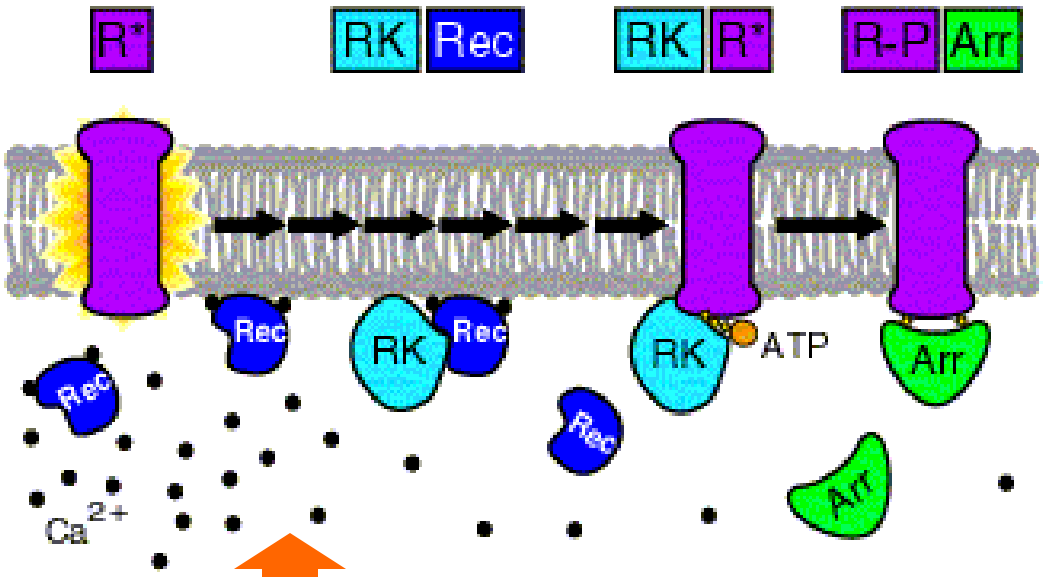
Inactivation steps of the phototransduction cascade



Removal of Ca^{2+} activates guanylate cyclase activating protein, GCAP. Activated GCAP binds to guanylate cyclase, stimulating production of cG.

Ca^{2+} feedback

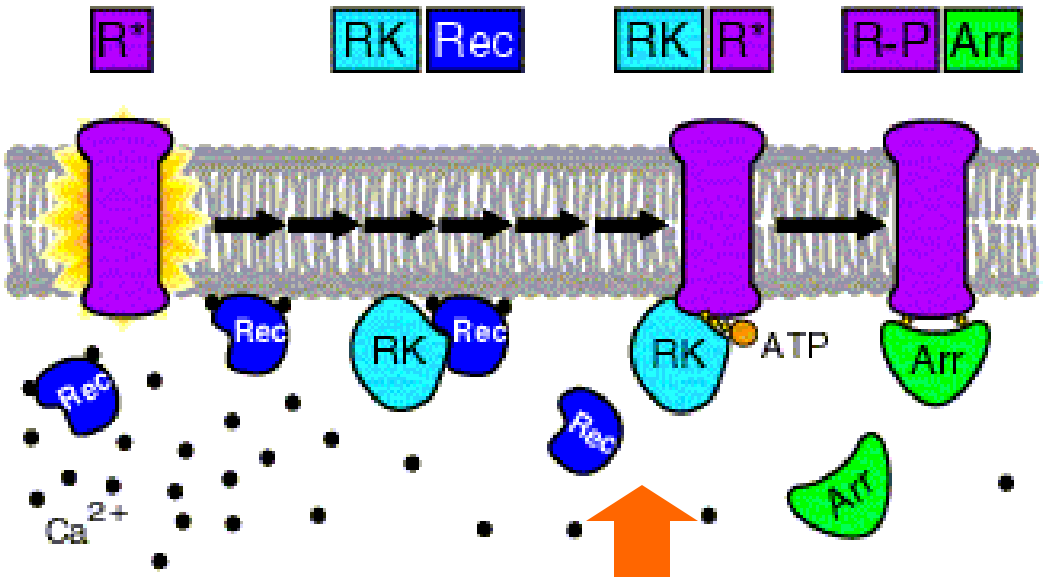
Credit: Pugh, Nikonov & Lamb



In the dark, when [Ca^{2+}] is high, most of recoverin (Rec) is in the calcium bound form at the membrane; Rec-2Ca^{2+} forms a complex bond with rhodopsin kinase (RK) blocking its activity.

Ca^{2+} feedback

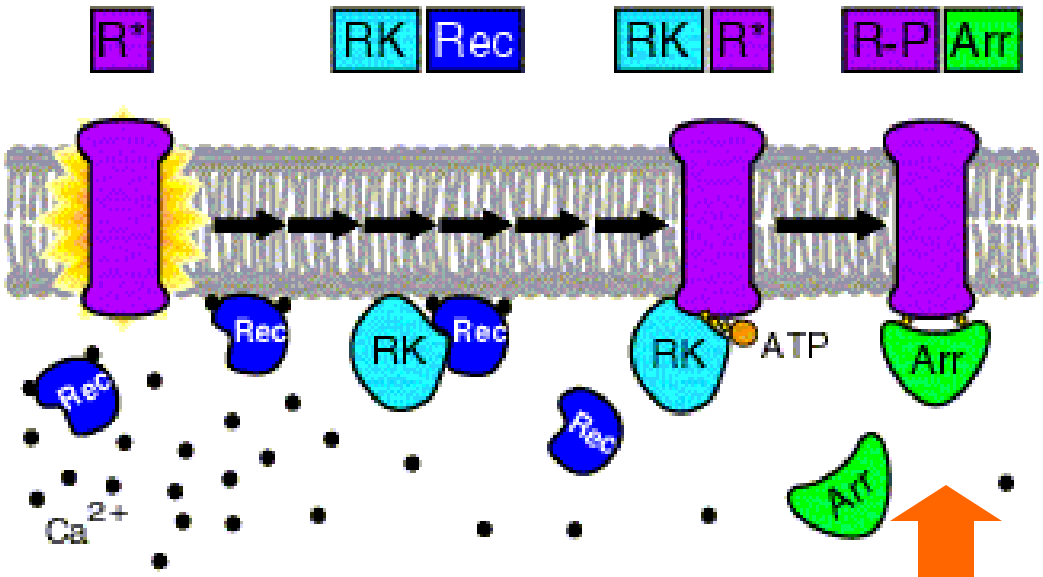
Credit: Pugh, Nikonov & Lamb



When [Ca^{2+}] drops, Ca^{2+} dissociates from Rec, which moves into solution. Free RK rapidly increases, increasing its interaction with R*, and leading to its rapid phosphorylation.

Ca^{2+} feedback

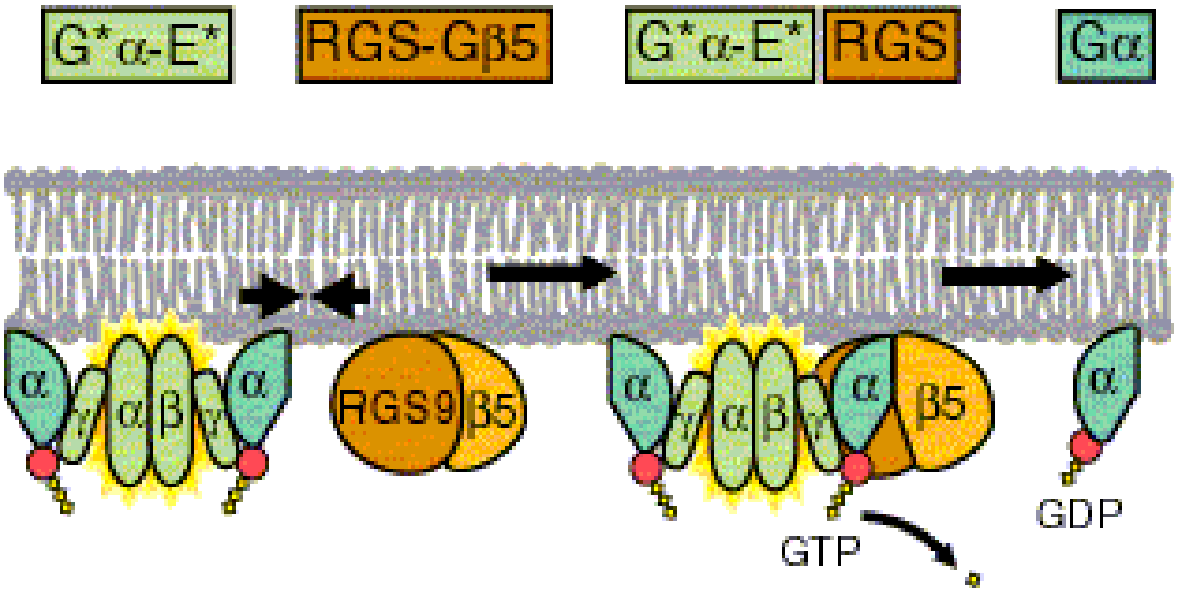
Credit: Pugh, Nikonov & Lamb



Arrestin (Arr) then binds quenching the activity of R*.

Ca^{2+} feedback

Credit: Pugh, Nikonov & Lamb

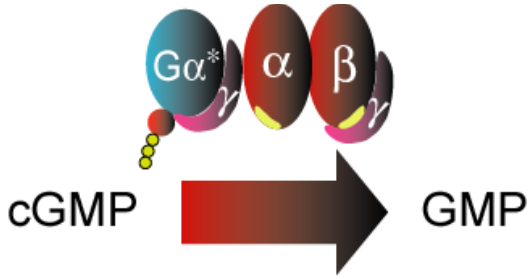


G*α-E* is inactivated when the terminal phosphate of its bound GTP is hydrolyzed, which occurs when the RGS9-Gβ5 protein binds to the complex.

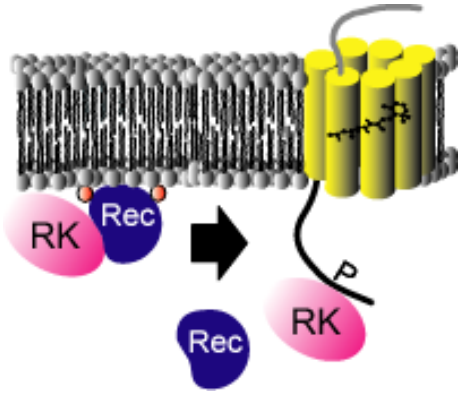
Credit: Pugh, Nikonov & Lamb

Summary of molecular adaptation mechanisms

Mechanisms that shorten the visual integration time

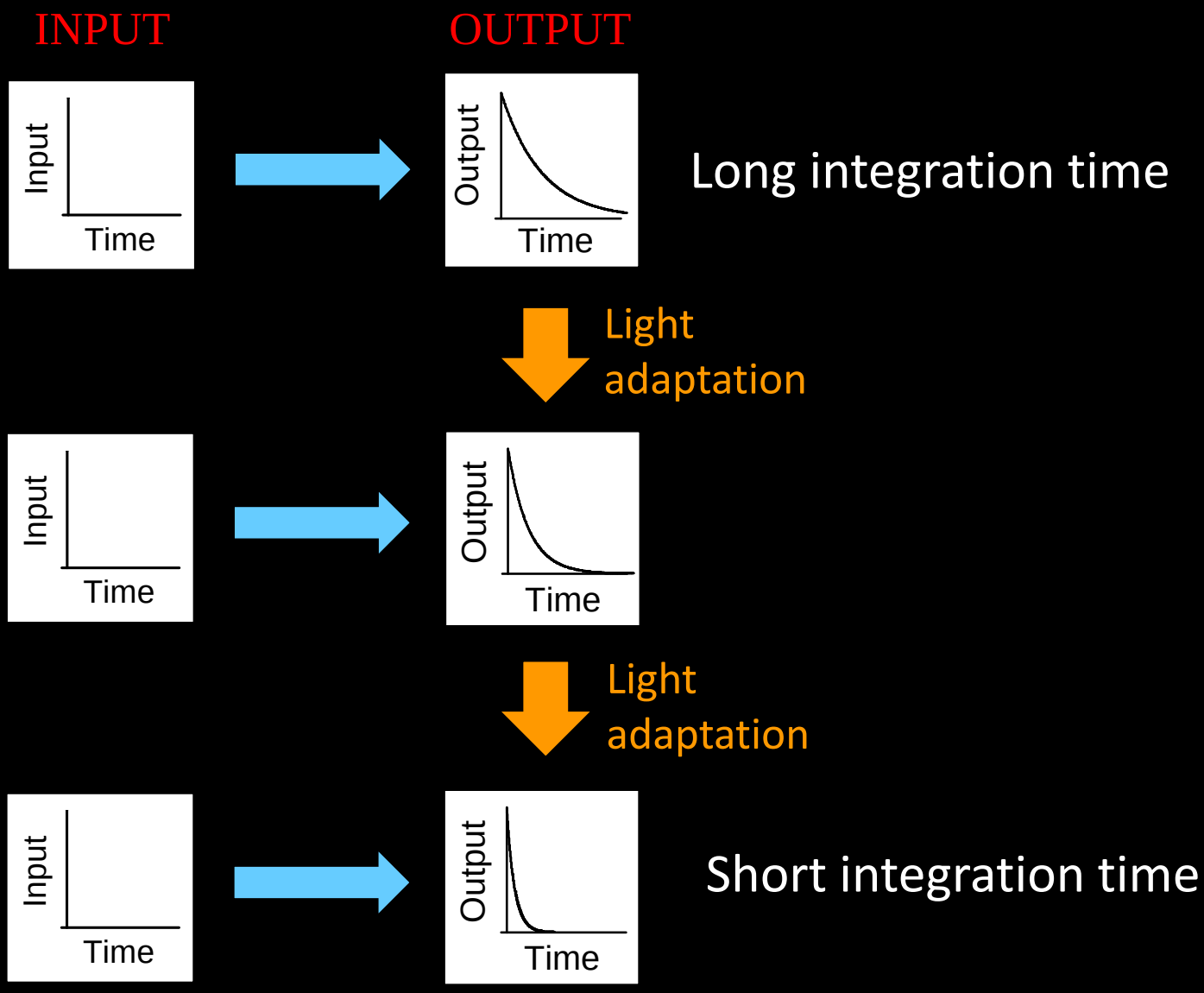


[G*α-PDE6*] dependent Increased rate of hydrolysis of cGMP to GMP



[Ca^{2+}] dependent activity of Rec

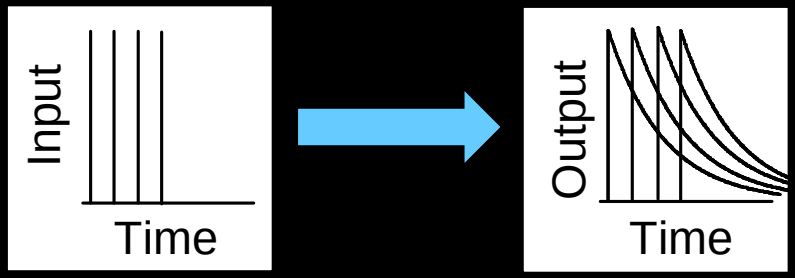
Changing the integration time of the system...



Shortening the integration time of the system and flicker sensitivity...

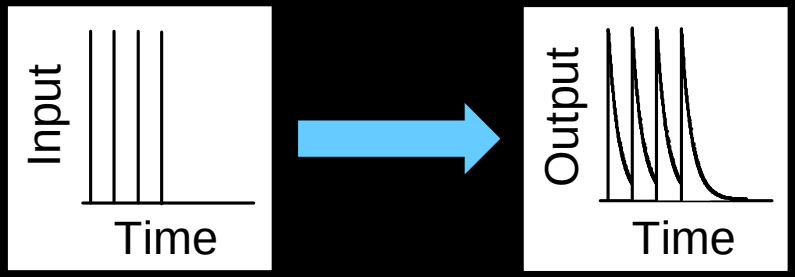
INPUT

OUTPUT



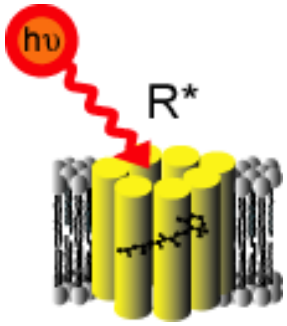
Long integration time

Light adaptation

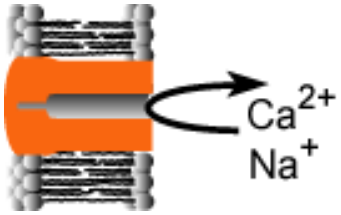


Short integration time

Mechanisms that decrease sensitivity

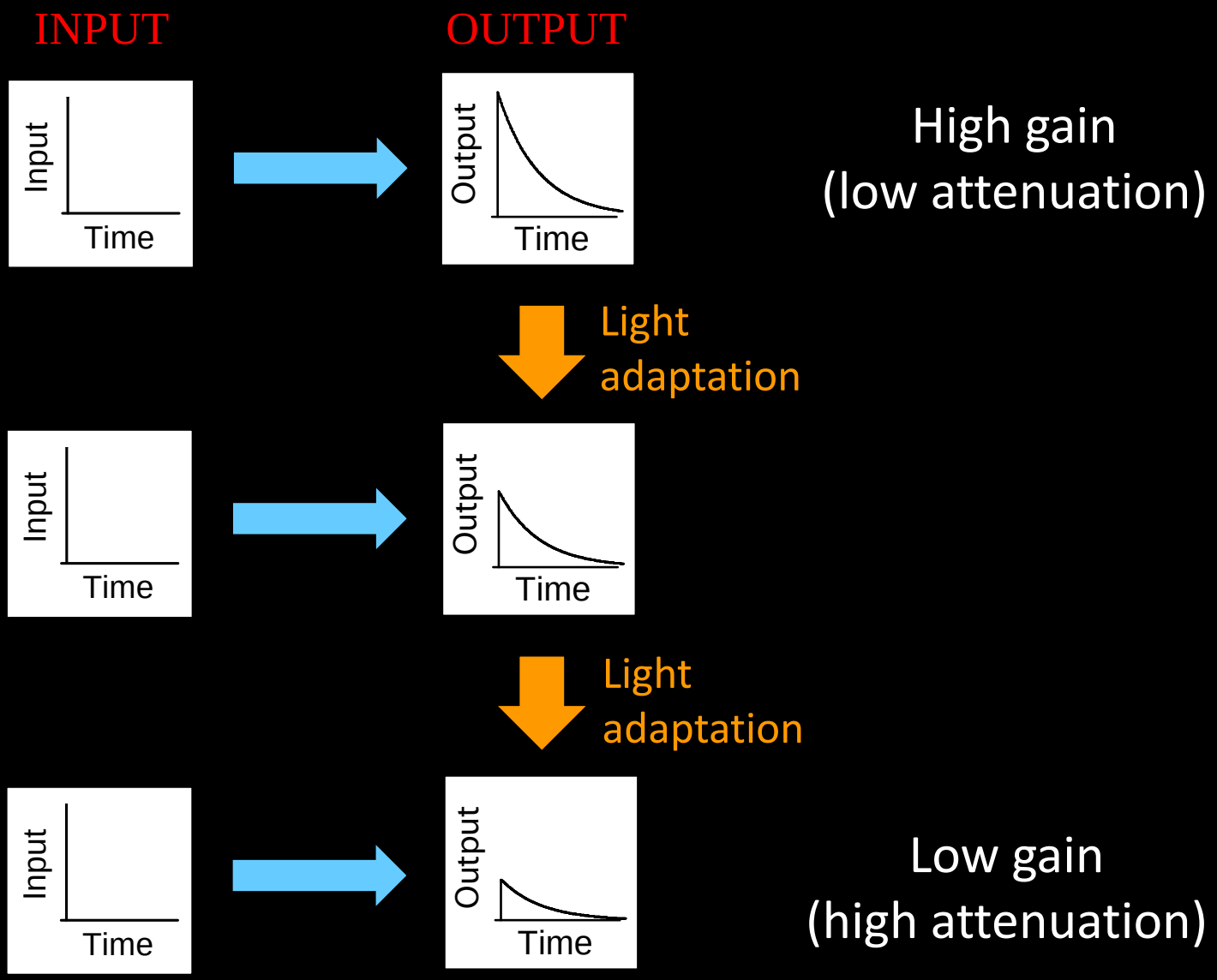


Photopigment bleaching (less photopigment available at high light levels)

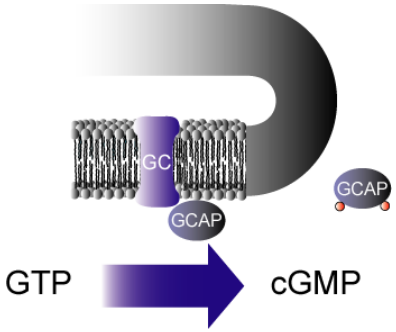


Reduction in the number of open CNG-gated channels

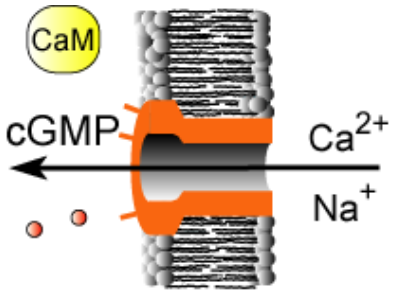
Changing the gain (attenuation) of the system...



Mechanisms that increase sensitivity (range extension)



[Ca^{2+}] dependent restoration of cGMP by GC



[Ca^{2+}] increase in CNG channels sensitivity to cGMP

Phototransduction – cones versus rods

Cones versus rods

Cones have different isoforms of:

Visual pigment, transducin, arrestin, PDE6, cGMP channel, and recoverin.

Quantitative differences. In cones:

(i) R* forms 4 times faster than for rods - faster onset of light response.

(ii) R* decays 10-50 times faster (lower amplification factor).

(iii) GTPase activating protein (RGS-Gβ5) expressed at much higher levels - shorter G*α (activated transducin) lifetime - faster recovery.

(iv) Clearance of Ca^{2+} from cone outer segments is several times faster than for rods.

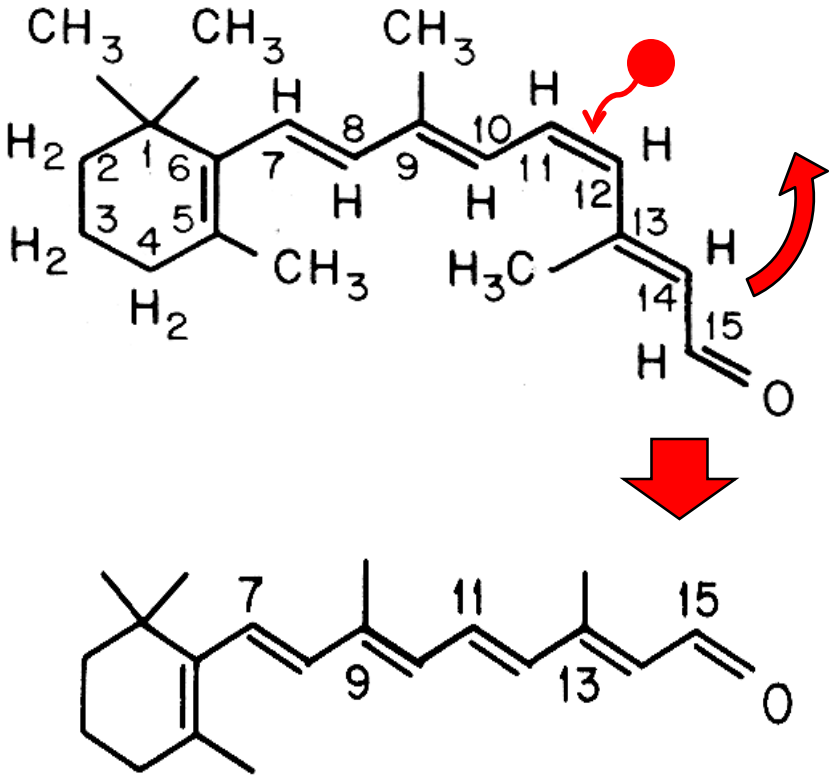
(v) cGMP channels in cones are twice as permeable to Ca^{2+} than in rods.

Cones versus rods

- Cones are 25 - 100 times less sensitive to single photons.
- They catch fewer photons (less visual pigment).
- They respond with faster kinetics (isoforms of transduction cascade).
- They have a much greater ability to adapt to background light.
- They do not saturate at normal environmental light levels.

Photopigments and spectral tuning

Chromophore



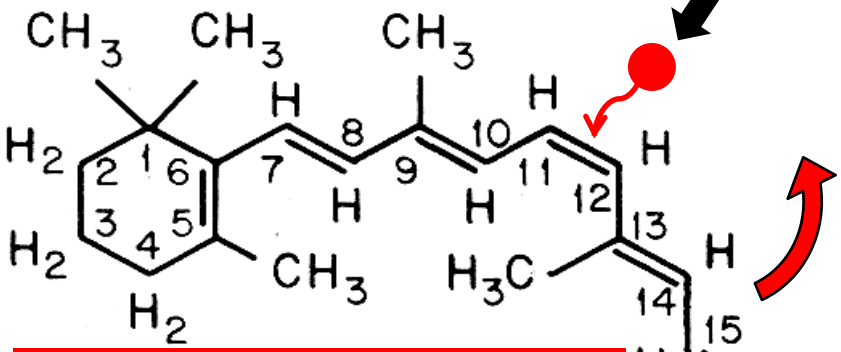
How can this process encode information about wavelength?

Can it?

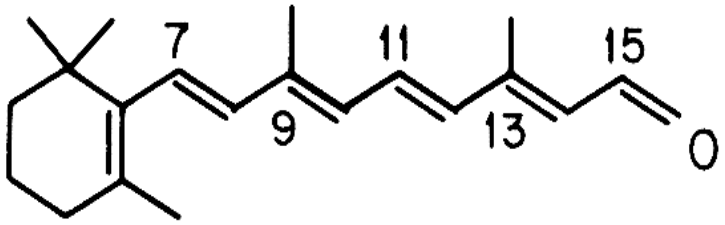
Chromophore

(*chromo-* colour, + *-phore,* producer)
Light-catching portion of any molecule

11-*cis* retinal

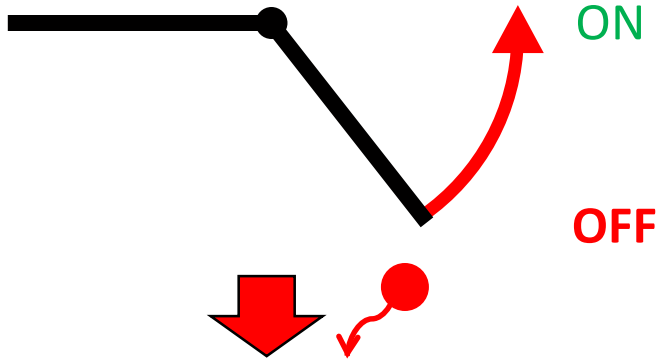


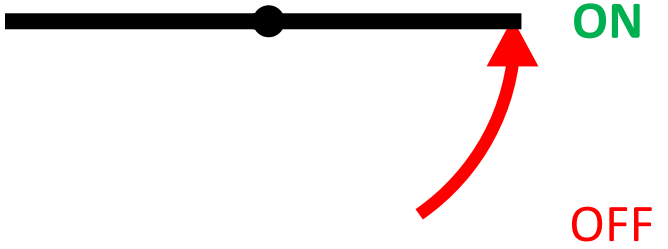
the energy of which initiates a conformational change to…



all-*trans* retinal

Think of the molecule as a photo-sensitive switch!



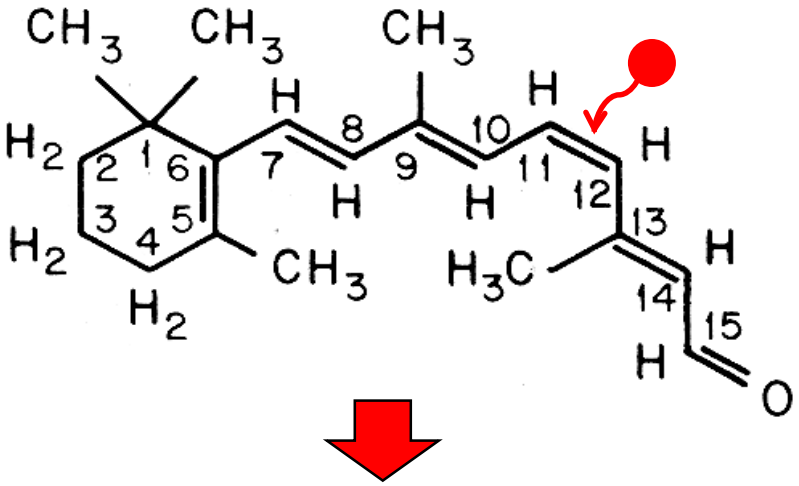


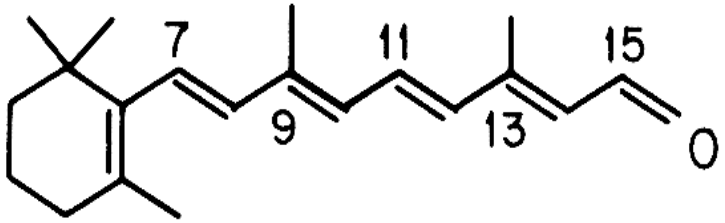
Chromophore

Crucially, the event is binary or “all or nothing”.

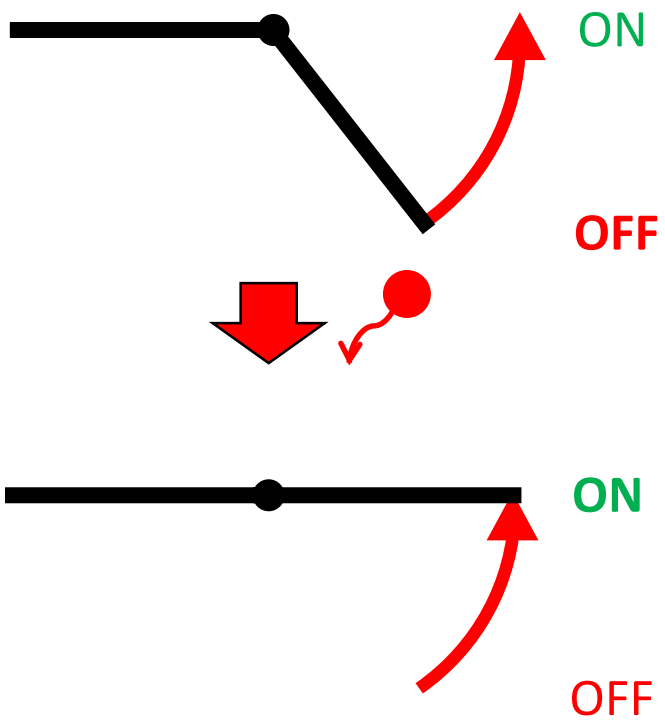
If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.

11-*cis* retinal





all-*trans* retinal

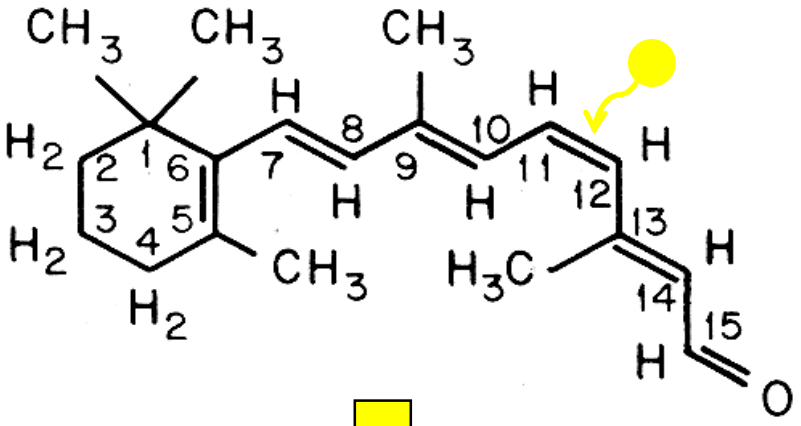


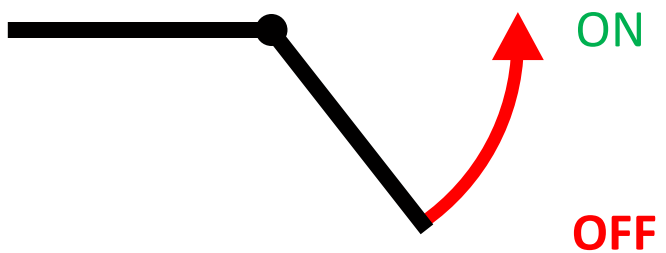
Chromophore

Crucially, the event is binary or “all or nothing”.

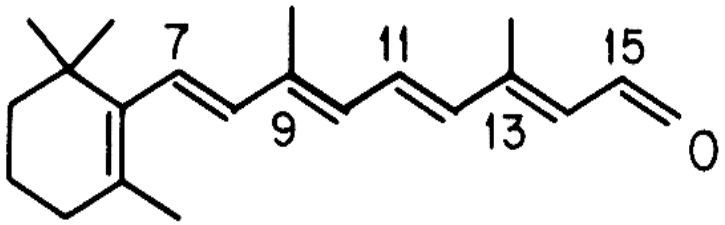
If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.

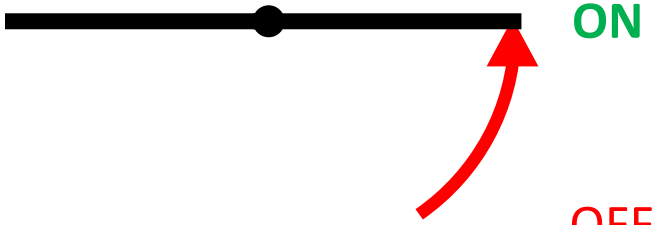
11-*cis* retinal





SAME EFFECT





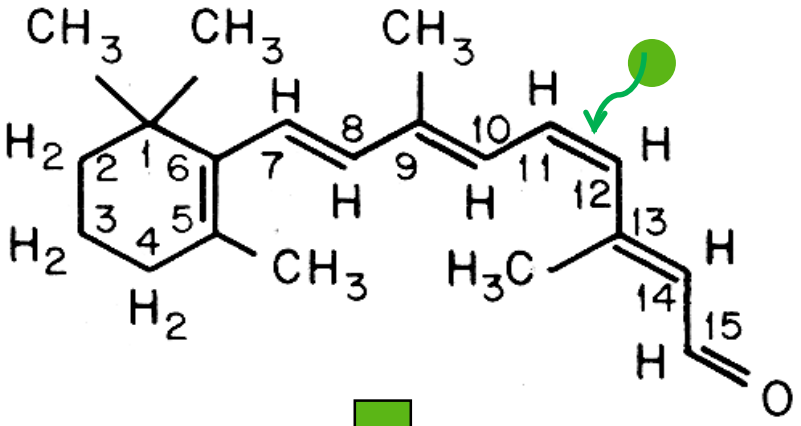
all-*trans* retinal

Chromophore

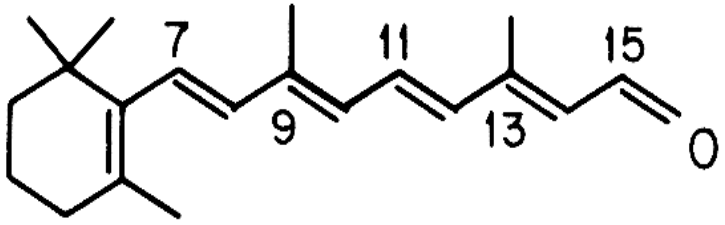
Crucially, the event is binary or “all or nothing”.

If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.

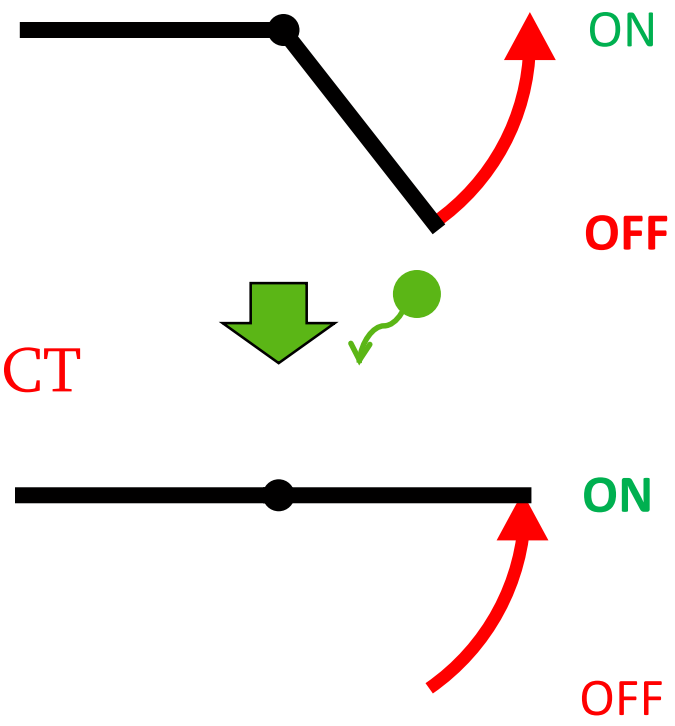
11-*cis* retinal



SAME EFFECT



all-*trans* retinal

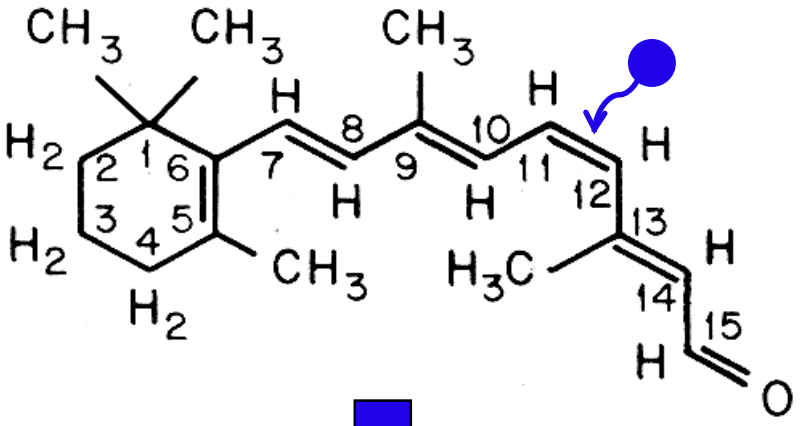


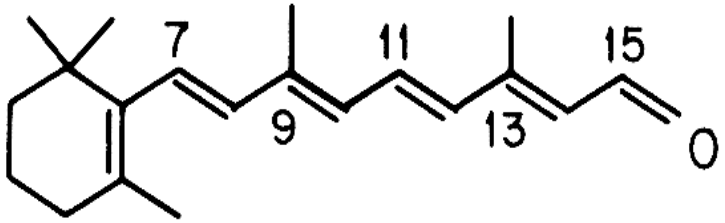
Chromophore

Crucially, the event is binary or “all or nothing”.

If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.

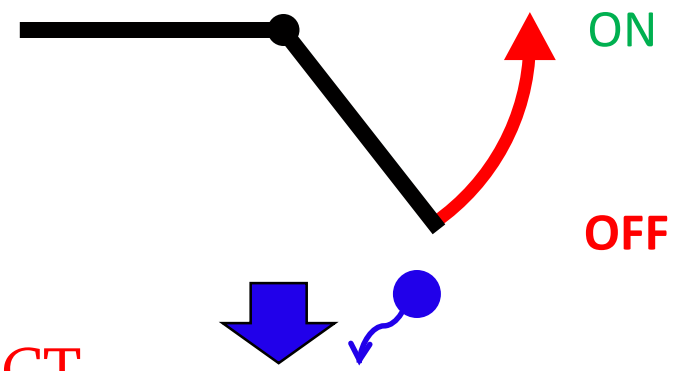
11-*cis* retinal

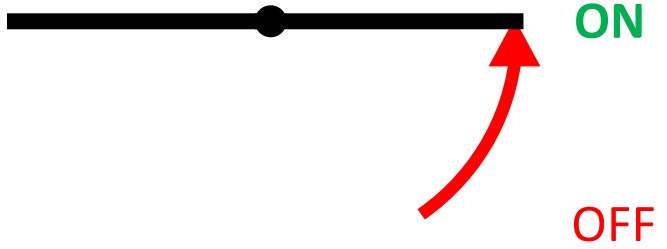




all-*trans* retinal

SAME EFFECT



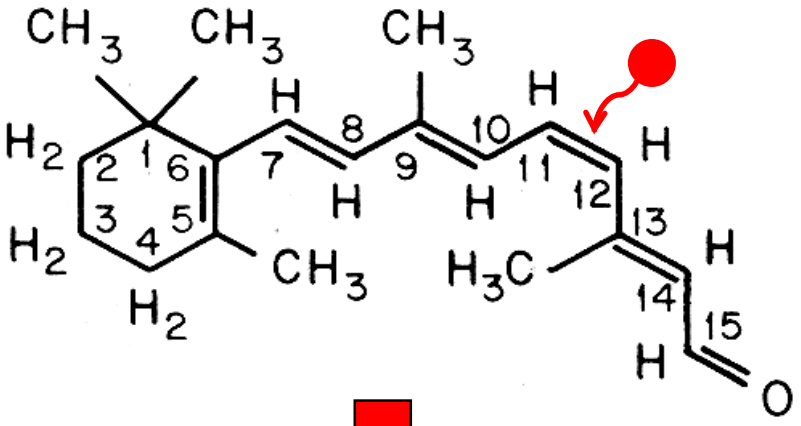


Chromophore

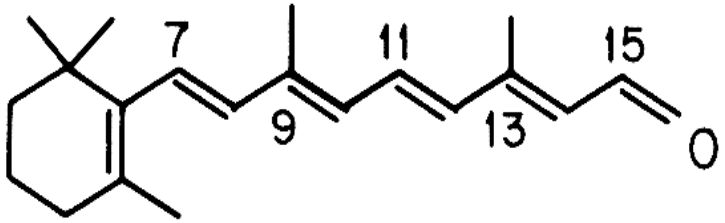
Crucially, the event is binary or “all or nothing”.

If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.

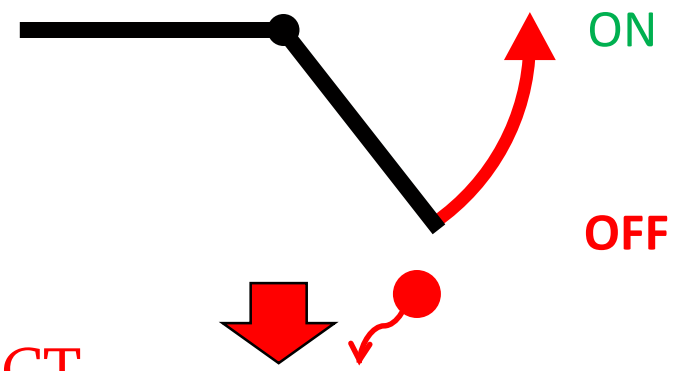
11-*cis* retinal

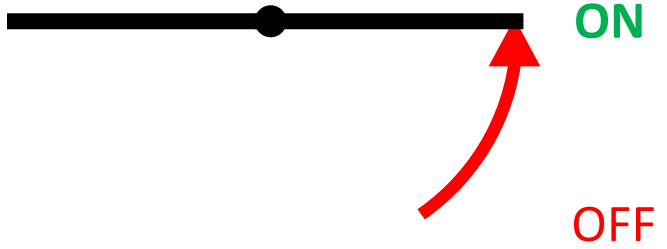


SAME EFFECT



all-*trans* retinal

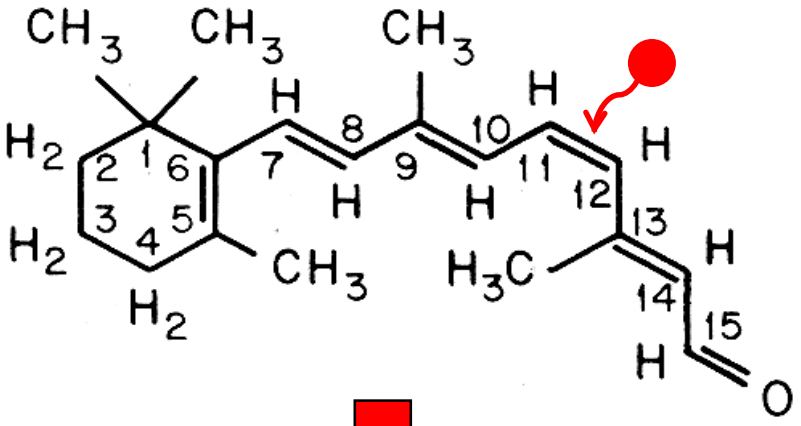


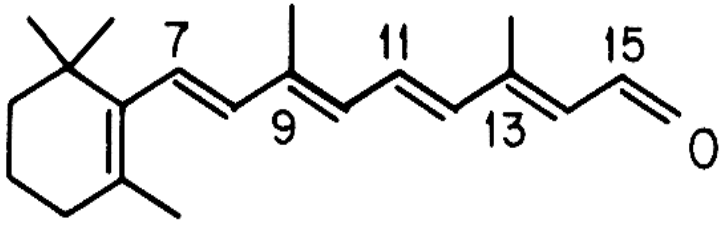


Chromophore

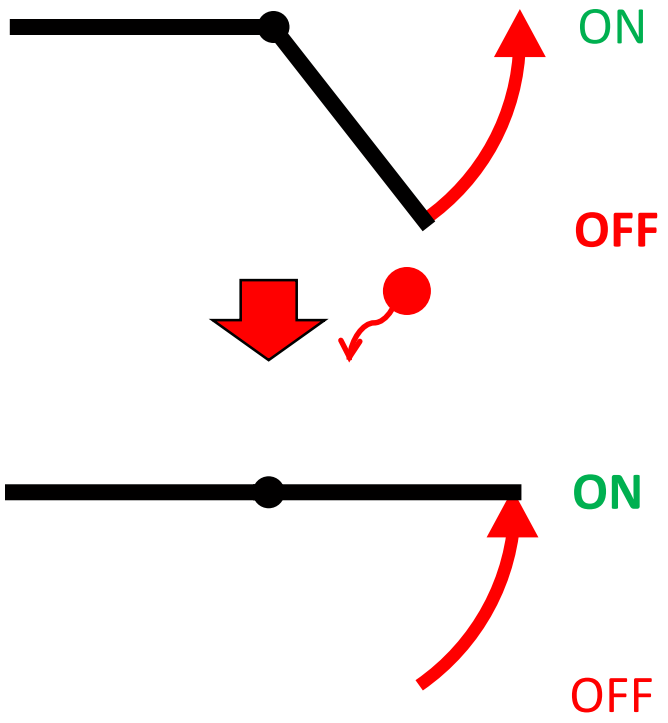
Can this process encode wavelength (colour)?

11-*cis* retinal





all-*trans* retinal

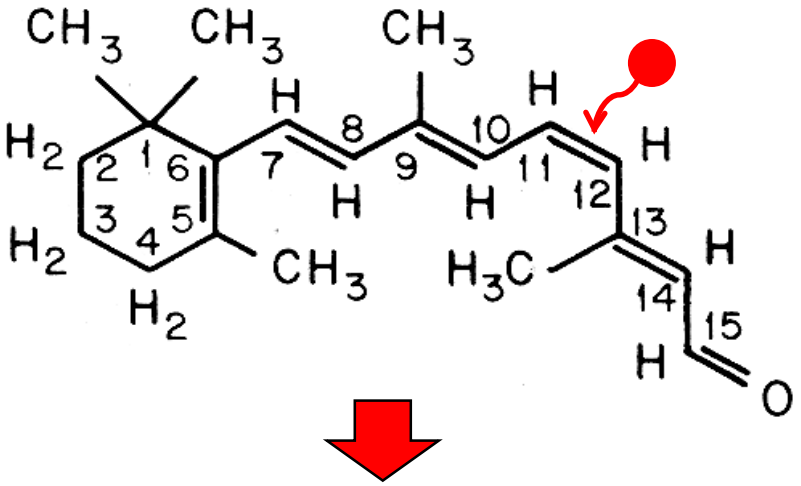


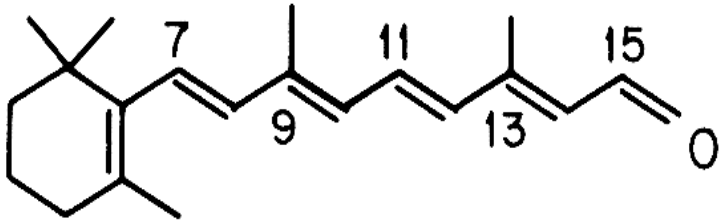
Chromophore

No, it cannot encode wavelength (colour)!

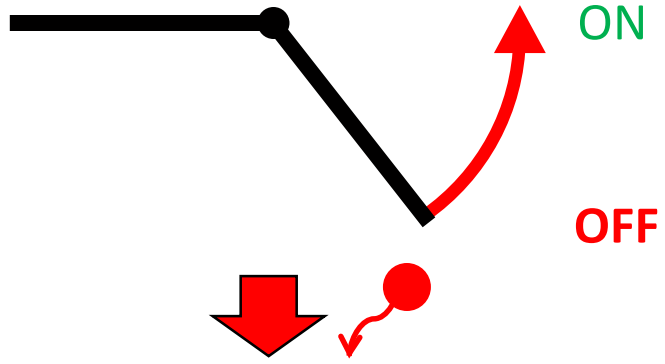
It is "UNIVARIANT"

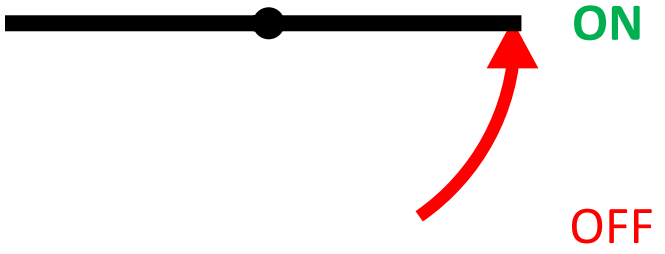
11-*cis* retinal





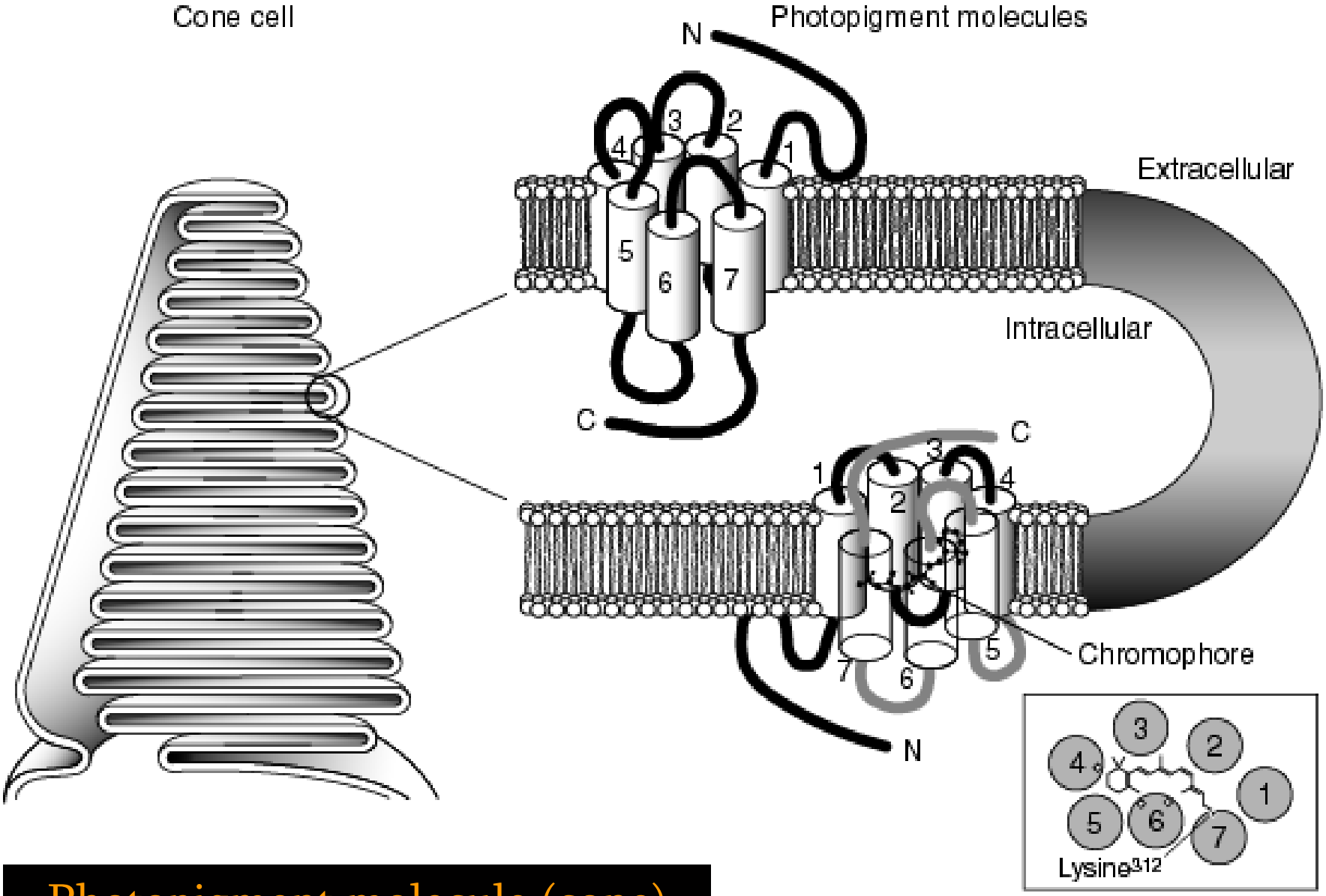
all-*trans* retinal





So, how do we see colours?

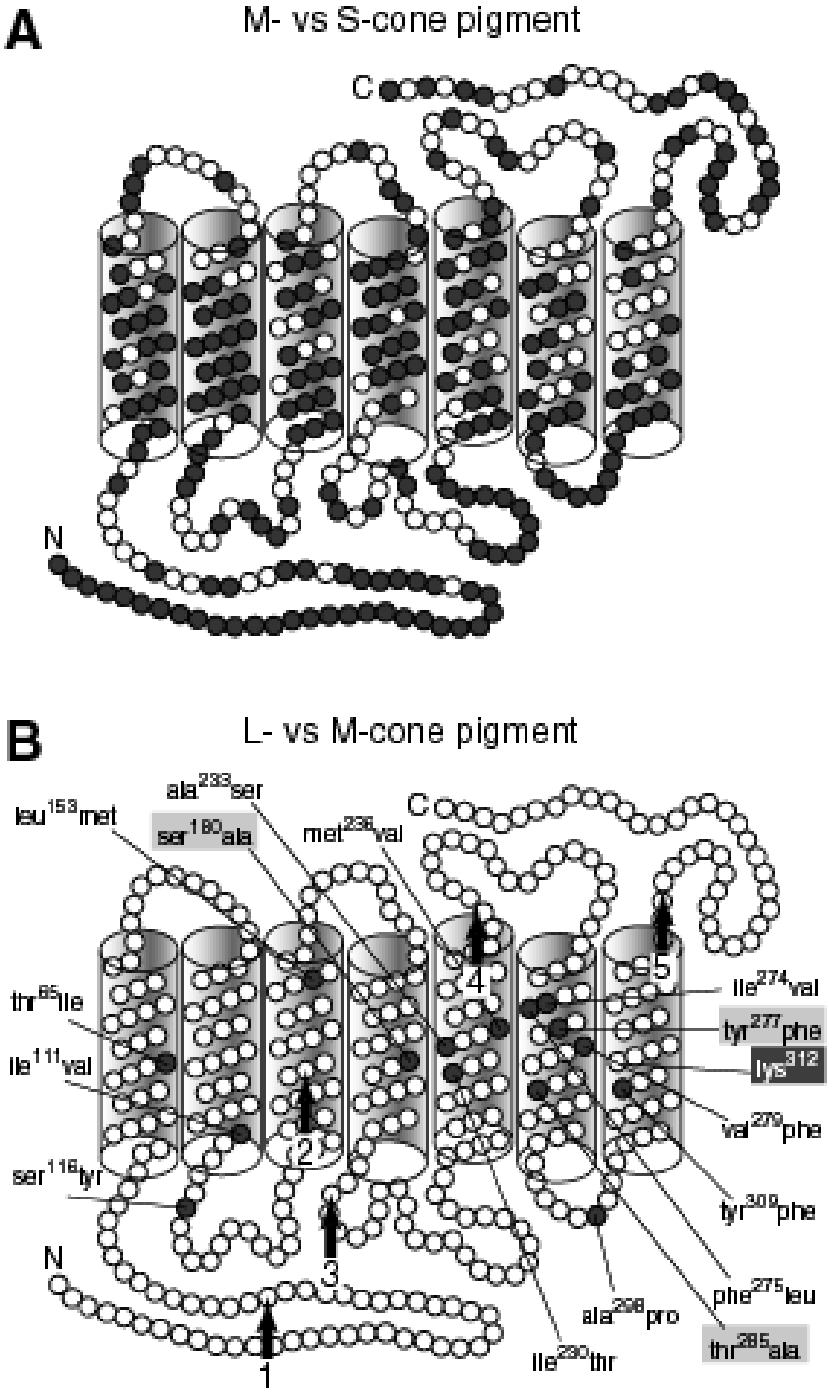
COVERED IN NEXT LECTURE…



Photopigment molecule (cone)

From Sharpe, Stockman, Jägle & Nathans, 1999

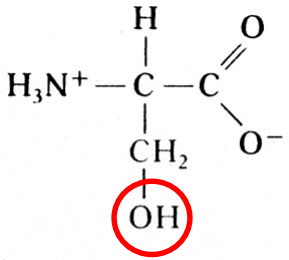
Opsin differences



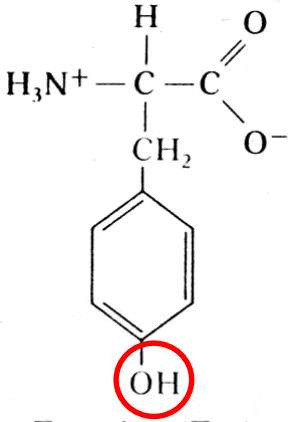
15 amino acid differences - 96% identical

From Sharpe, Stockman, Jägle & Nathans, 1999

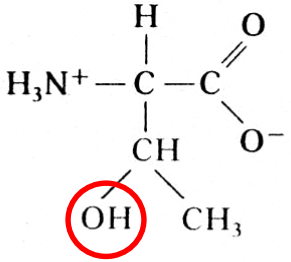
POLAR



Serine (Ser)



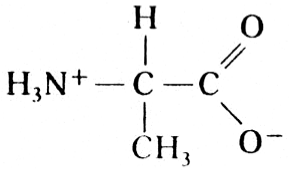
Tyrosine (Tyr)



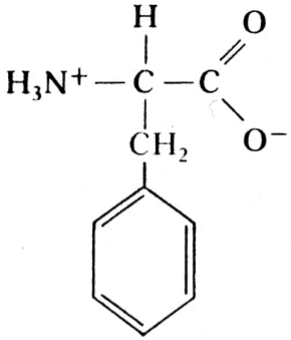
Threonine (Thr)

LWS
all with OH group

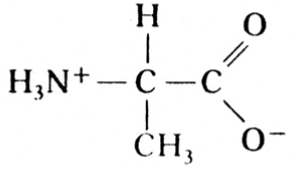
NON-POLAR



Alanine (Ala)



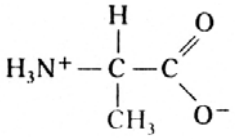
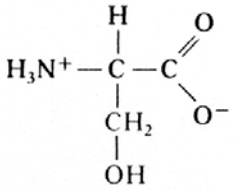
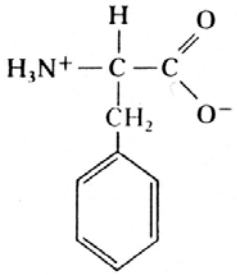
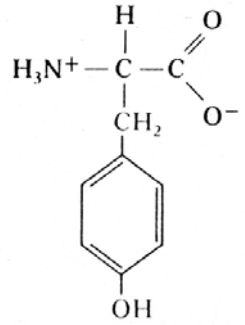
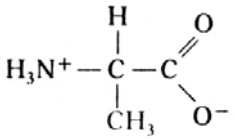
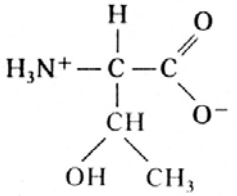
Phenylalanine (Phe)



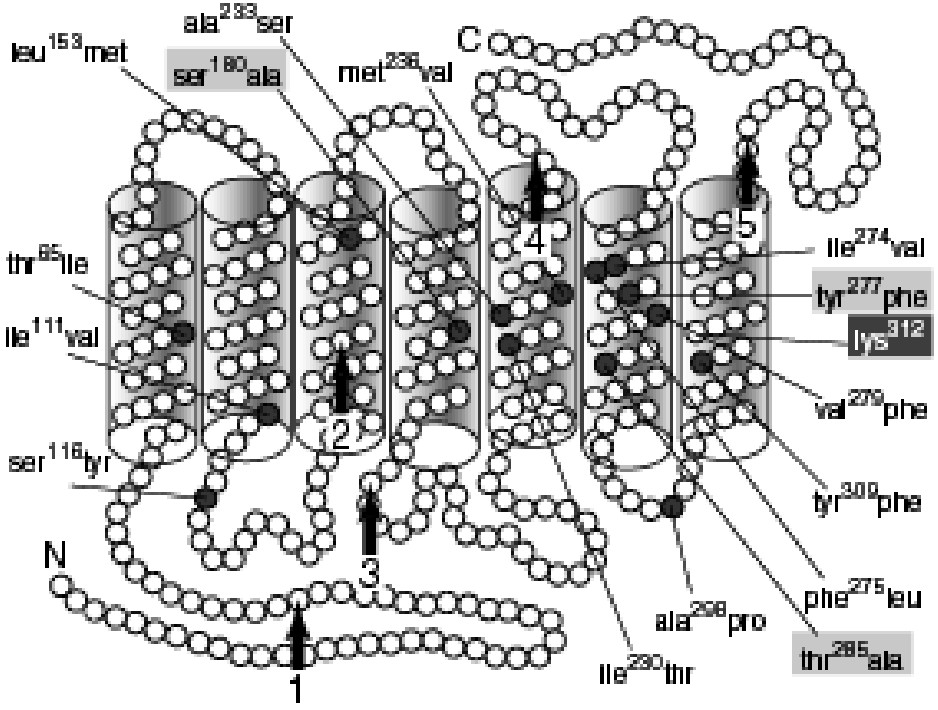
Alanine (Ala)

MWS

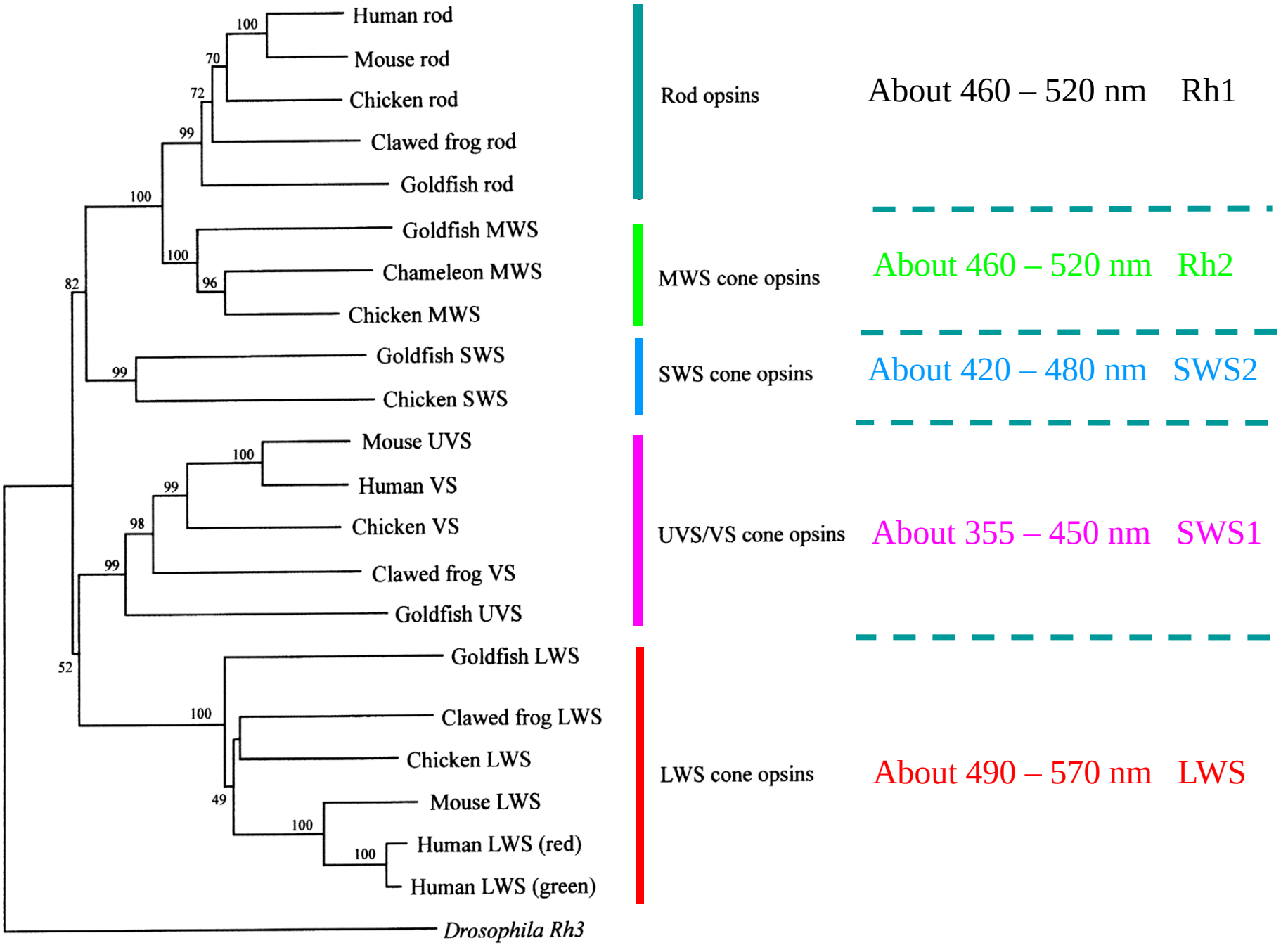
180 277 285

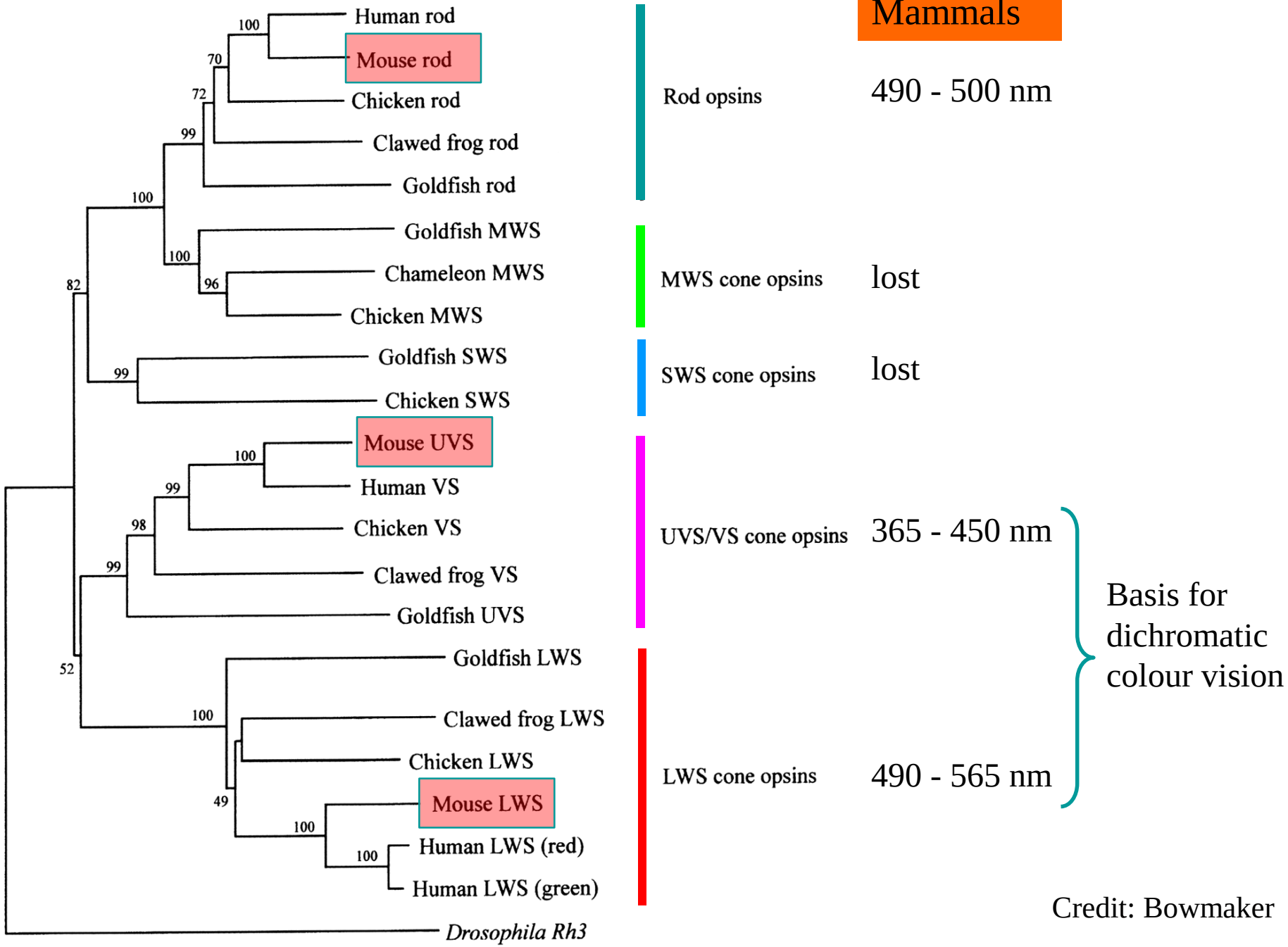
Tuning Site	MWS	LWS	
180	alanine	serine $^{OH-}$	~ 5 nm
			
277	phenylalanine	tyrosine $^{OH-}$	~ 25 nm
			
285	alanine	threonine $^{OH-}$	
			

Why are there so few differences between the M- and L-cone opsin genes?

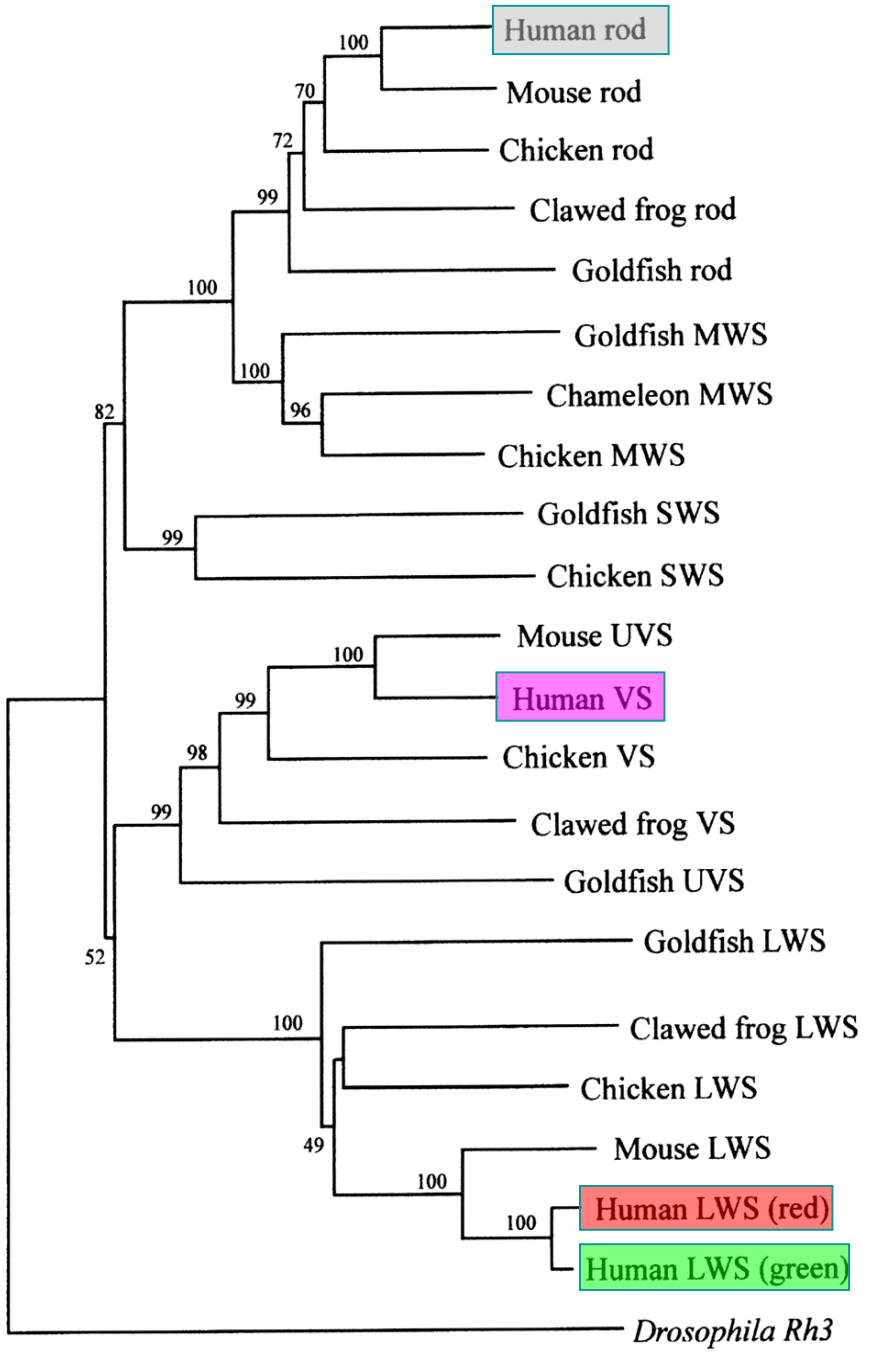


Phylogenetic tree of visual pigments





Credit: Bowmaker



Rod opsins

MWS cone opsins

SWS cone opsins

UVS/VS cone opsins

LWS cone opsins

Humans

490 - 500 nm

lost

lost

365 - 450 nm

Basis for trichromatic colour vision

Gene duplication

Credit: Bowmaker

The emergence of two longer wavelength (M- and L-cones) is thought to have occurred relatively recently in primate evolution.

Why is it important?

No red-green discrimination

Source: Hans Irtel

Red-green discrimination

Source: Hans Irtel

Four human photoreceptors have different spectral sensitivities