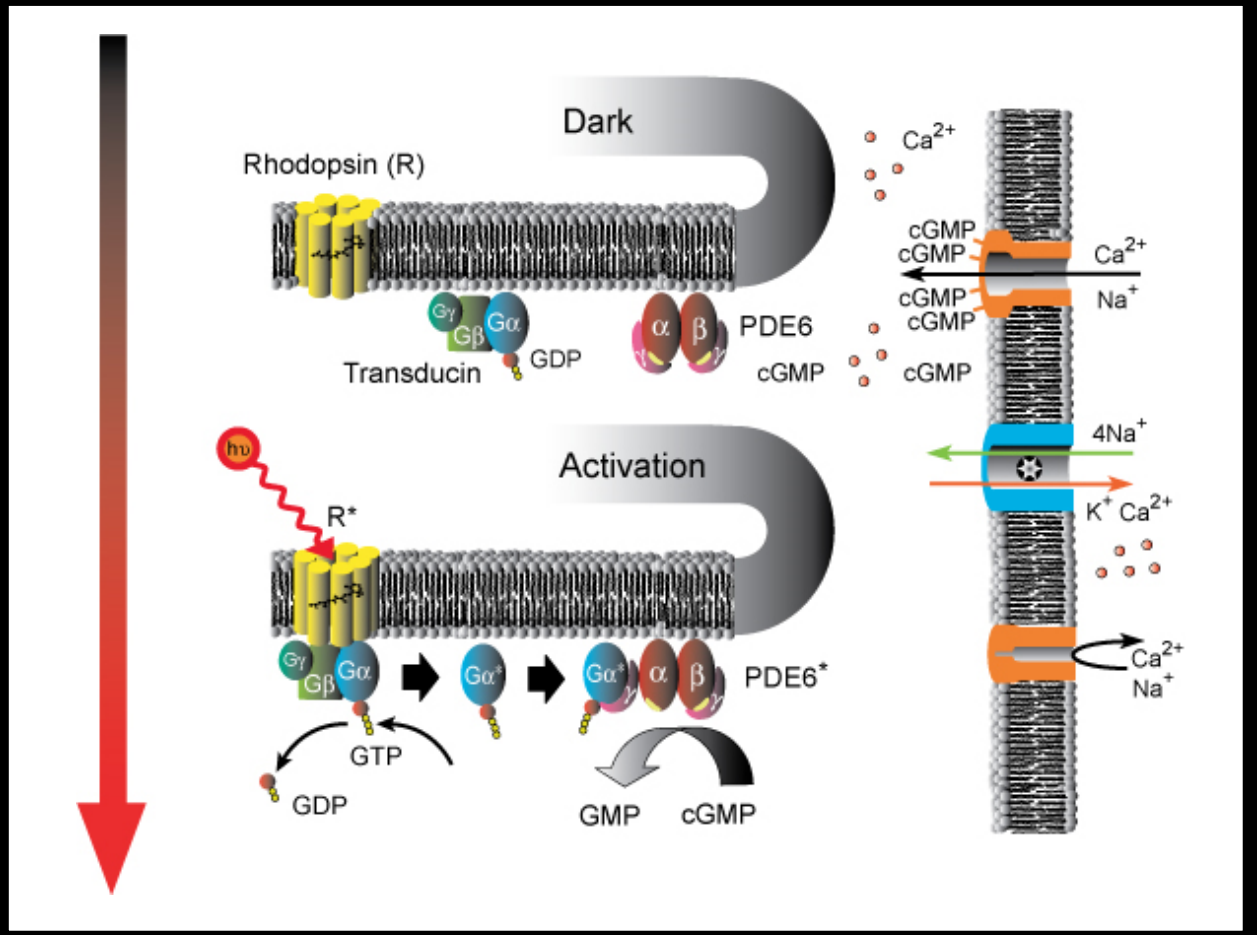
Photoreceptors and phototransduction

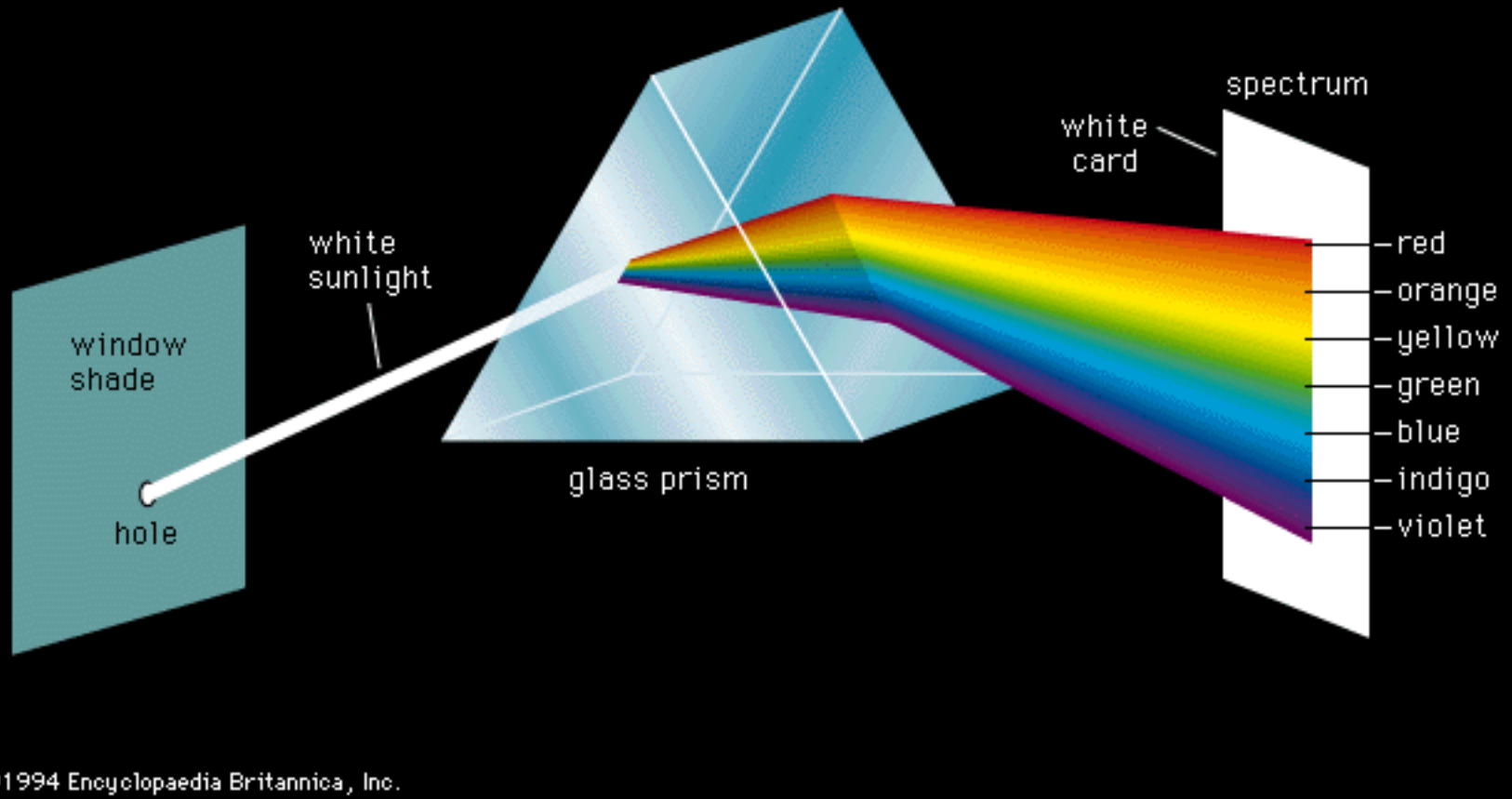


Andrew Stockman

Background

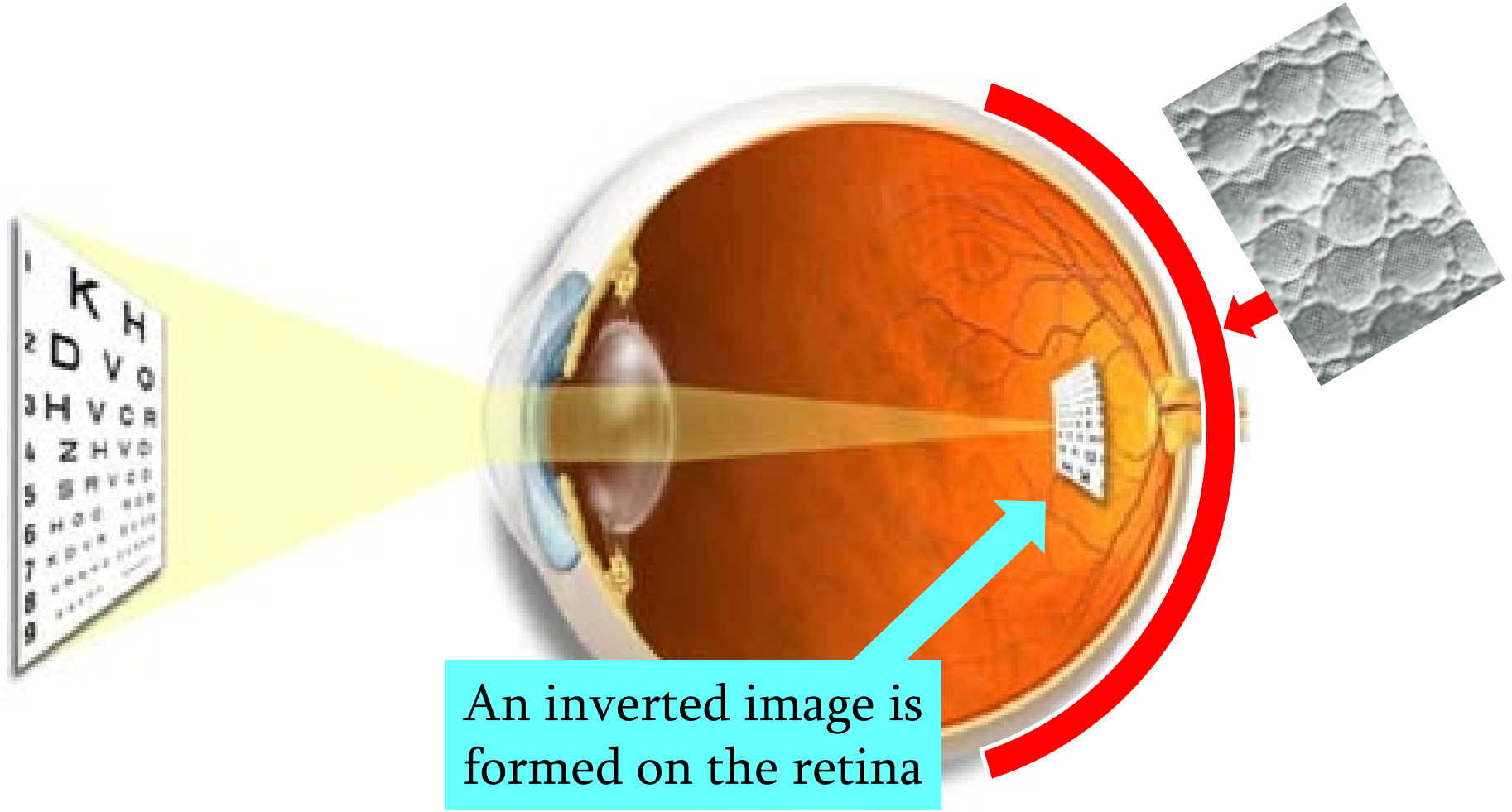
Light

400 - 700 nm is important for vision



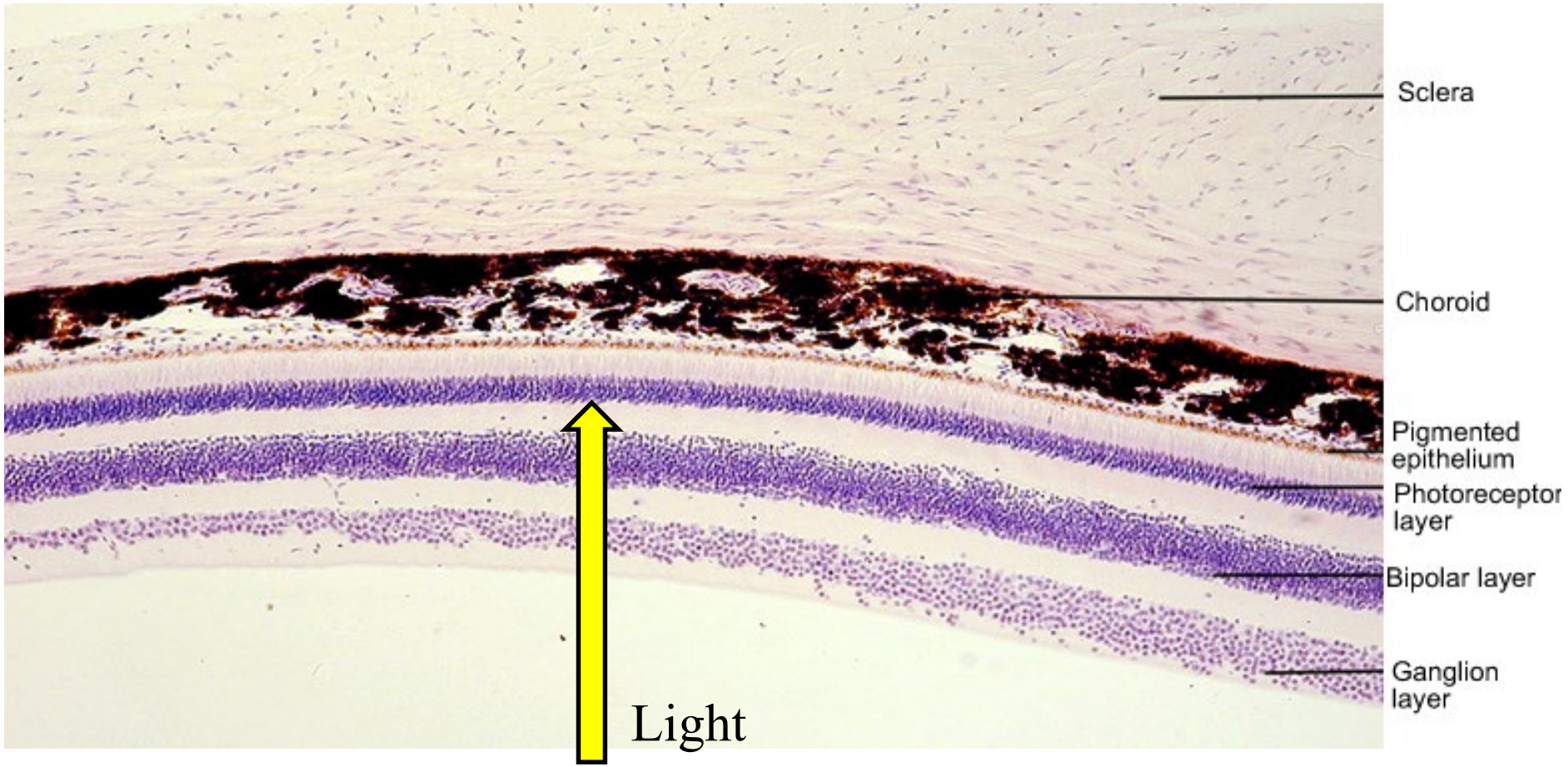
The eye

The retina is carpeted with light-sensitive rods and cones



An inverted image is formed on the retina

Retinal cross-section



Retina 200 ×

Rods and cones

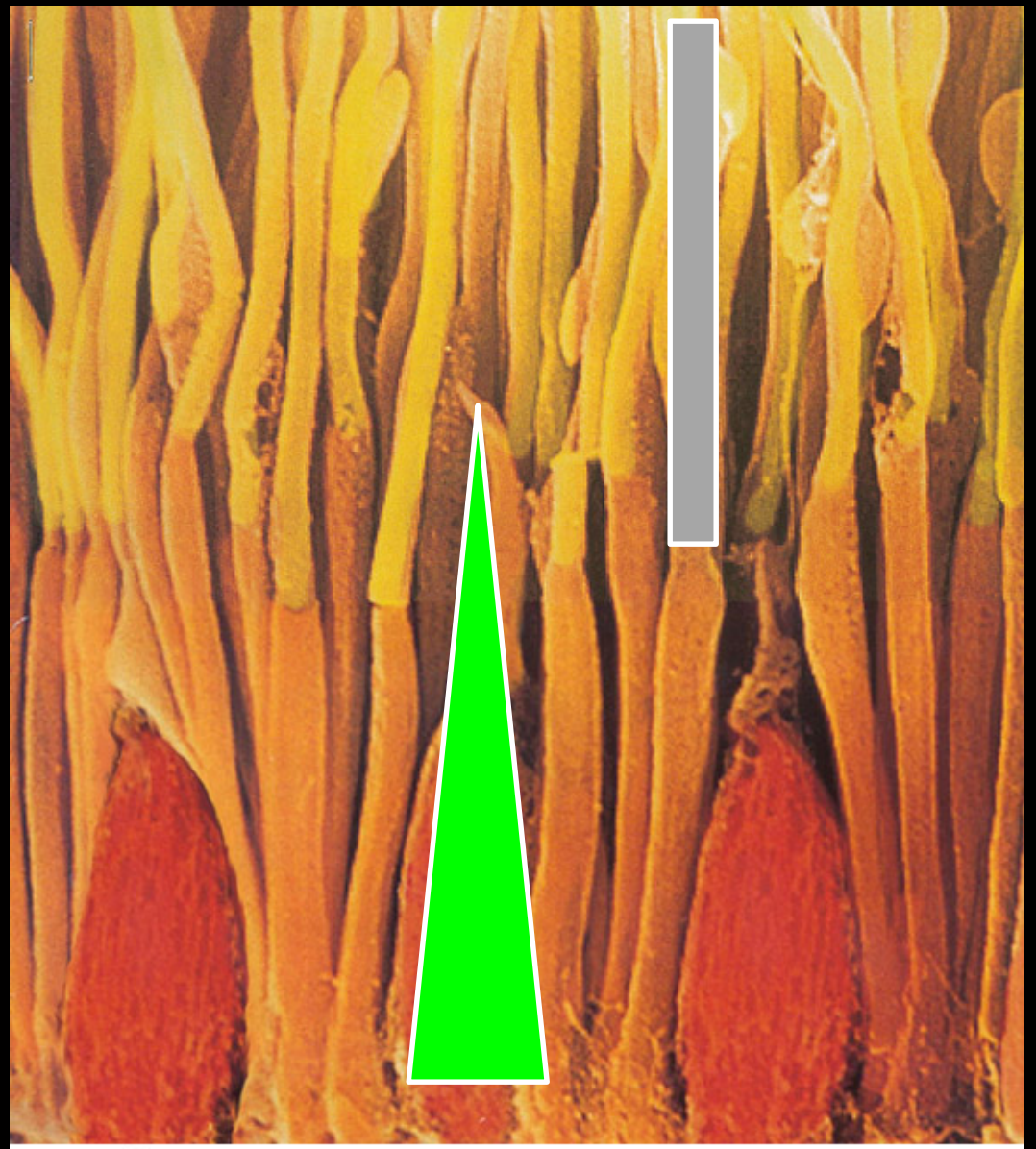
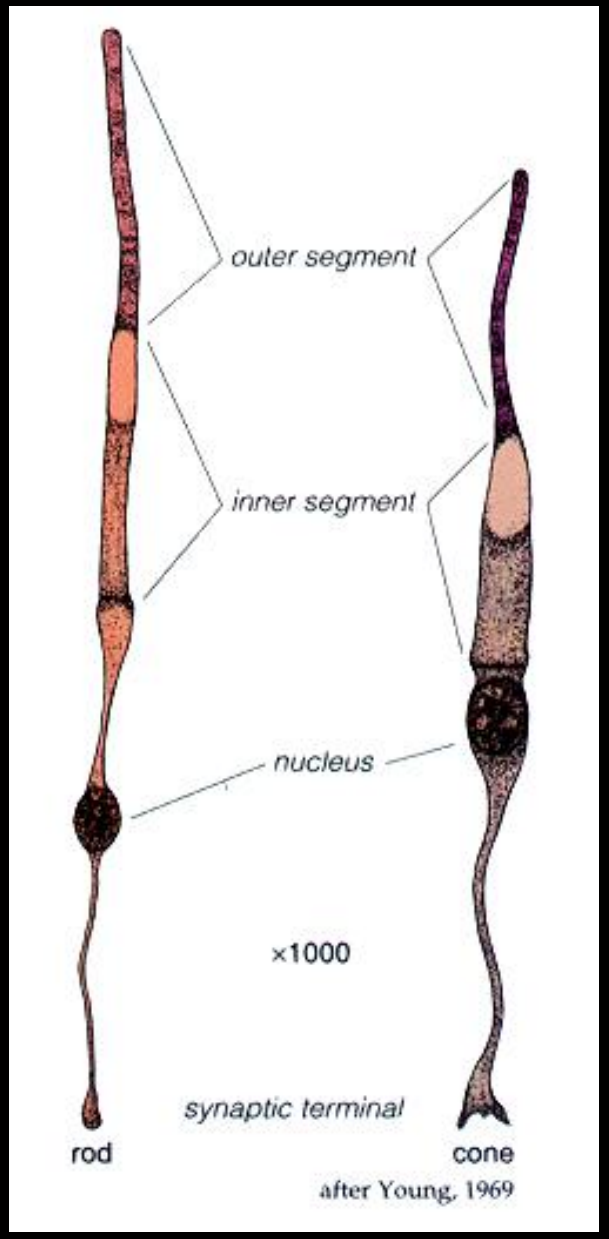


Fig1b. Scanning electron micrograph of the rods and cones of the primate retina. Image adapted from one by Ralph C. Eagle/Photo Researchers, Inc.



Human photoreceptors

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

Rod

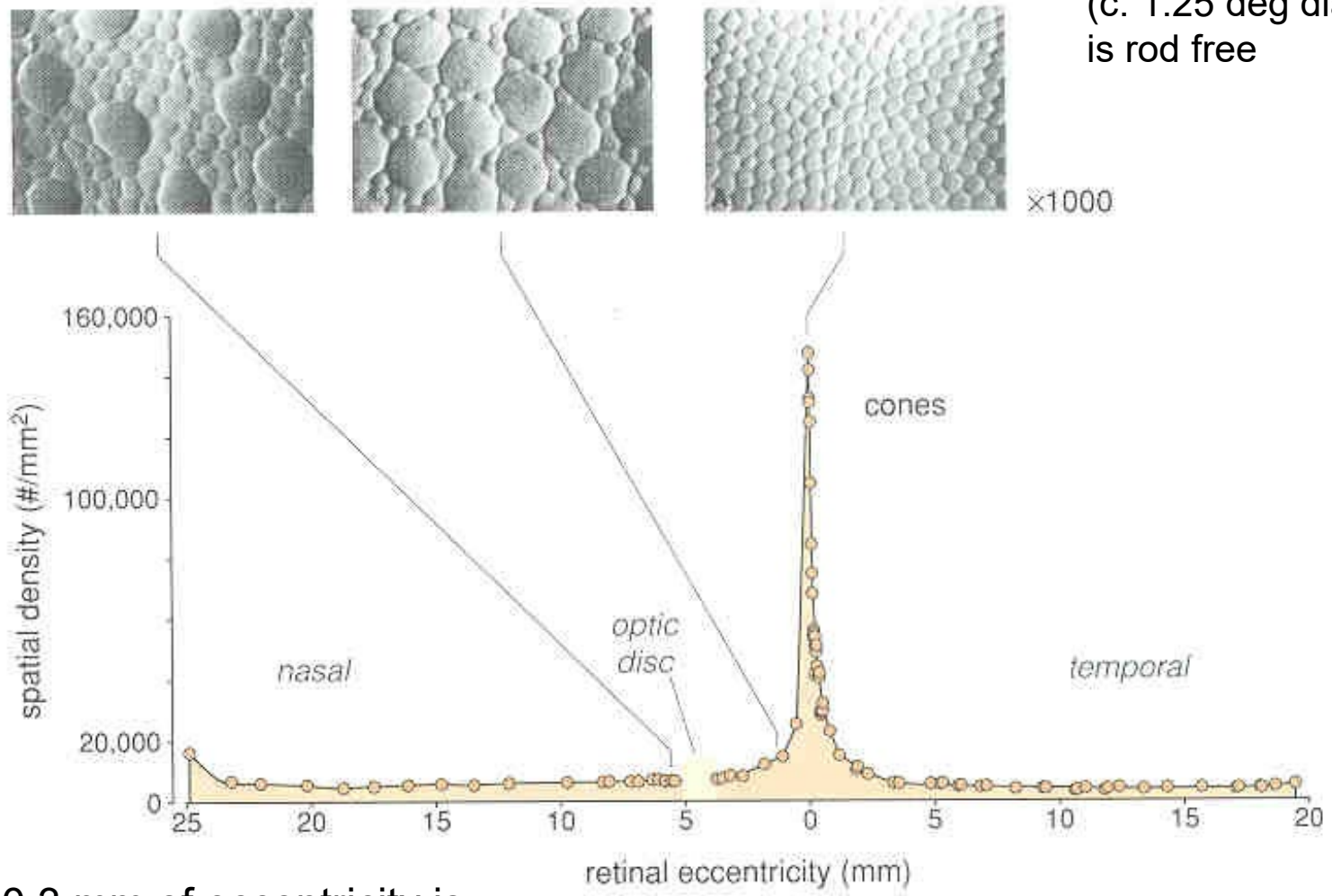
Long-wavelength-sensitive (L) or "red" cone

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

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

Human photoreceptor mosaics

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

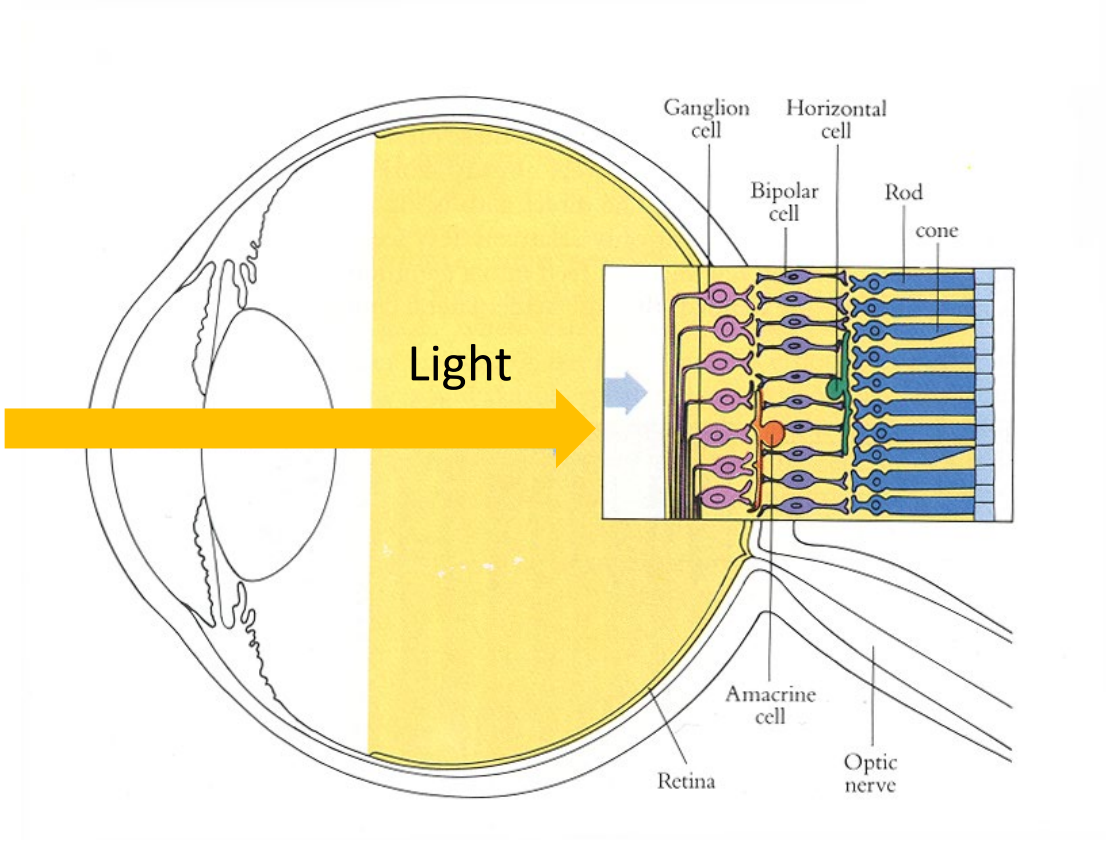


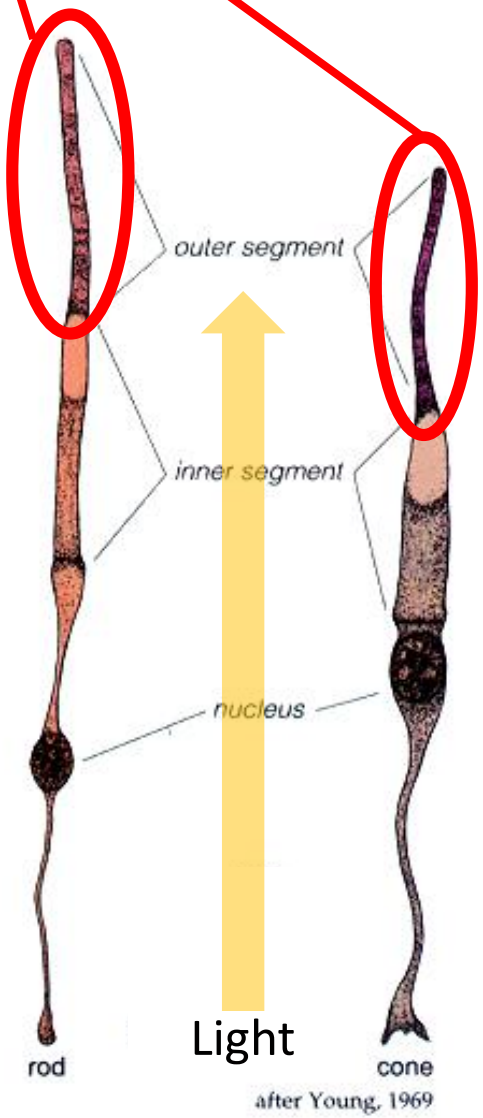
0.3 mm of eccentricity is about 1 deg of visual angle

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

Light and the eye

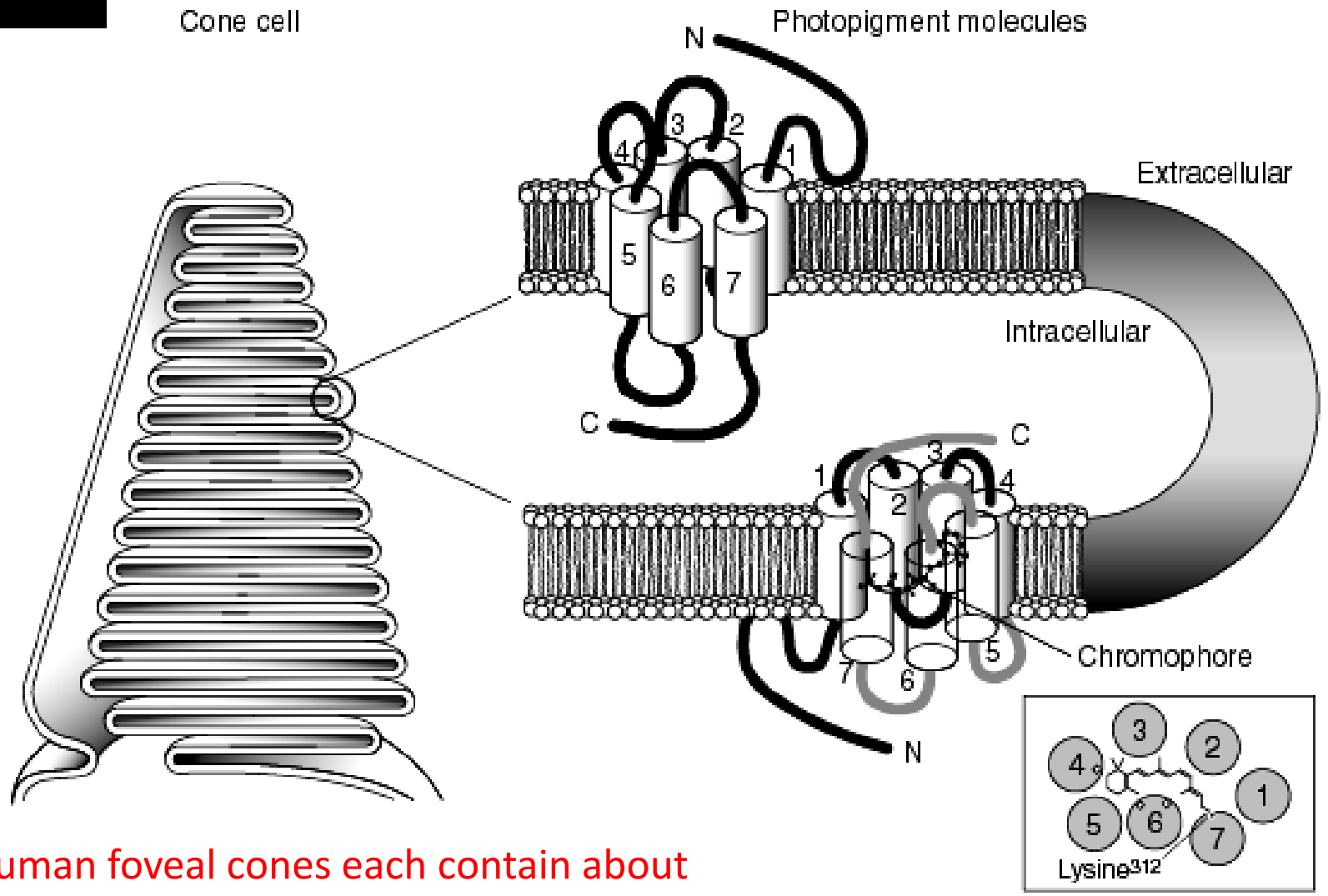
The light-sensitive photopigment lies inside the rod and cone outer segments.





Arrangement of visual pigment molecules

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

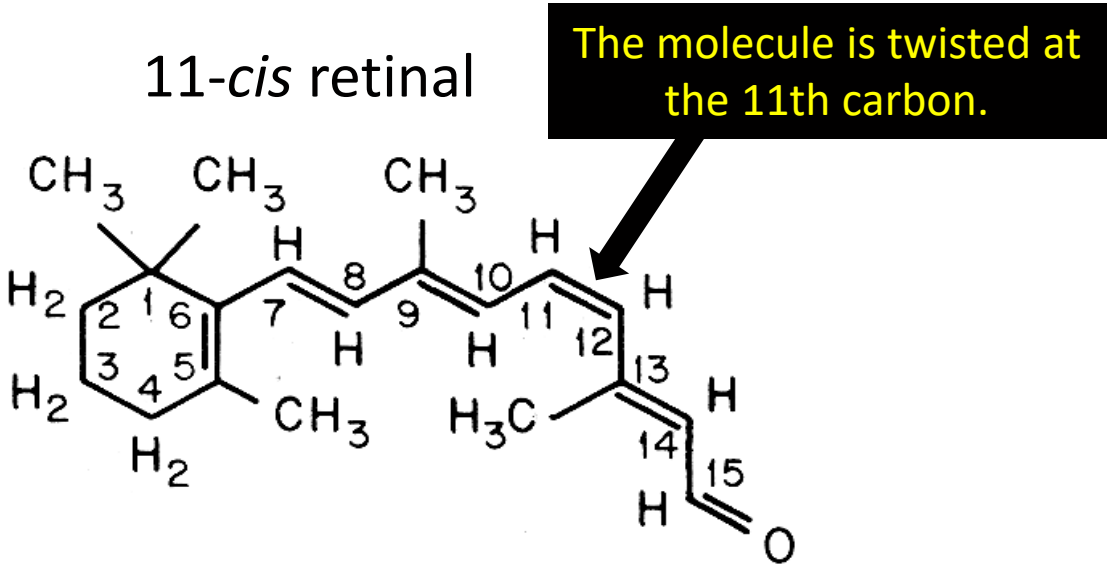


Human foveal cones each contain about 10^{10} visual pigment molecules

Chromophore

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

11-*cis* retinal



Chromophore

(*chromo-* colour, + *-phore,* producer)
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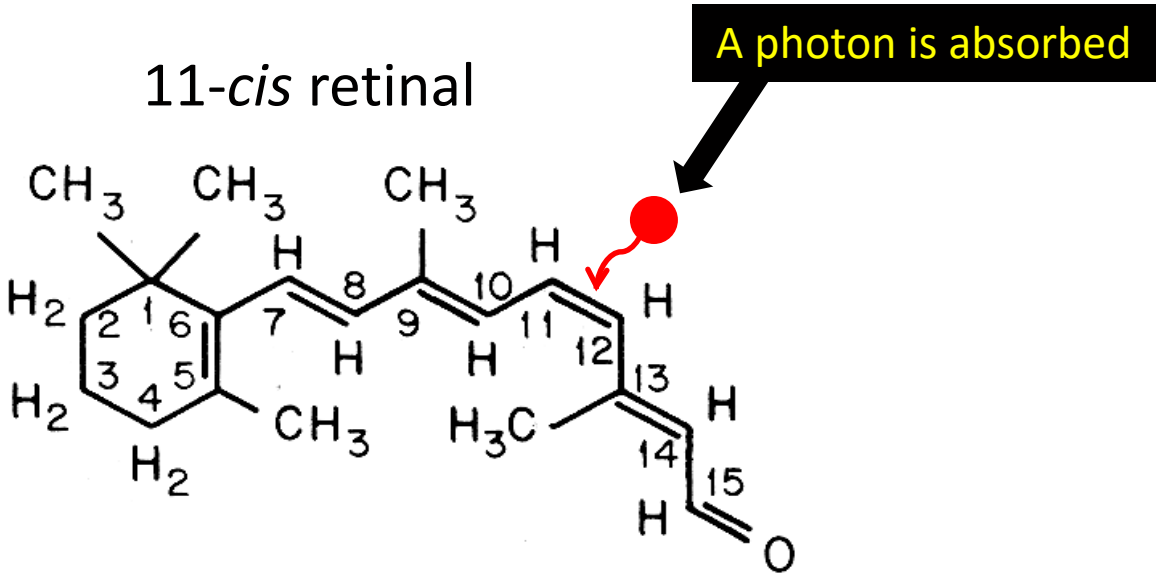
11-*cis* retinal



Chromophore

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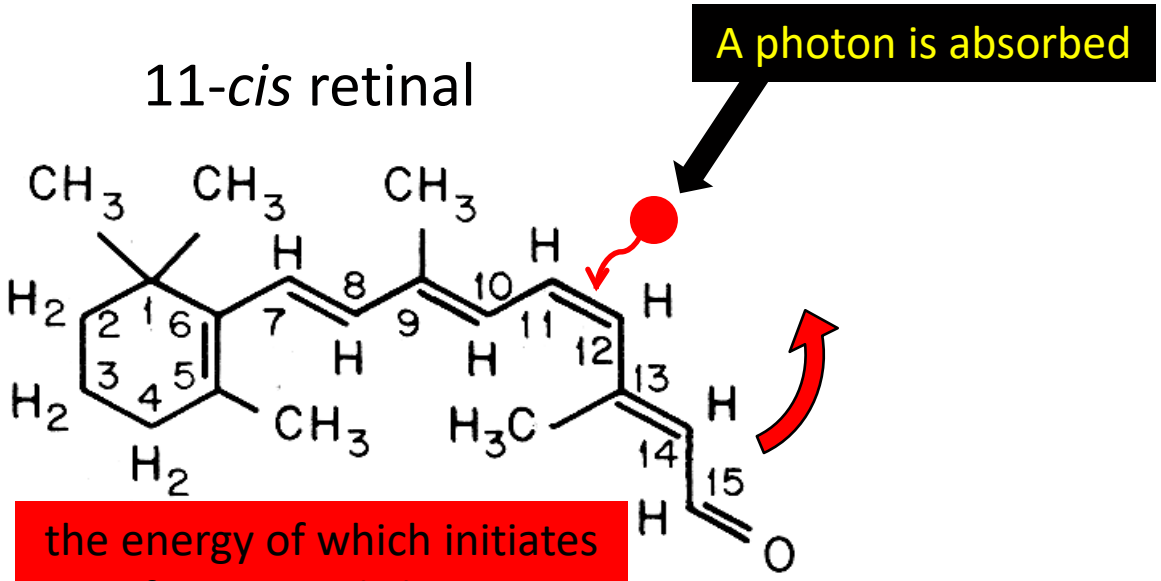
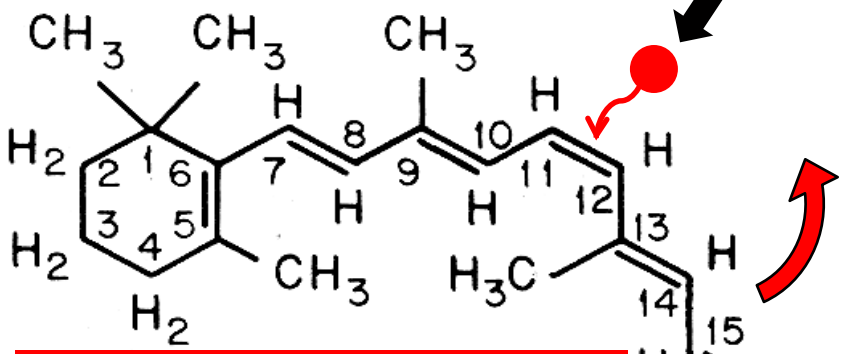


the energy of which initiates a conformational change to…

Chromophore

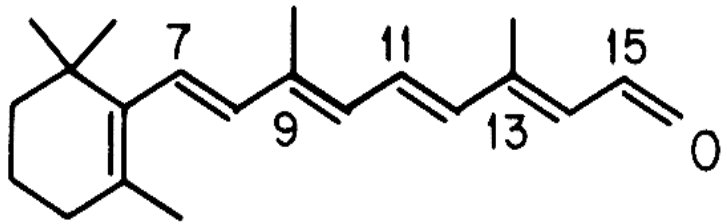
(*chromo-* colour, + *-phore,* producer)
Light-catching portion of any molecule

11-*cis* retinal



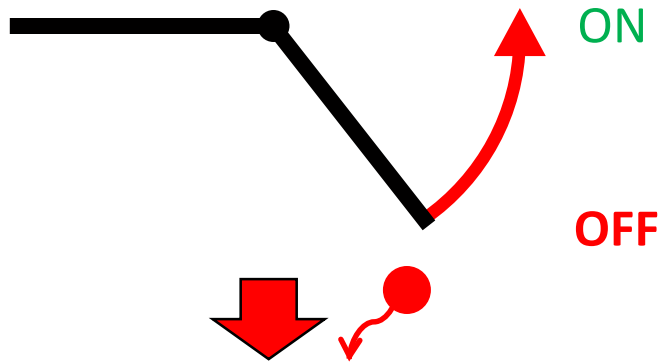
A photon is absorbed

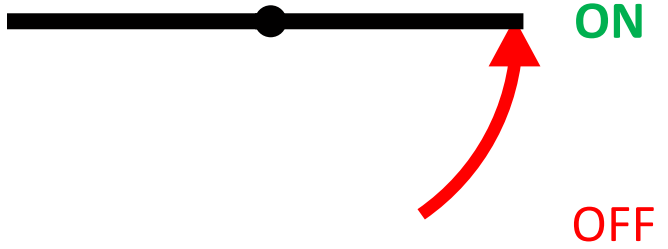
the energy of which initiates a conformational change to…



all-*trans* retinal

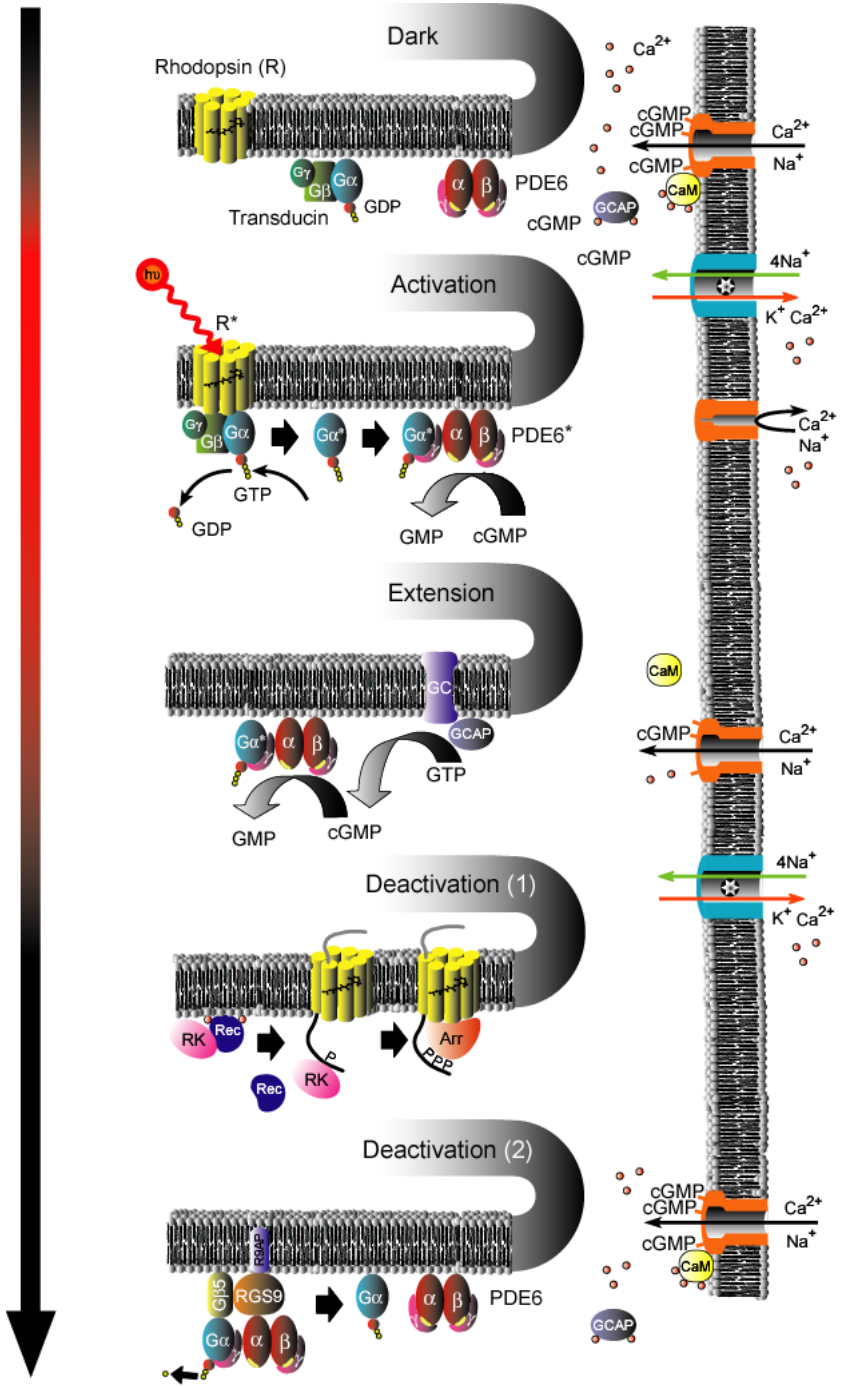
Think of the molecule as a photo-sensitive switch!





Phototransduction

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

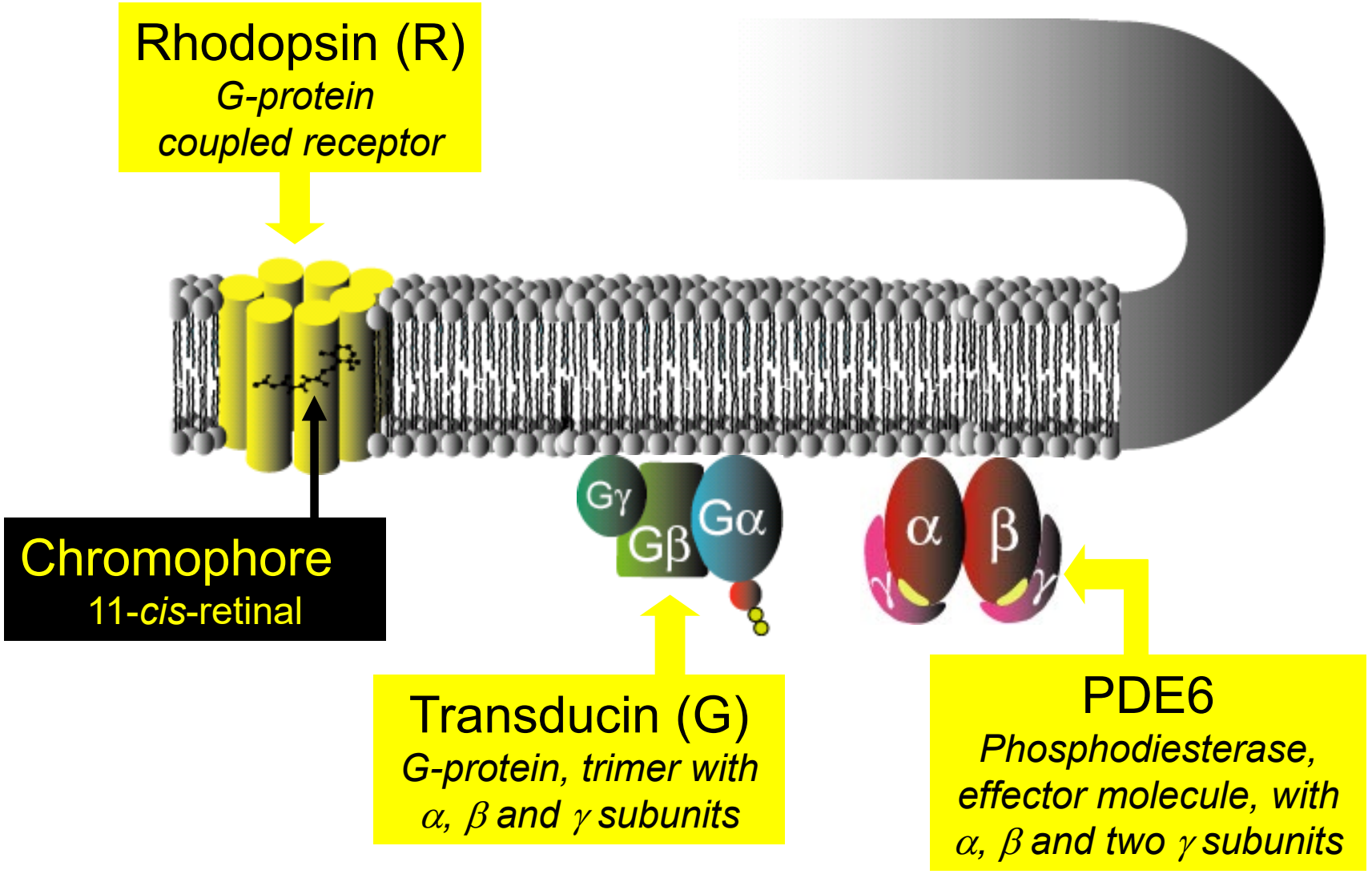


Phototransduction

- Activation
- Range extension
- Deactivation

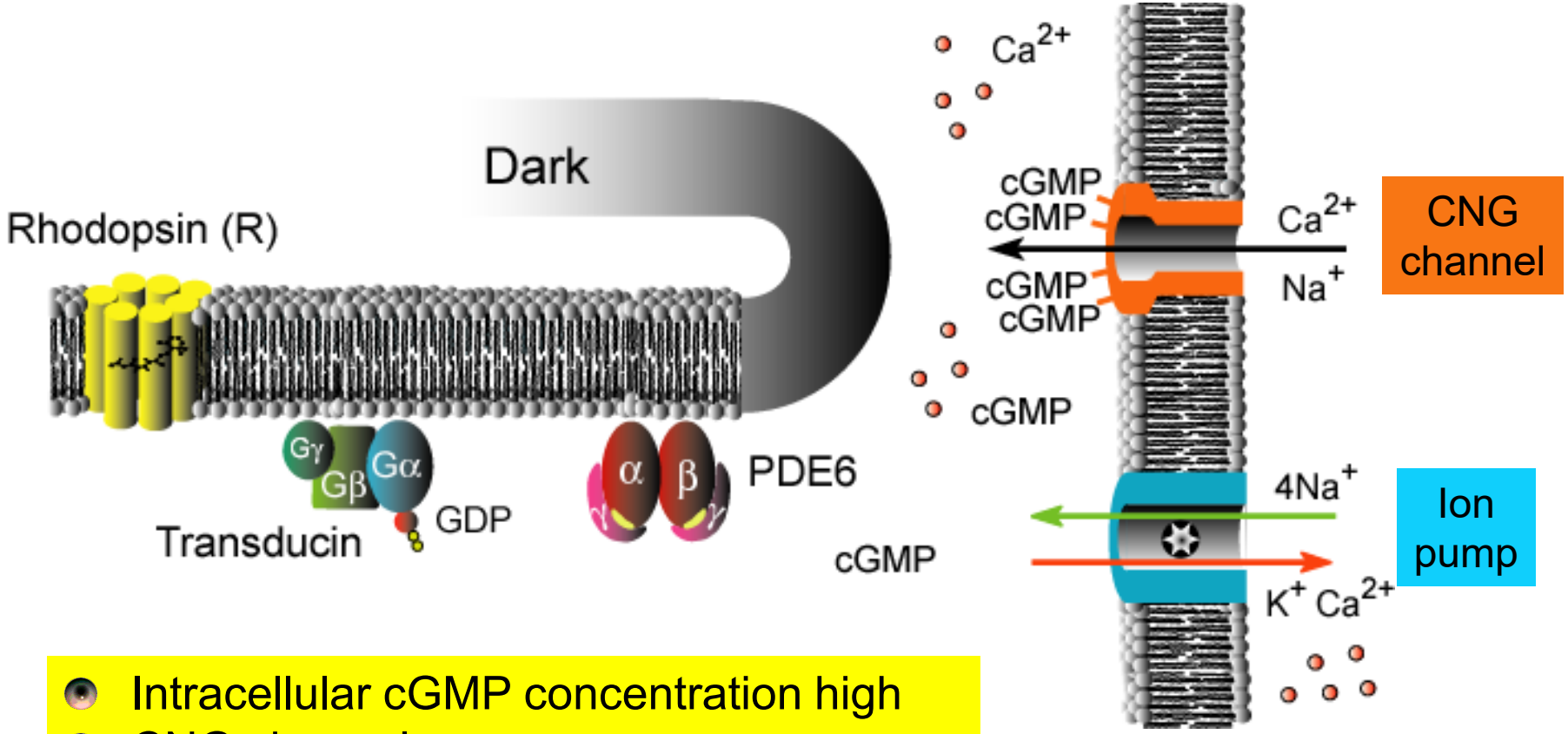
Inspired by:
Pugh, Nikonov, & Lamb (1999). *Current Opinion on Neurobiology, 9*, 410-418.
Burns & Arshavsky (2005). *Neuron, 48*, 387-401.

Main molecular players in the cascade



In the Dark…

In the Dark

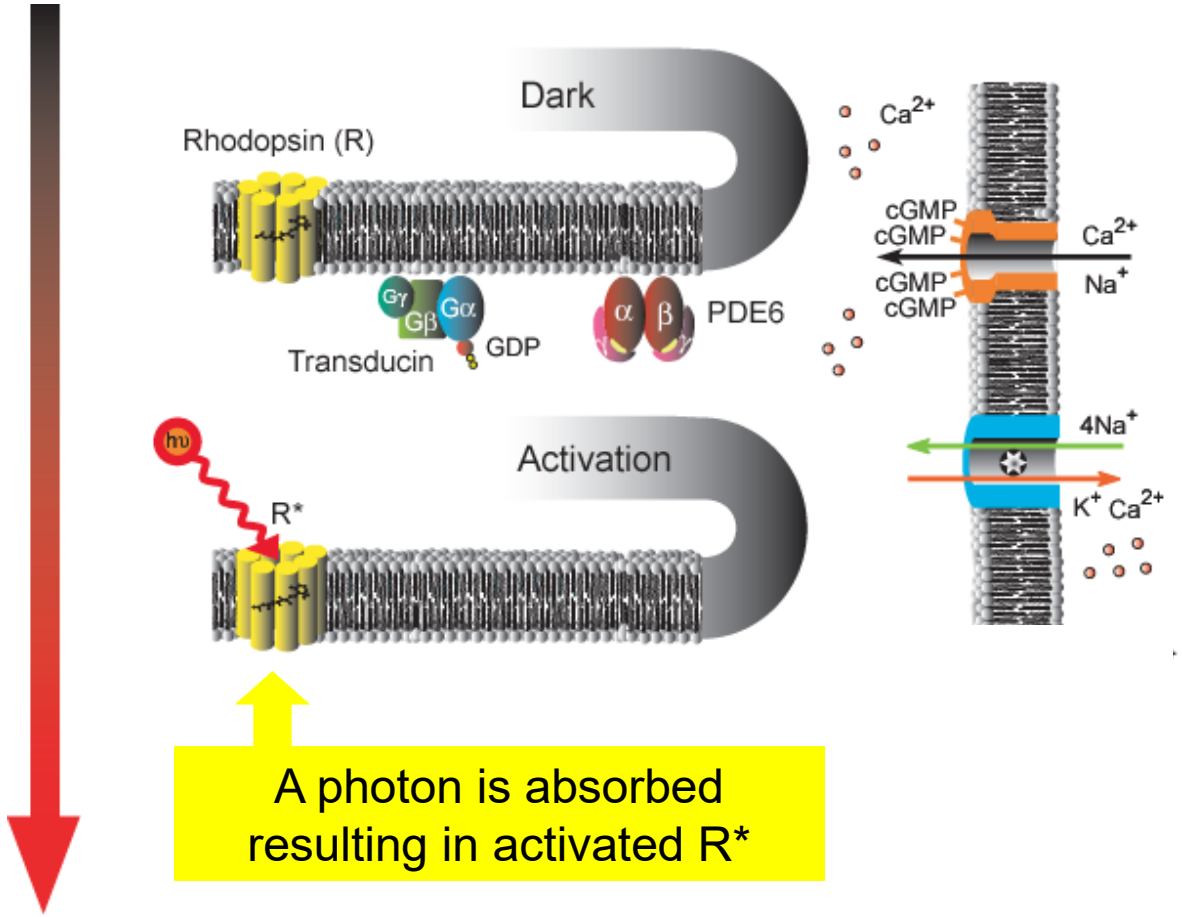


- Intracellular cGMP concentration high
- CNG channels open

CNG = Cyclic Nucleotide Gated channel

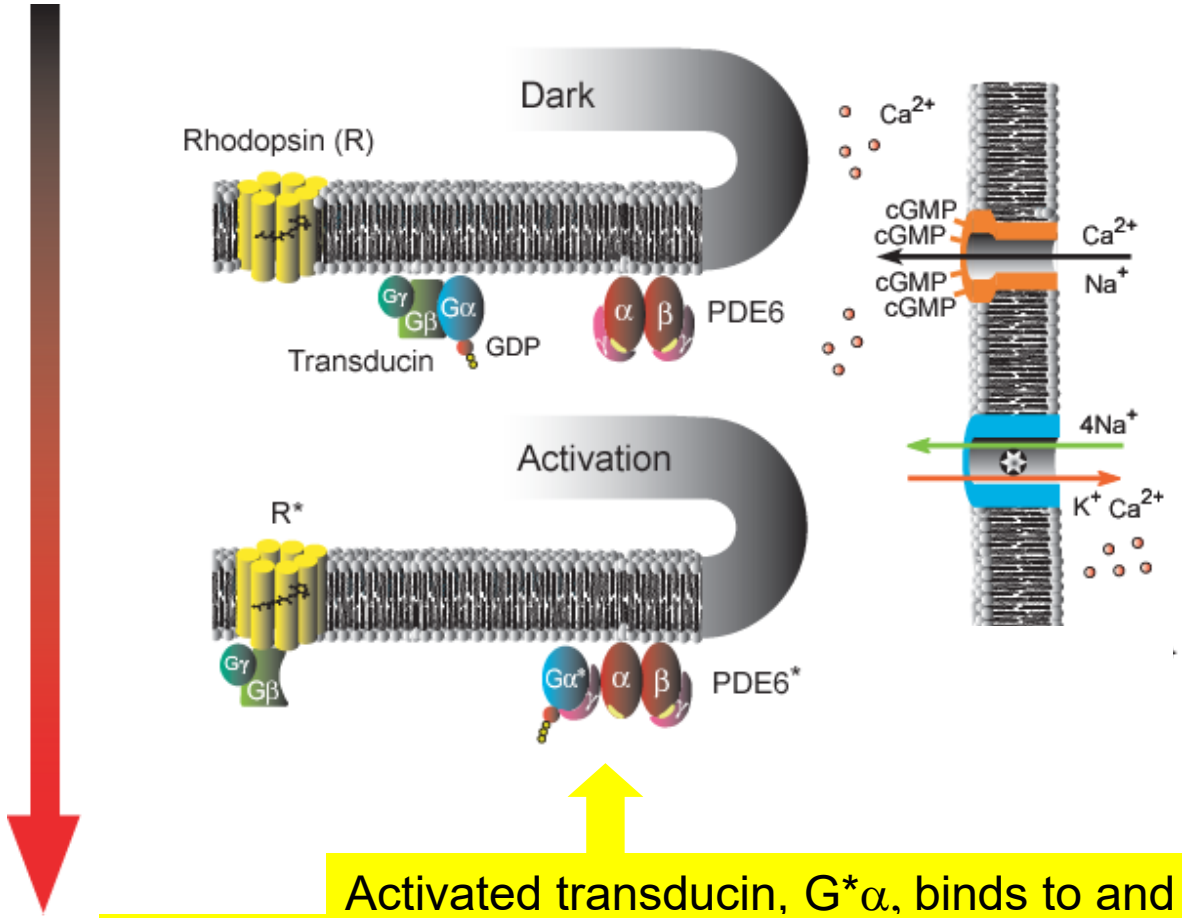
Activation steps

Activation steps



A photon is absorbed resulting in activated R*

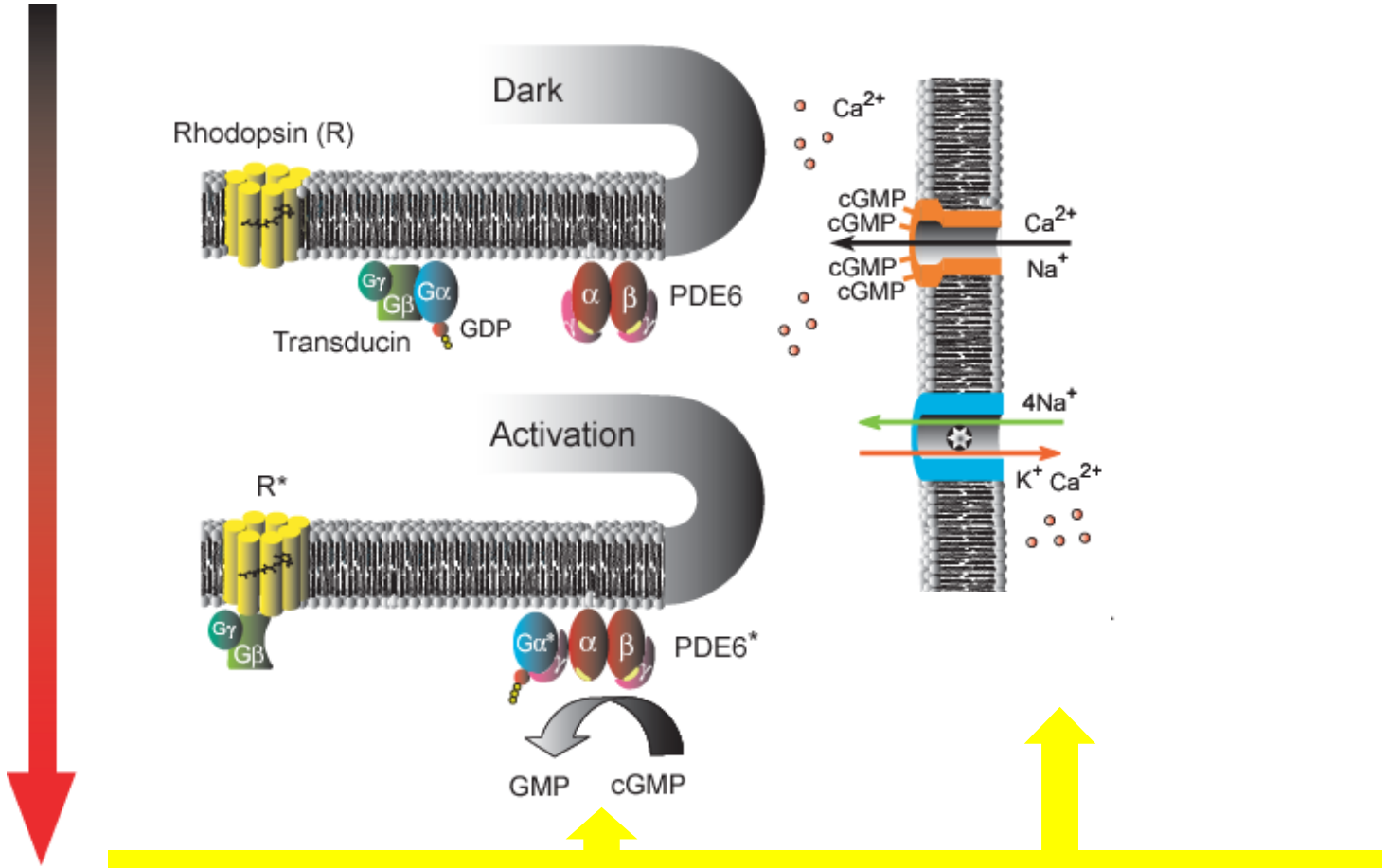
Activation steps



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



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!

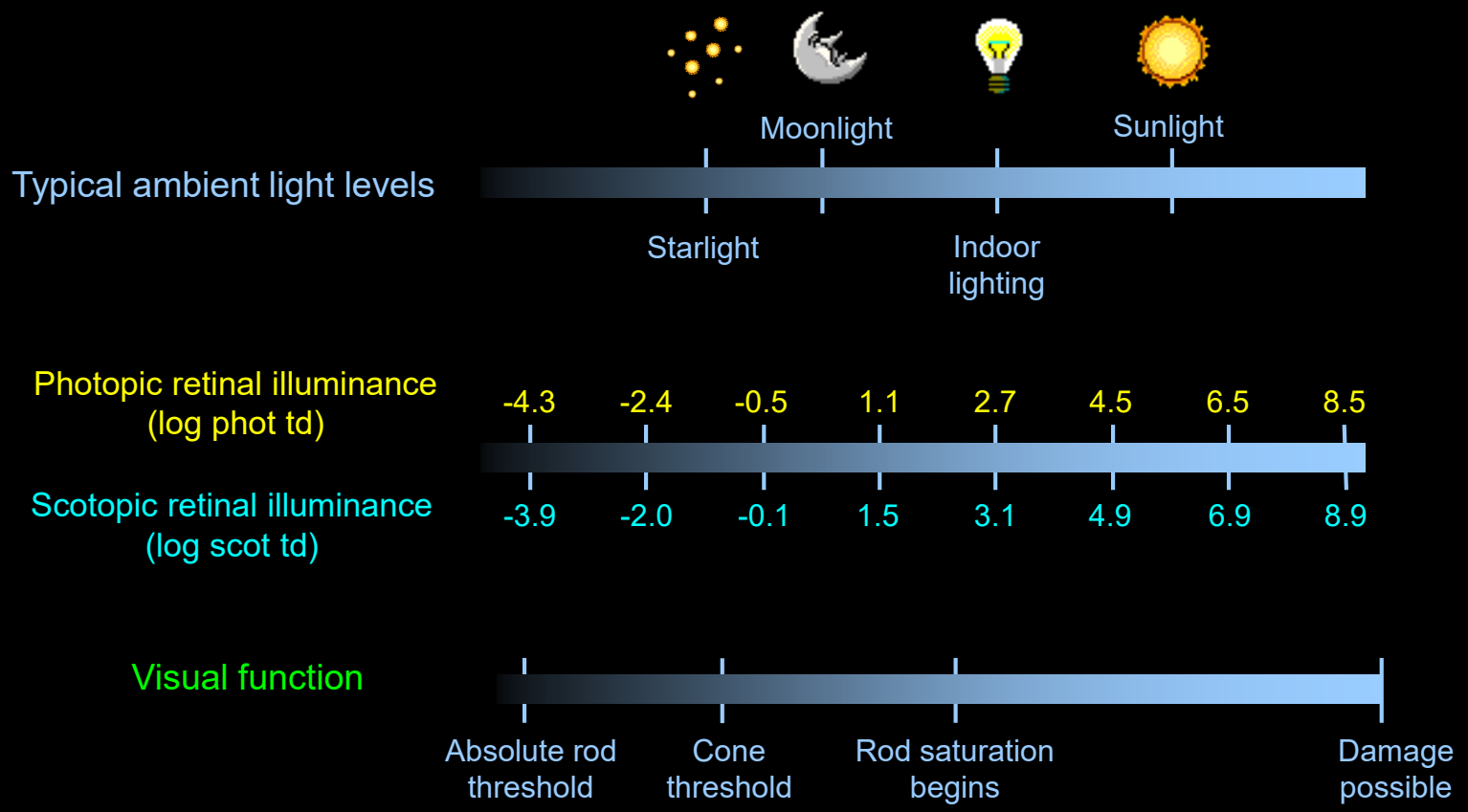
Amplification is beneficial at low light levels, but what negative effects might amplification have at high light levels?

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

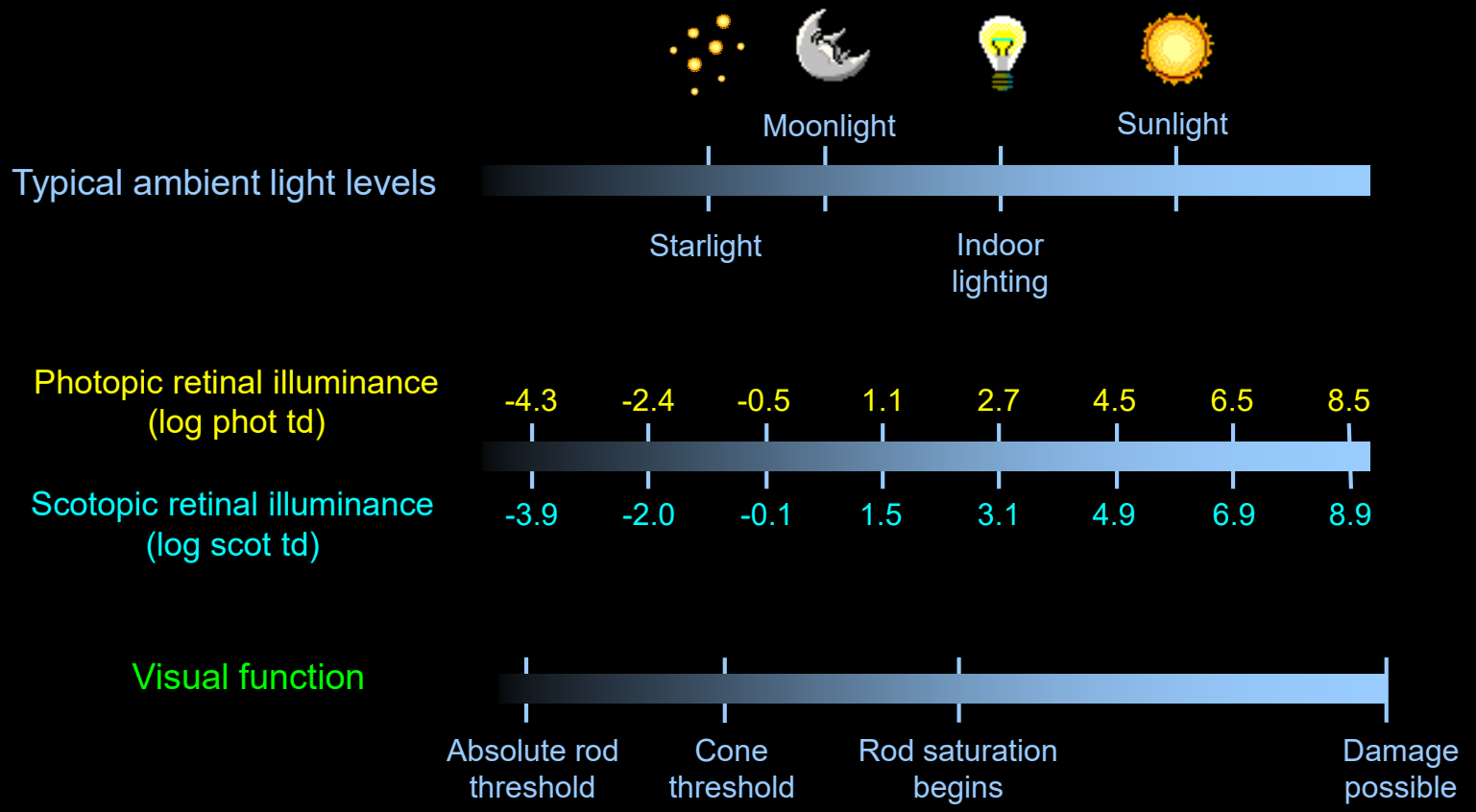
Range extension and light adaptation

Why is light adaptation or sensitivity regulation important?

- Because 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.



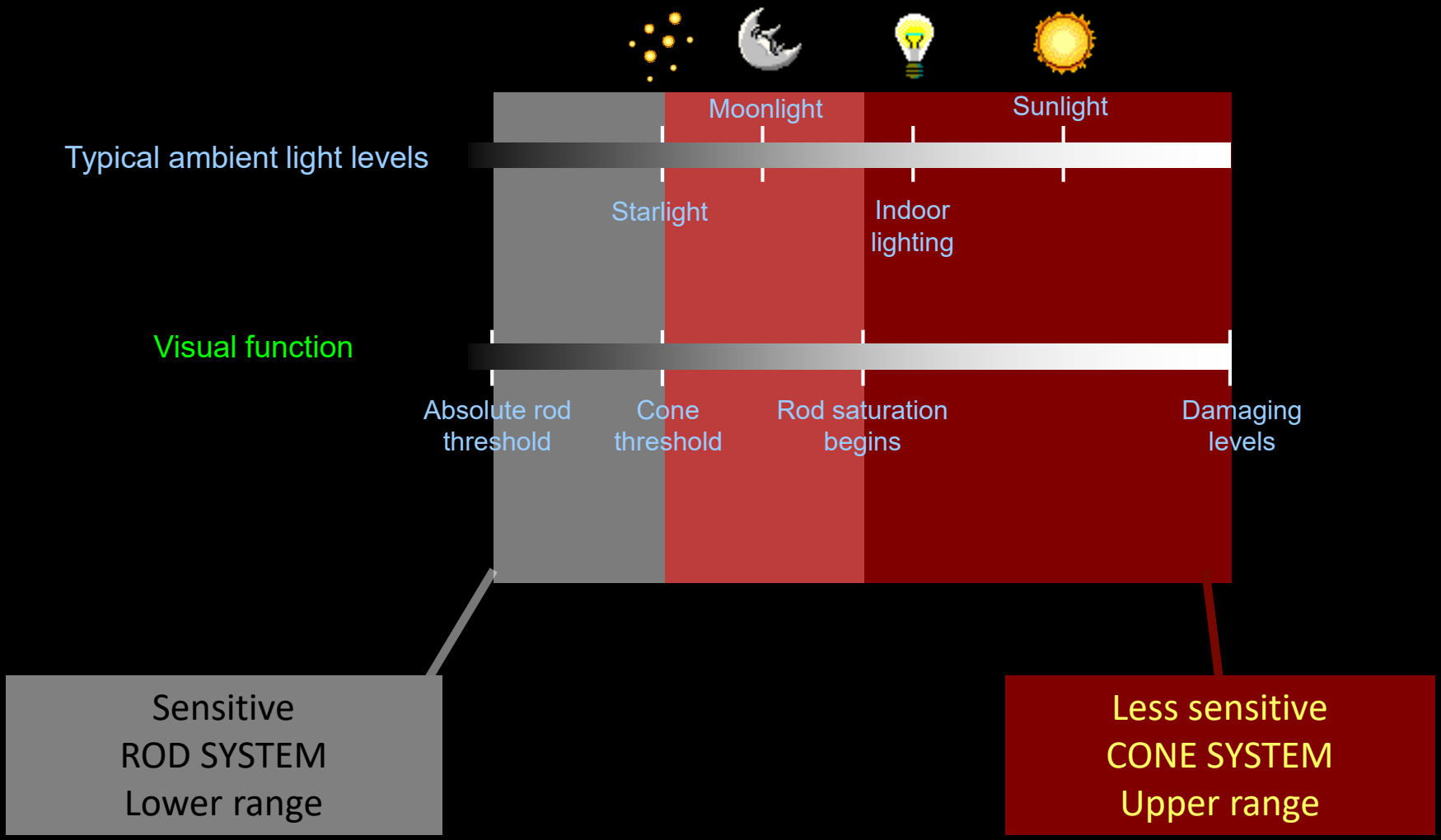
It must do so despite the fact that that a typical postreceptoral neuron can operate over a range of only c. 10^3.



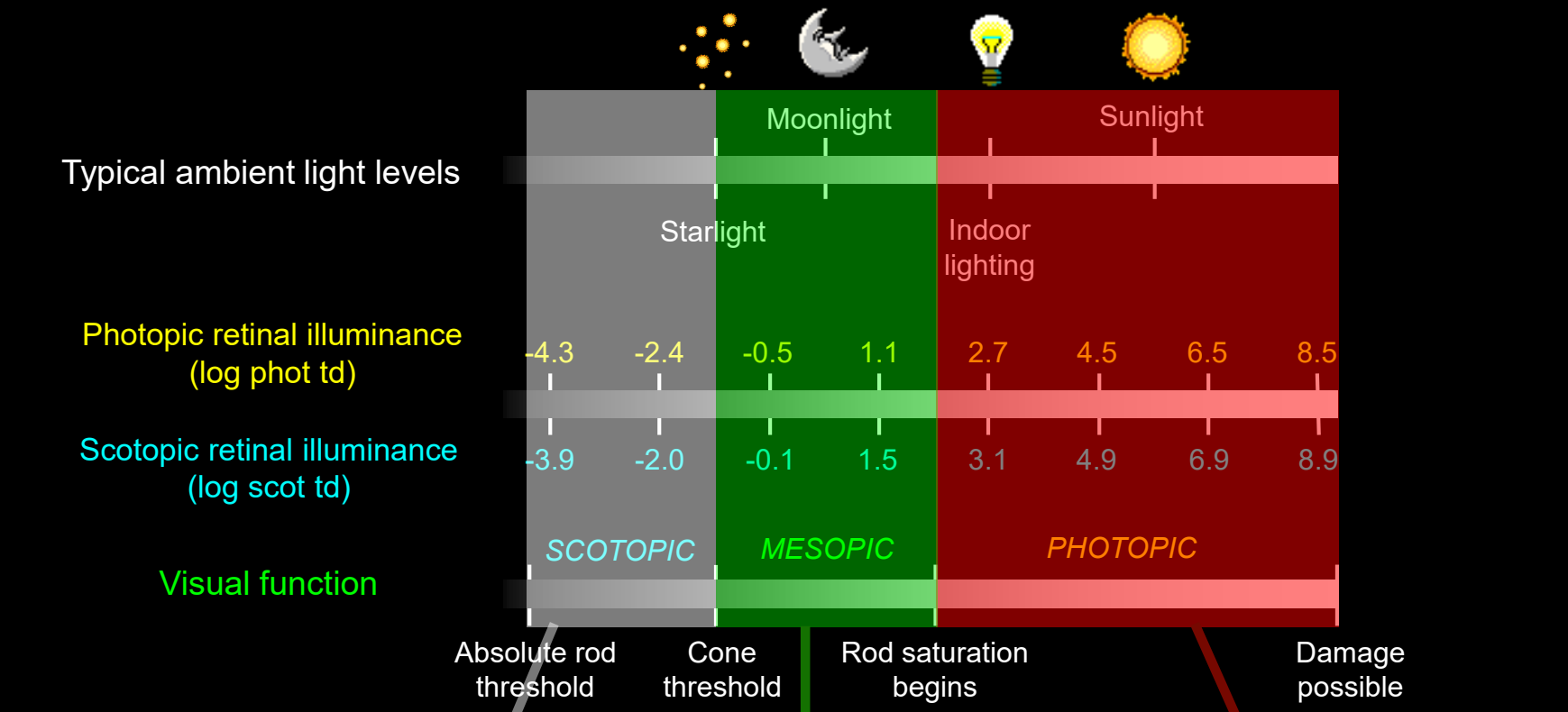
Rods and cones

Rods that are optimized for low light levels

Cones that are optimized for higher light levels



Rods and cones



Scotopic levels
(below cone threshold)
where rod vision functions alone.
A range of c. 10^3

Mesopic levels
where rod and cone vision function together.
A range of c. 10^3

Photopic levels
(above rod saturation)
where cone vision functions alone.
A range of > 10^6

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.

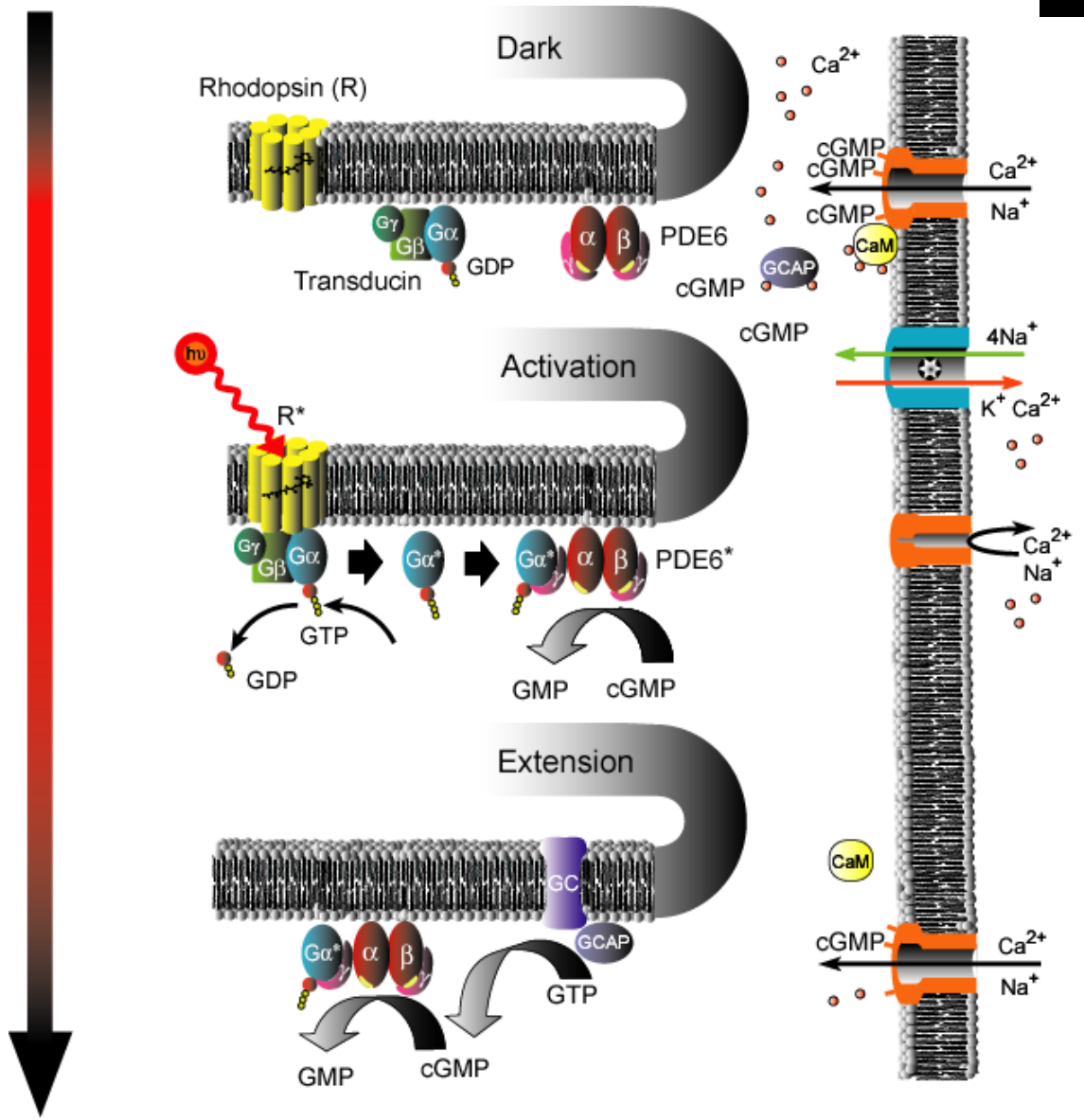
How can this achieved within the transduction cascade?

Adaptation and sensitivity…

At low light levels the sensitivity is very high: 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”).

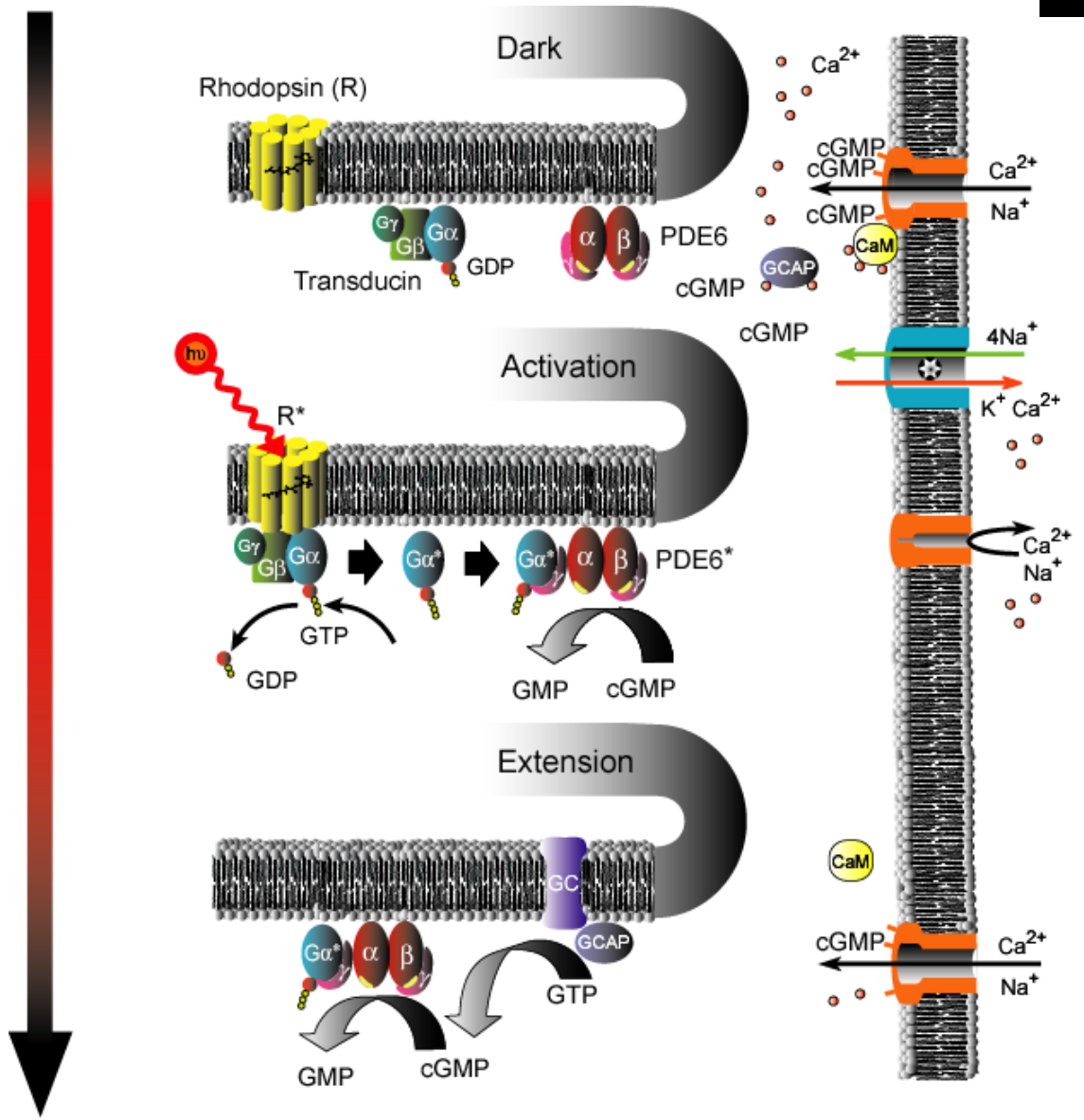
Range extension (1)



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

Ca^{2+} feedback

Range extension (2)



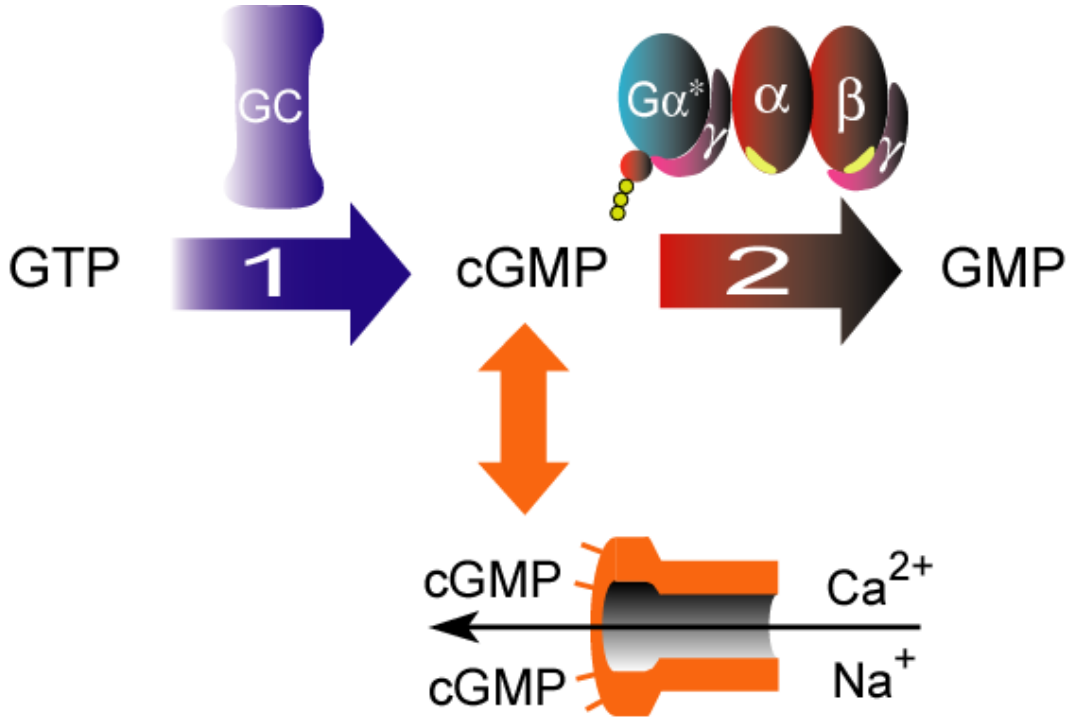
Reduction in [Ca^{2+}] causes Calmodulin (CaM) to dissociate from the CNG channels raising the affinity of the channels for cGMP

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

Ca^{2+} feedback

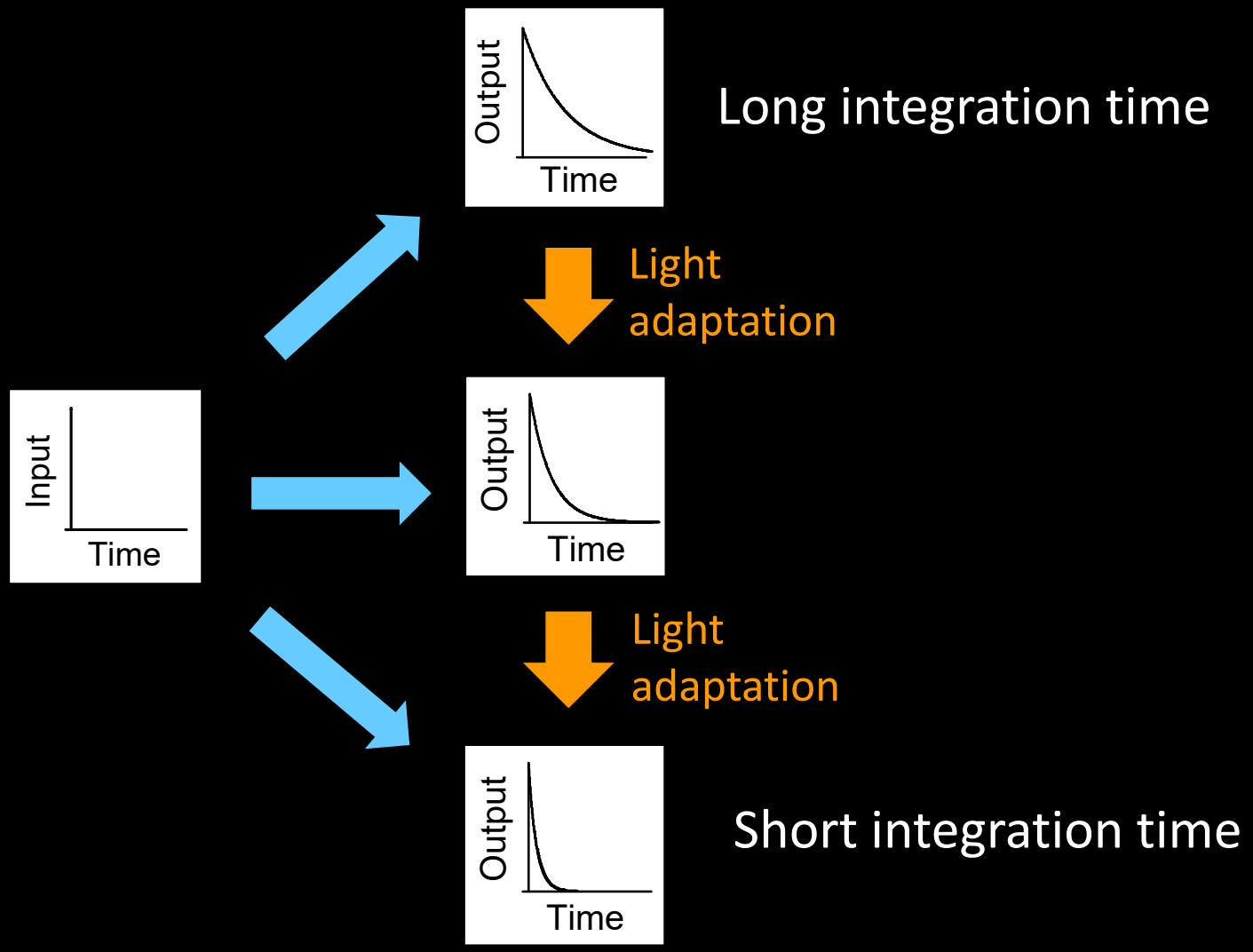
Adaptation: Speeding up the visual response

Increase in concentration of G*α-PDE6* in light speeds up rate of reaction 2 and speeds up the visual response

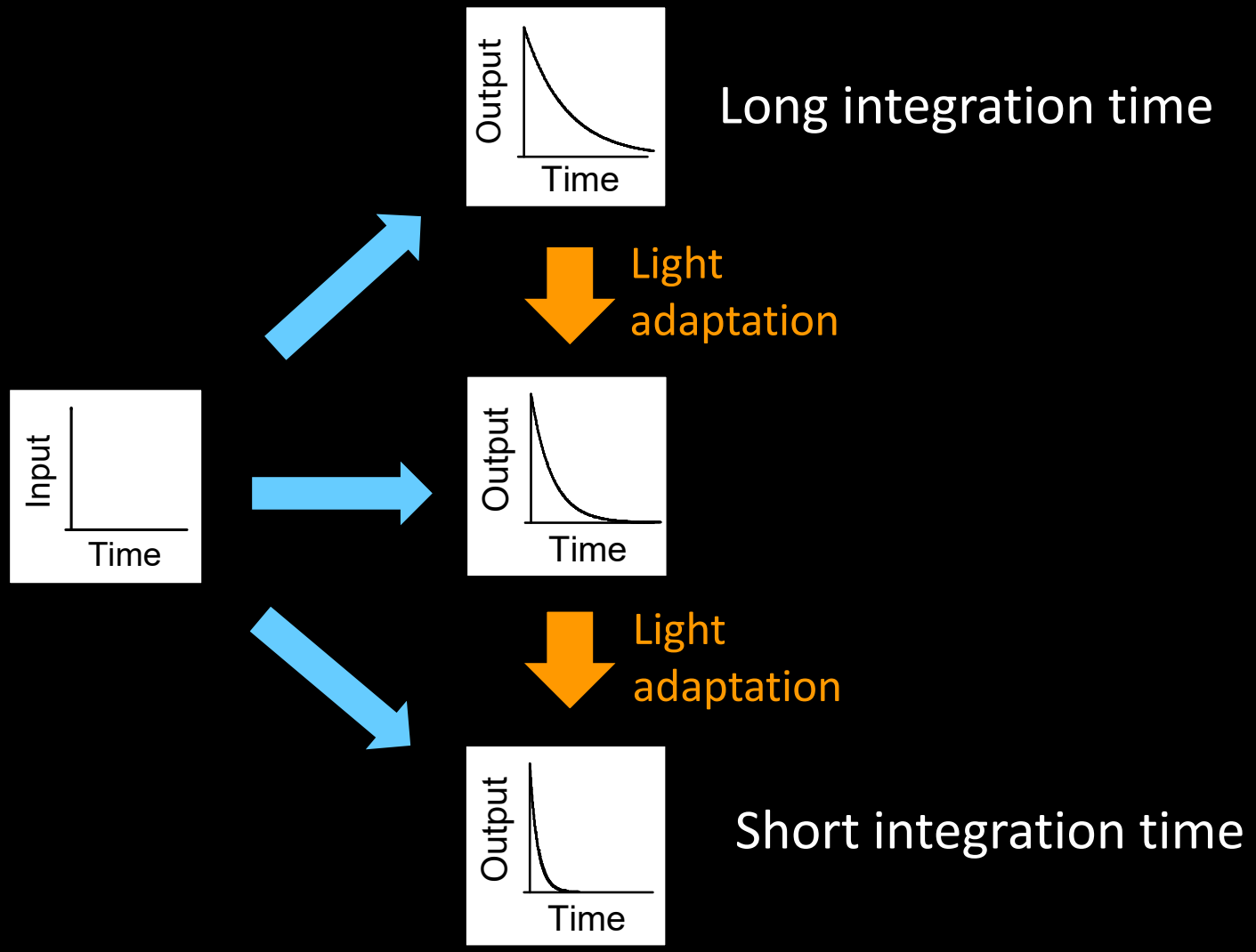


How does speeding up the visual response help light adaptation?

It reduces the integration time of the system...



What are the benefits of this type of adaptation?

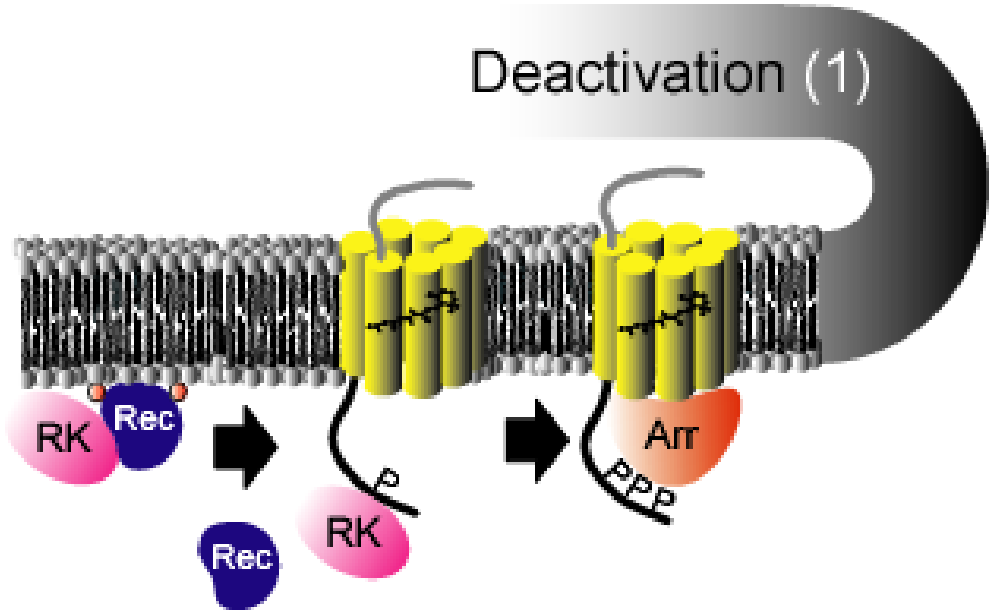


Deactivation

Speeding up deactivation also decreases temporal integration.

Deactivation steps

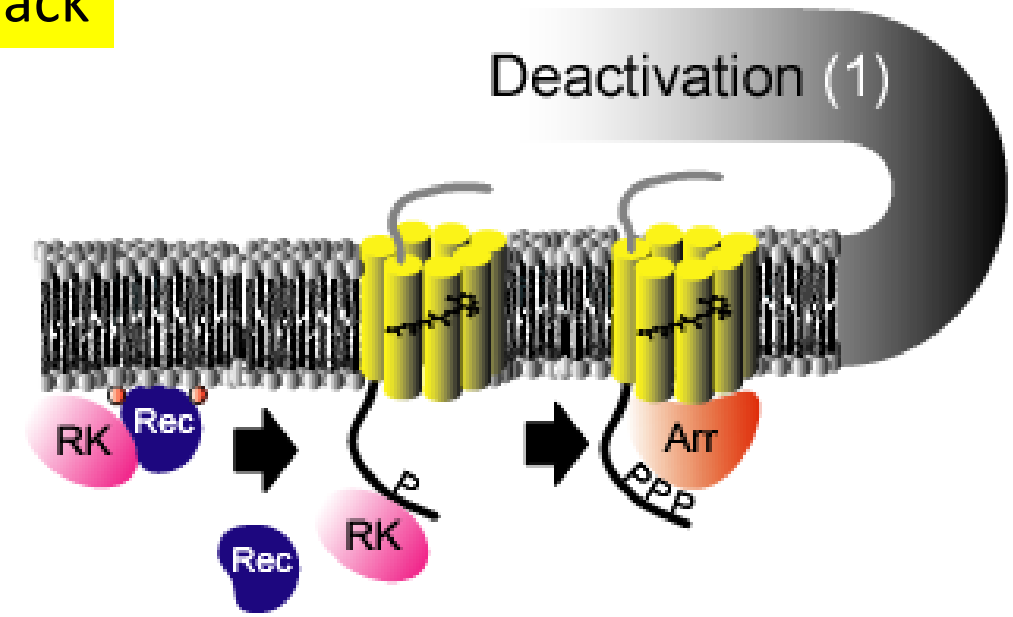
Ca^{2+} feedback



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

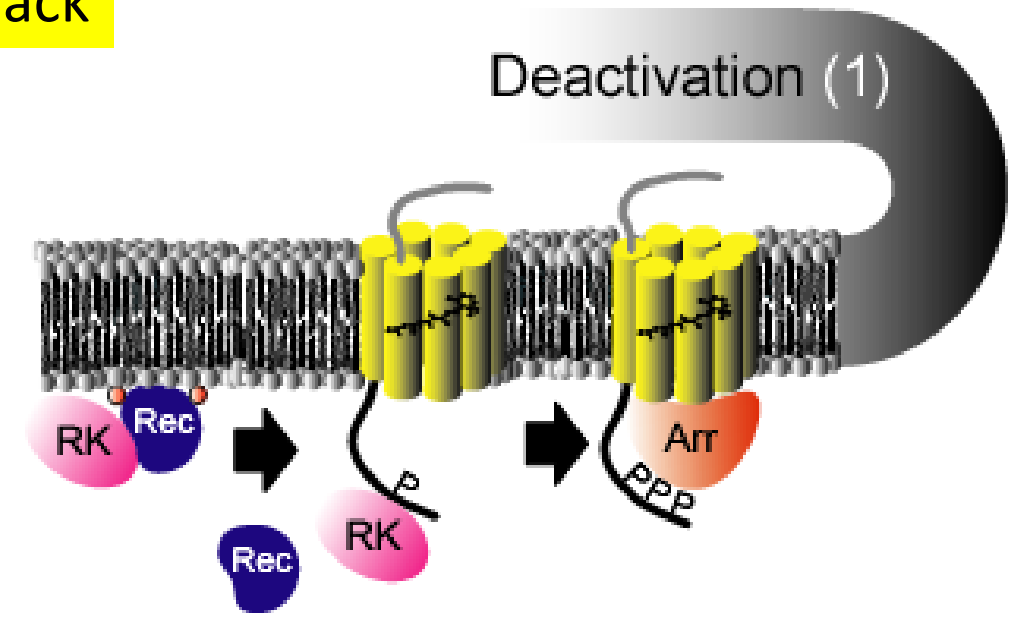
Ca^{2+} feedback



Free RK multiply phosphorylates R*

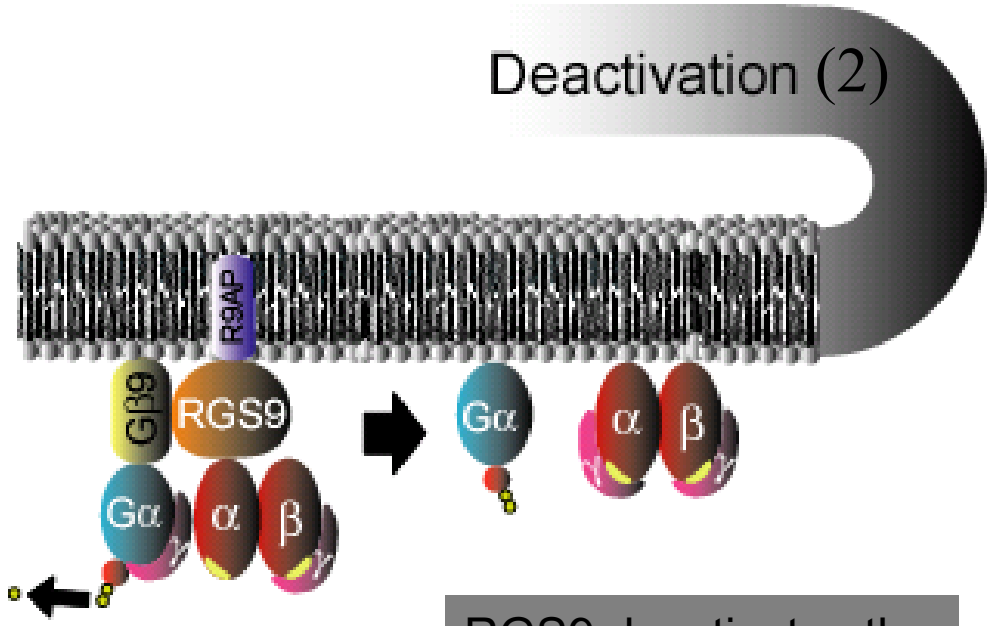
Deactivation steps

Ca^{2+} feedback



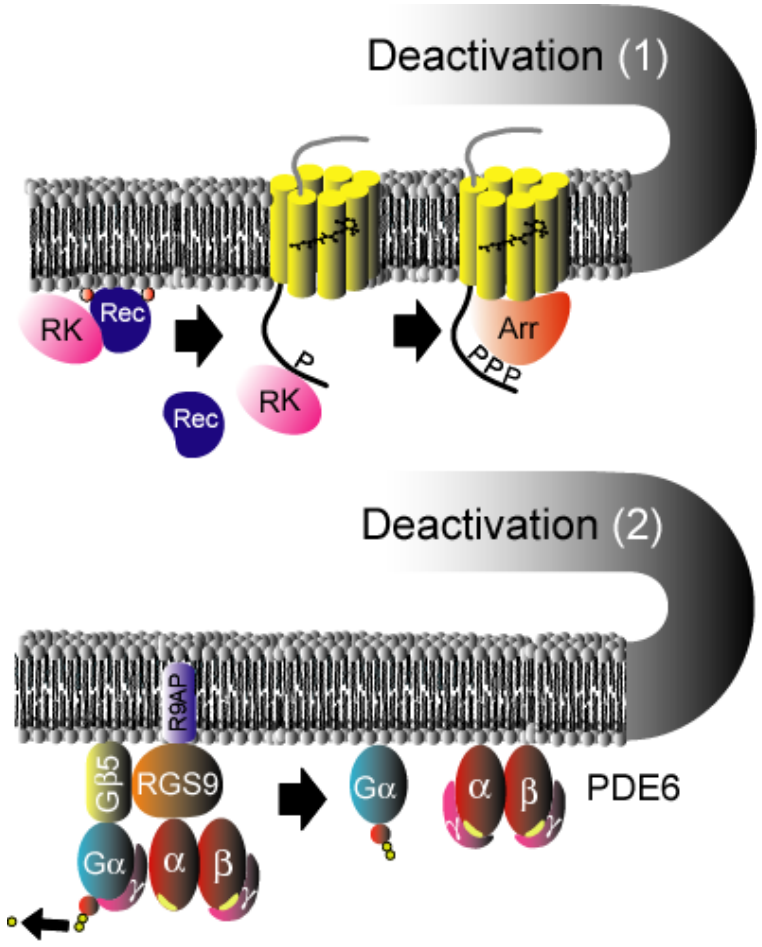
Arrestin (Arr) quenches the phosphorylated R*

Deactivation steps



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

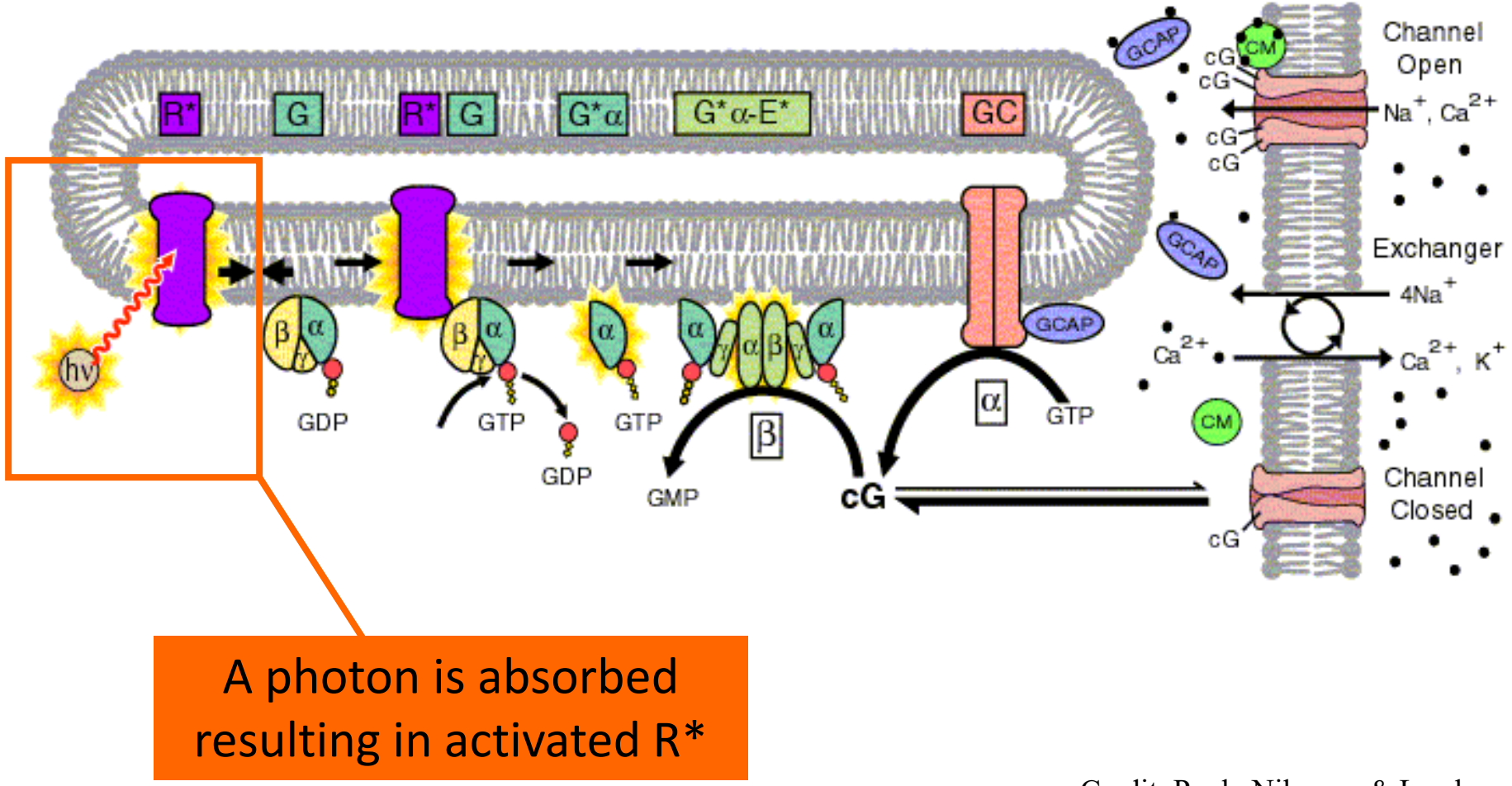
Deactivation steps



Second run through…

Phototransduction cascade activation stages

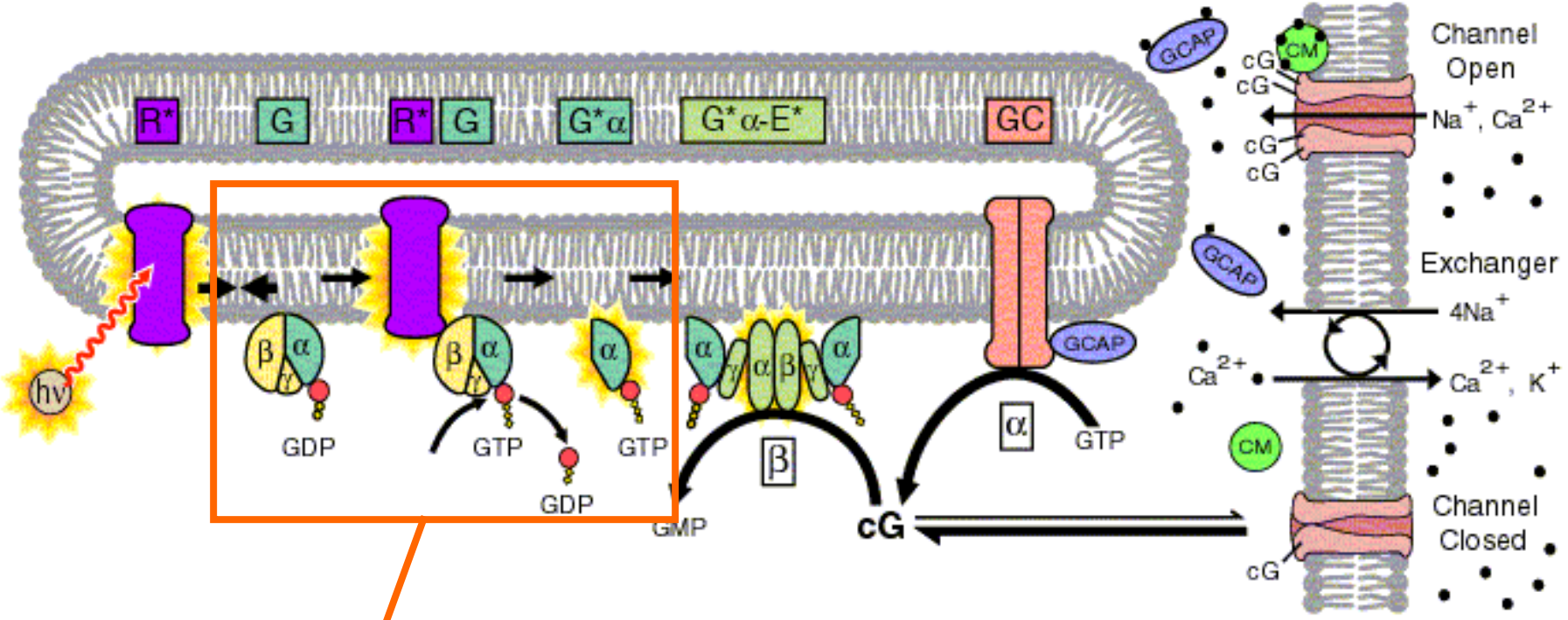
Activation steps of the phototransduction cascade



A photon is absorbed resulting in activated R*

Credit: Pugh, Nikonov & Lamb

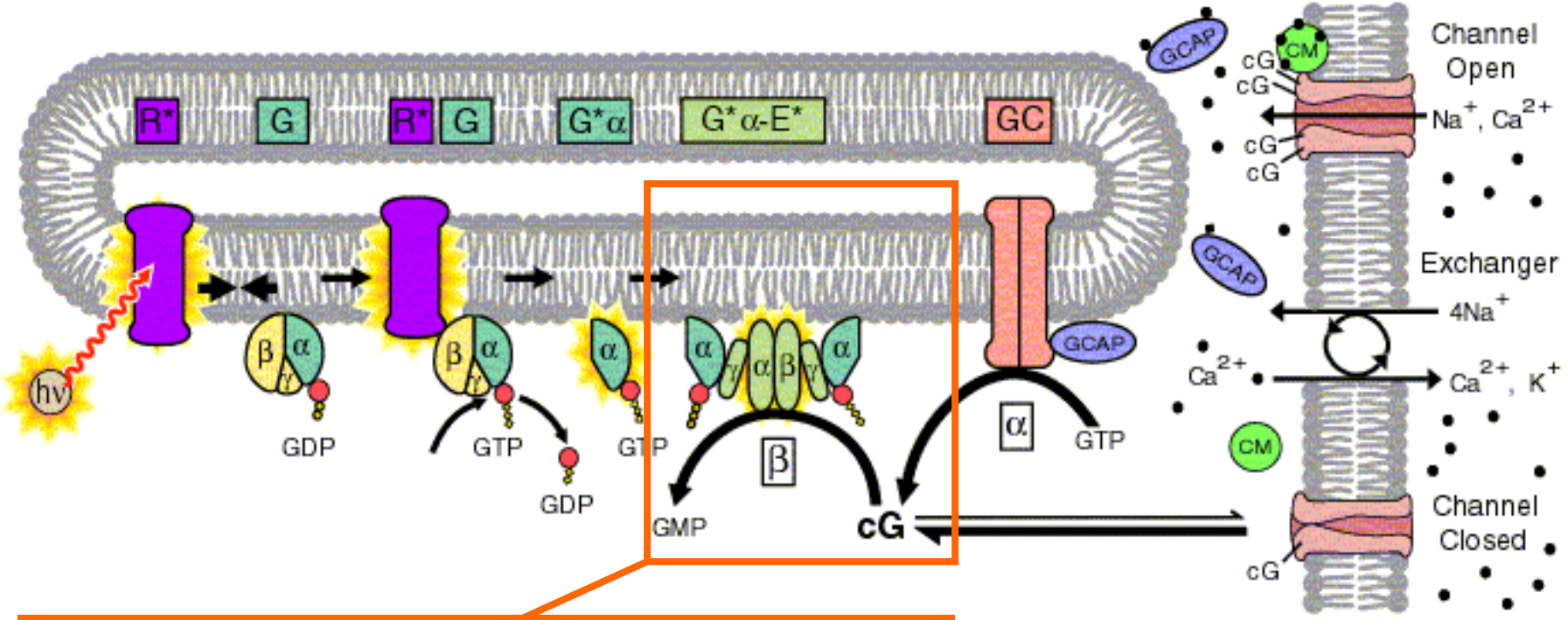
Activation steps of the phototransduction cascade



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

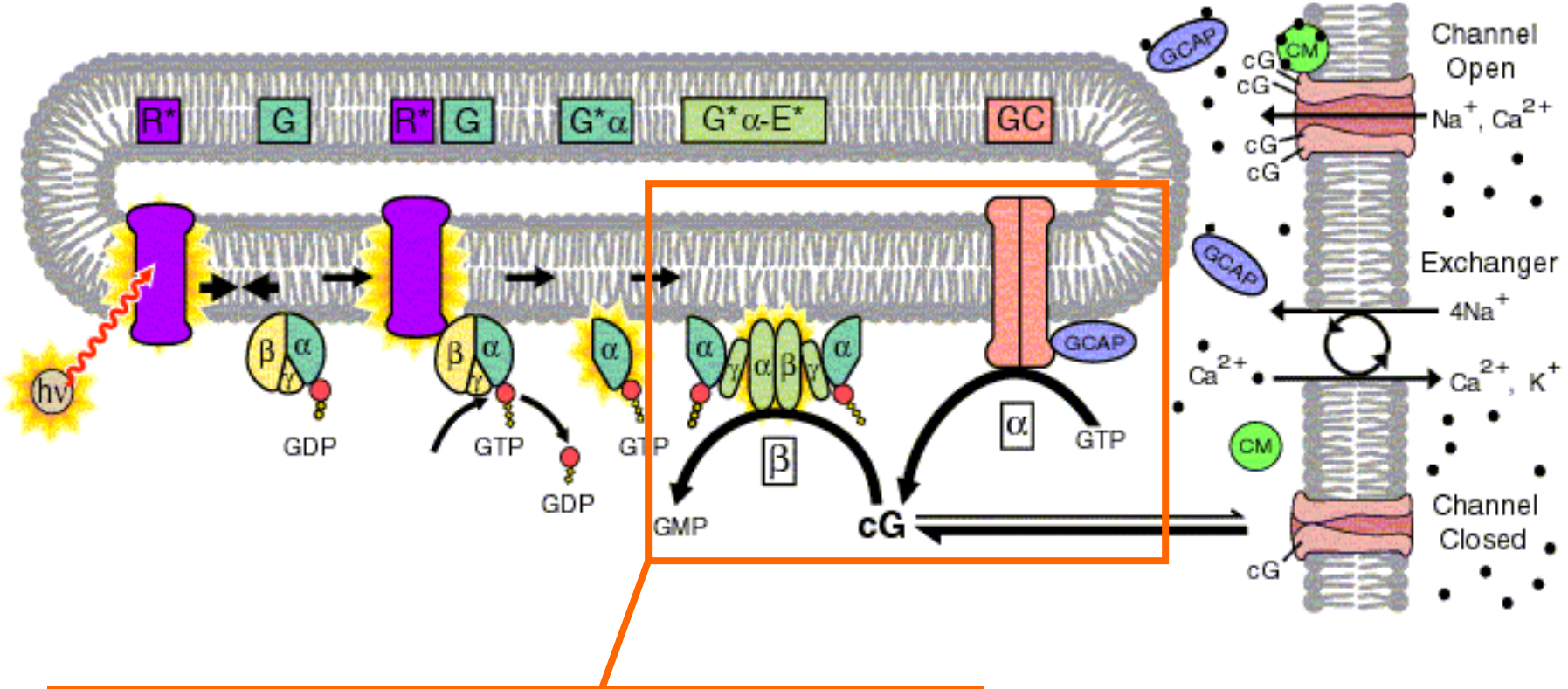
Activation steps of the phototransduction cascade



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

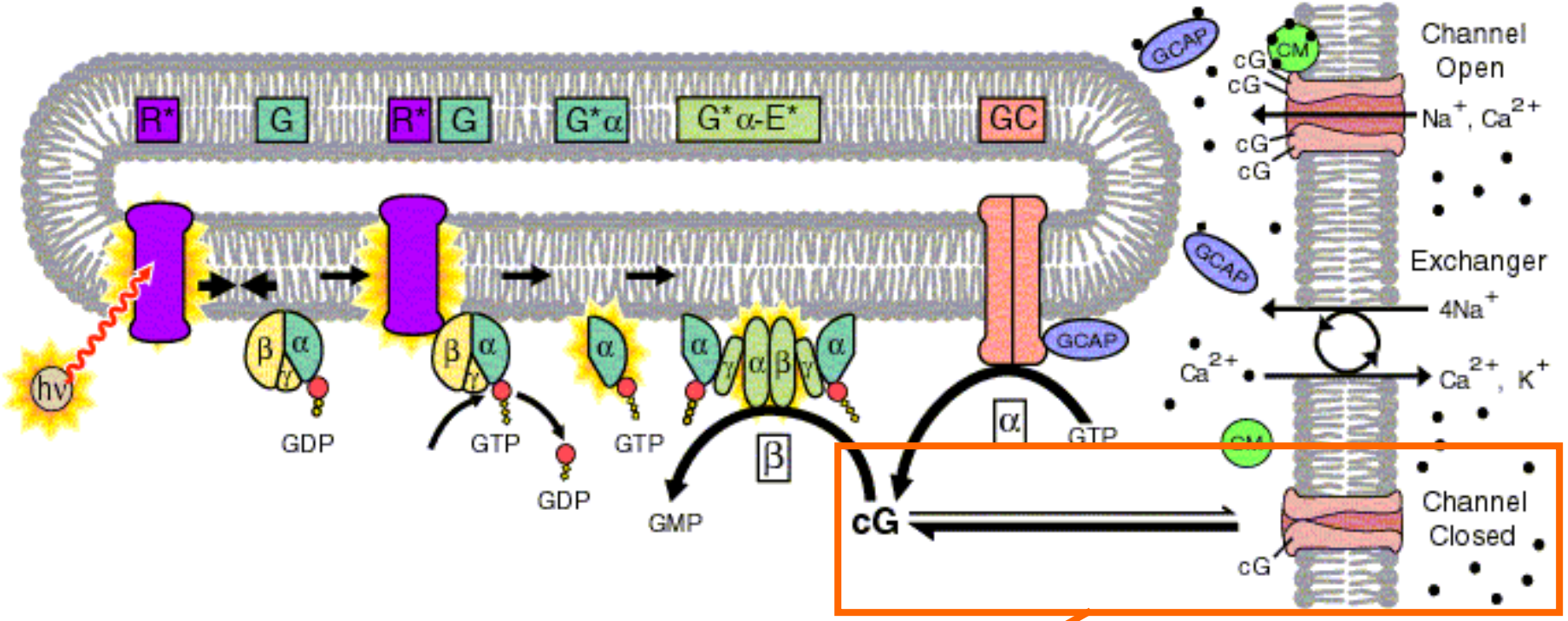
Activation steps of the phototransduction cascade



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

Credit: Pugh, Nikonov & Lamb

Activation steps of the phototransduction cascade

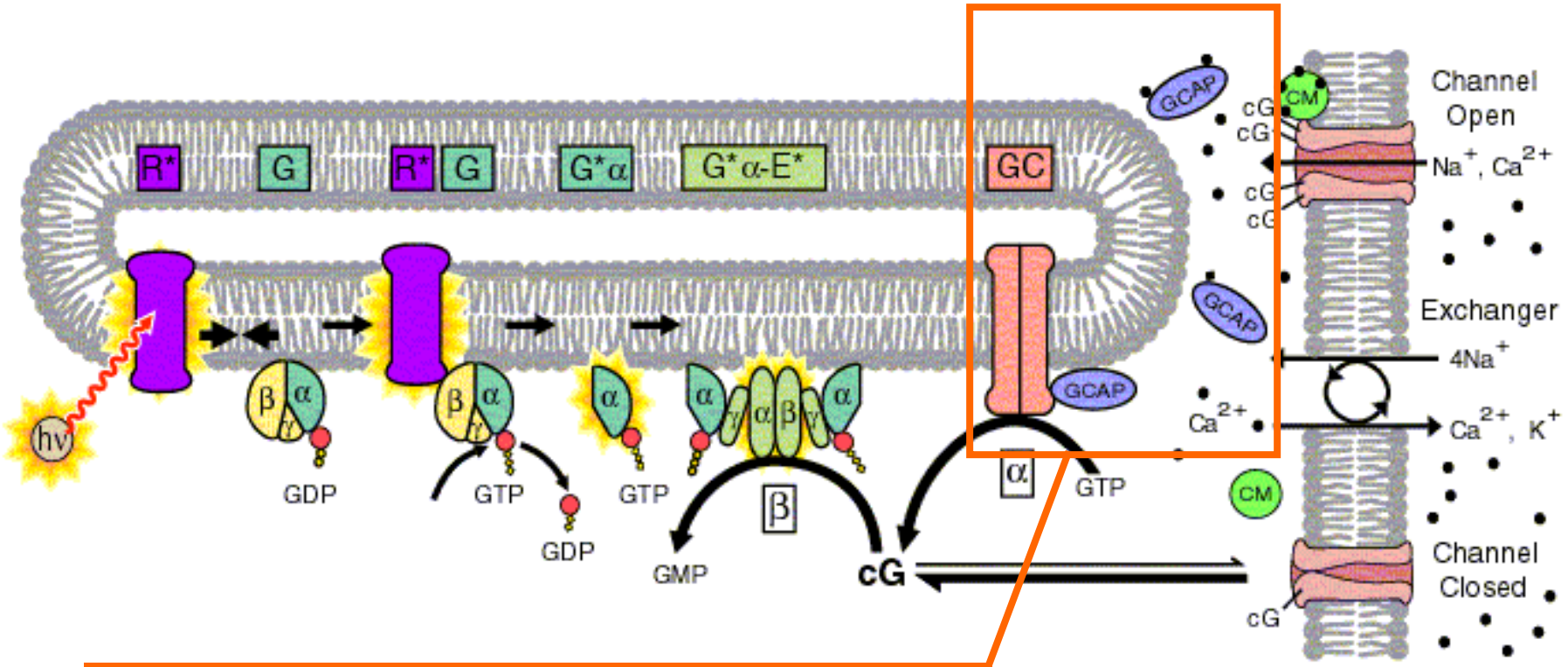


A drop in cGMP leads to closure of cGMP gated channels, blocking the entry of Na^+ and Ca^{2+} into the outer segment. The ion exchanger continues to function lowering [Ca^{2+}] in the outersegment.

Credit: Pugh, Nikonov & Lamb

Phototransduction cascade inactivation steps

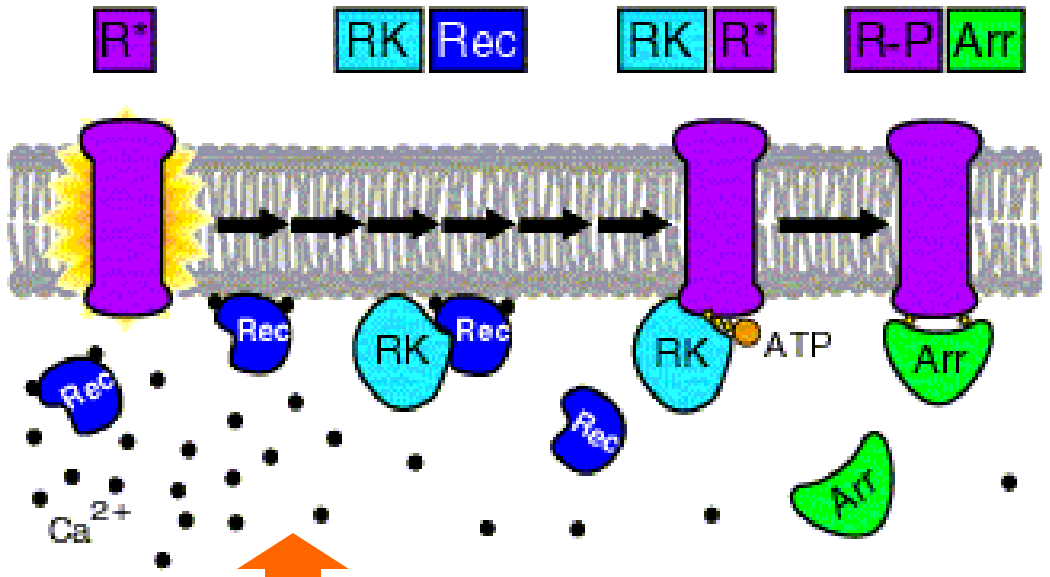
Inactivation steps of the phototransduction cascade



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

Ca^{2+} feedback

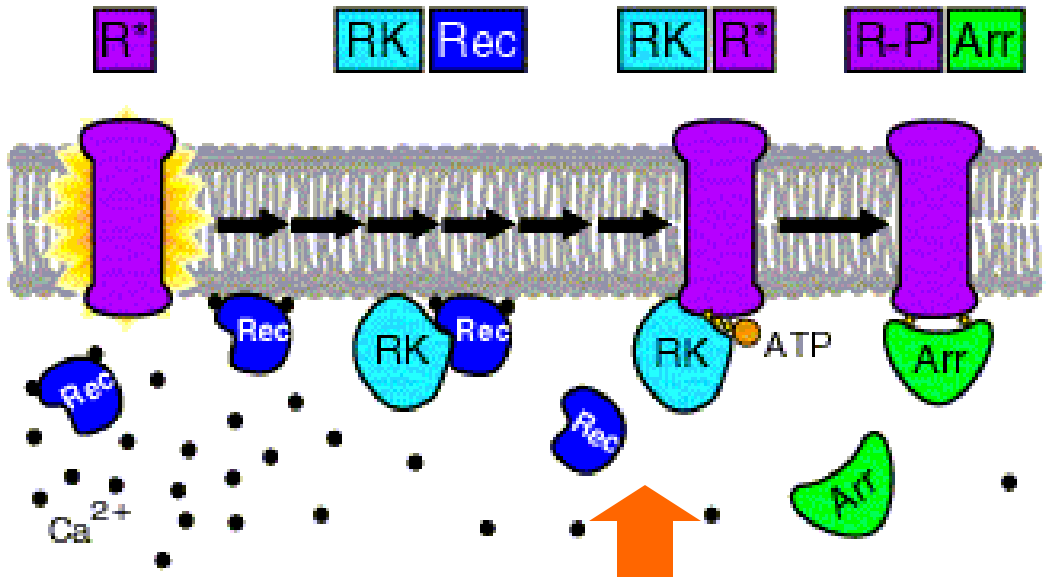
Credit: Pugh, Nikonov & Lamb



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

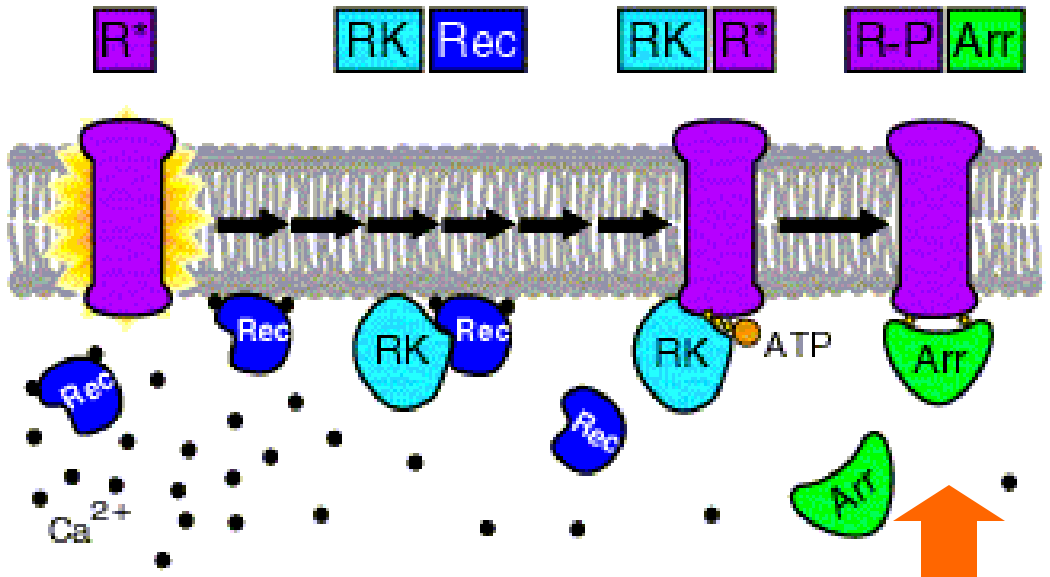
Credit: Pugh, Nikonov & Lamb



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

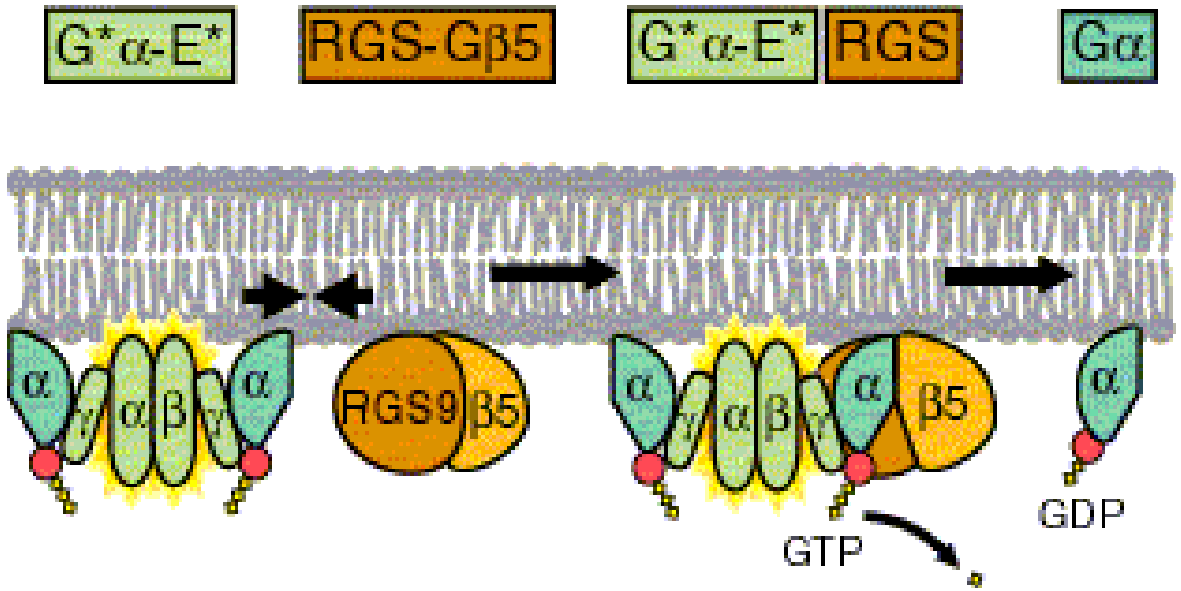
Credit: Pugh, Nikonov & Lamb



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

Ca^{2+} feedback

Credit: Pugh, Nikonov & Lamb



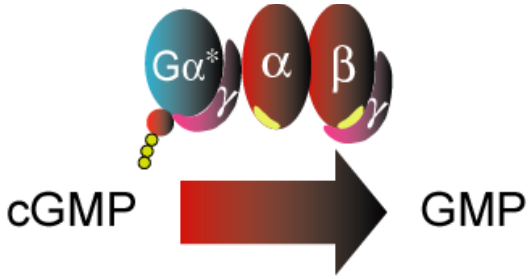
G*α-E* is inactivated when the terminal phosphate of its bound GTP is hydrolyzed, which occurs when the RGS9-Gβ5 protein binds to the complex.

Credit: Pugh, Nikonov & Lamb

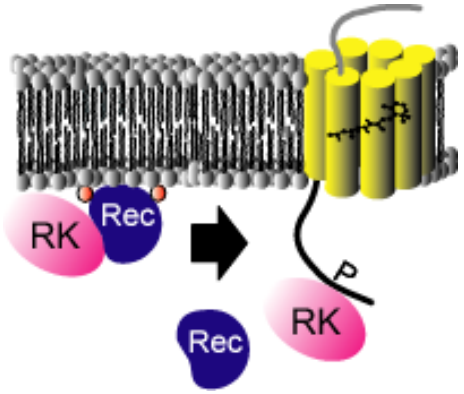
Summary of molecular adaptation mechanisms

We'll come back to these in the Sensitivity Regulation lecture

Mechanisms that shorten the visual integration time

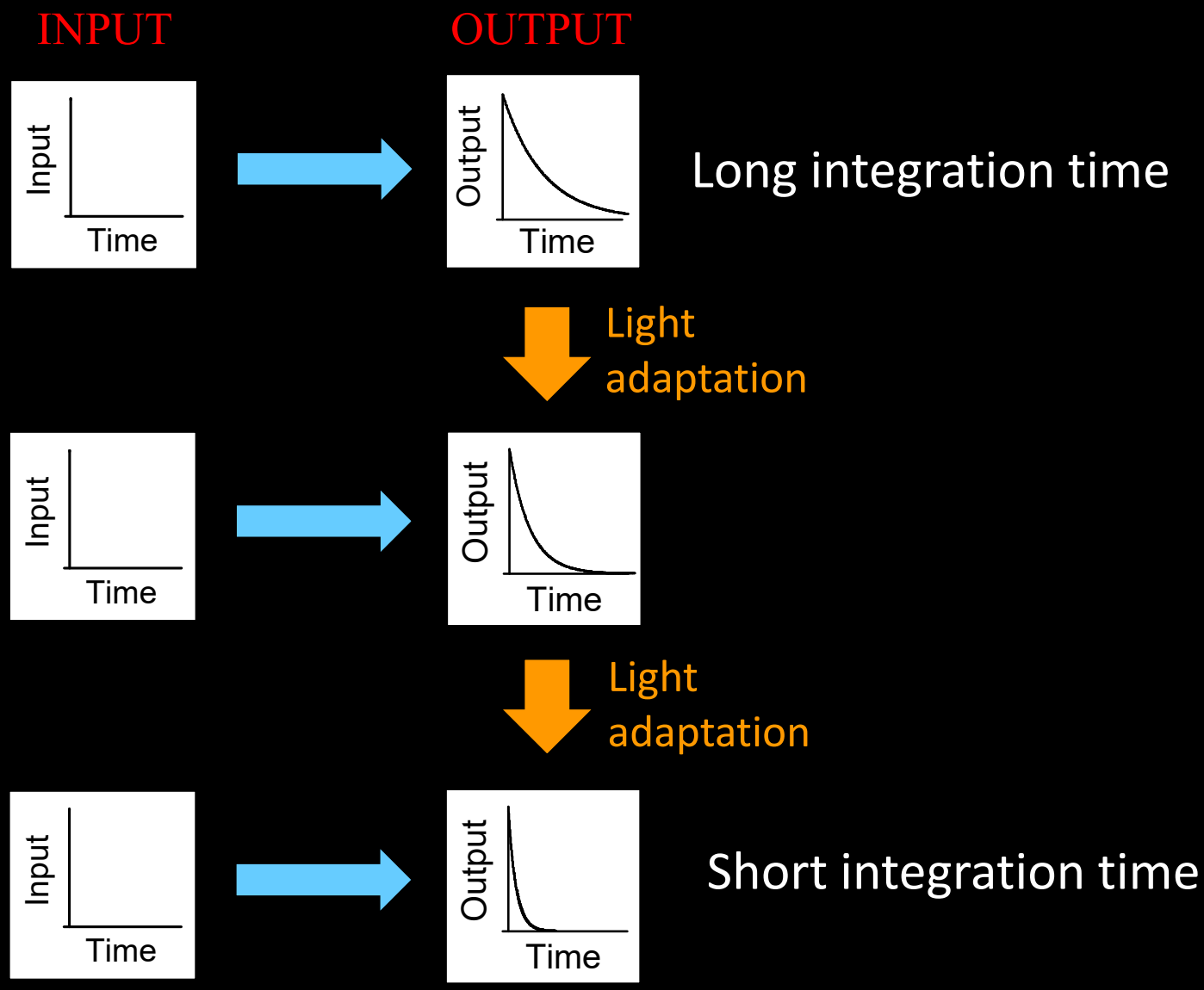


[G*α-PDE6*] dependent Increased rate of hydrolysis of cGMP to GMP



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

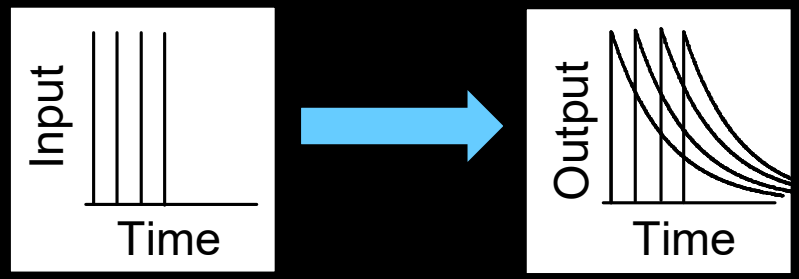
Changing the integration time of the system...



Shortening the integration time of the system increases sensitivity to higher flicker rates...

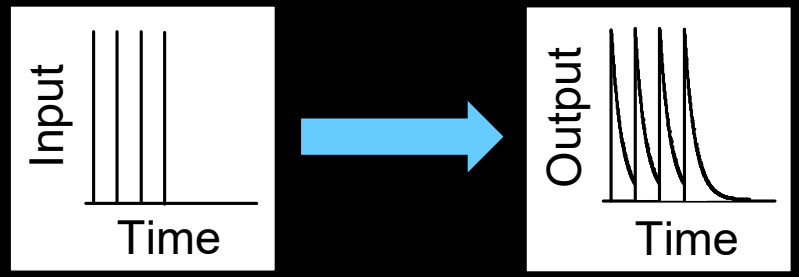
INPUT

OUTPUT



Long integration time

Light adaptation



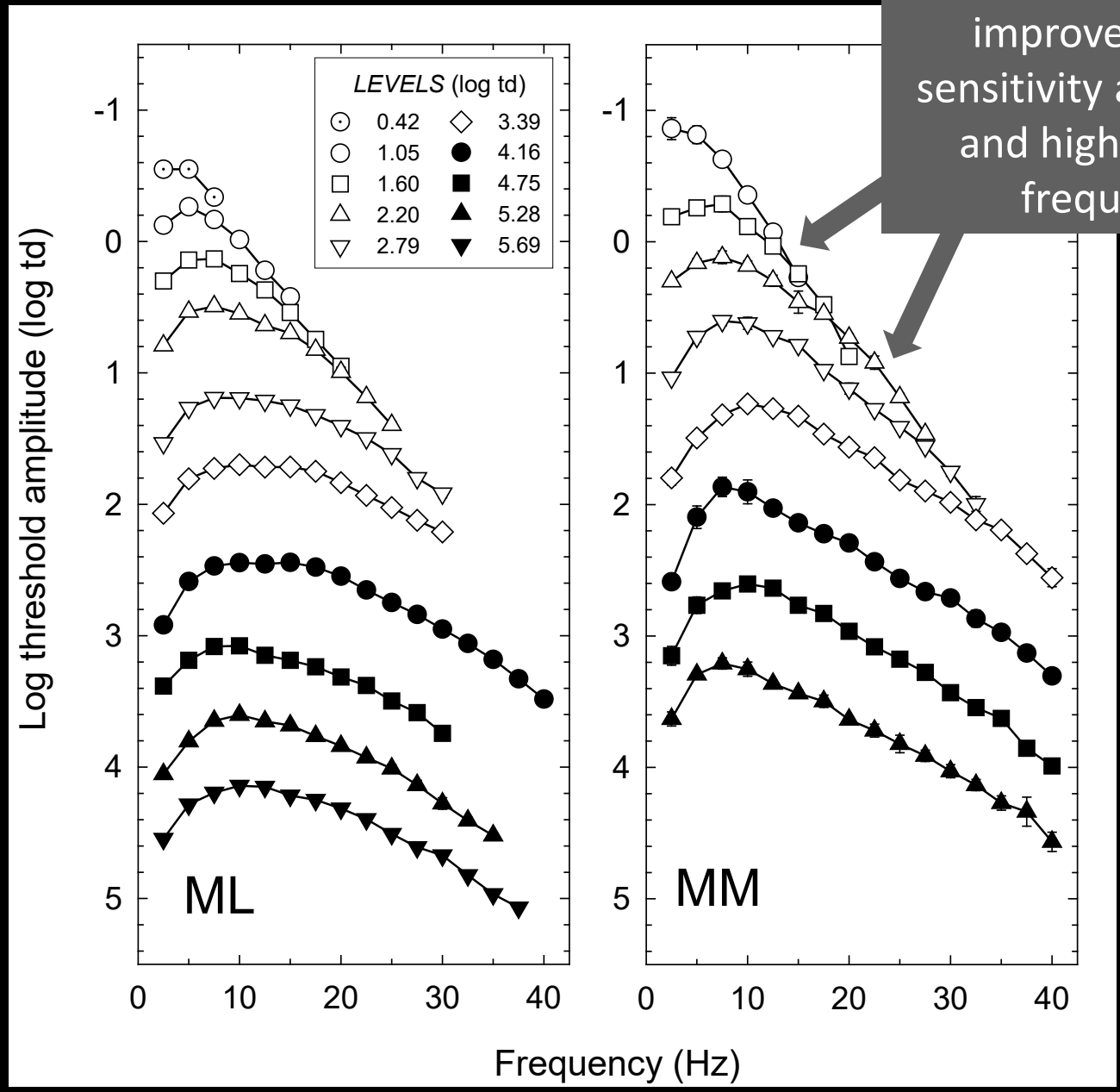
Short integration time

Human temporal response

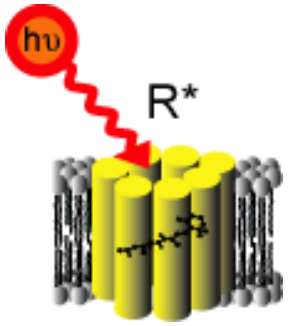
- An excellent way of characterizing the effects of light adaptation psychophysically is to measure changes in the temporal response.

- Focus on changes in temporal sensitivity.

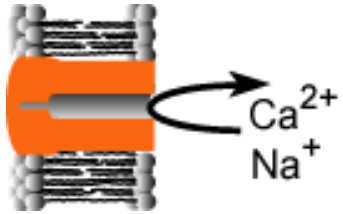
Changes in temporal sensitivity



Mechanisms that simply decrease sensitivity

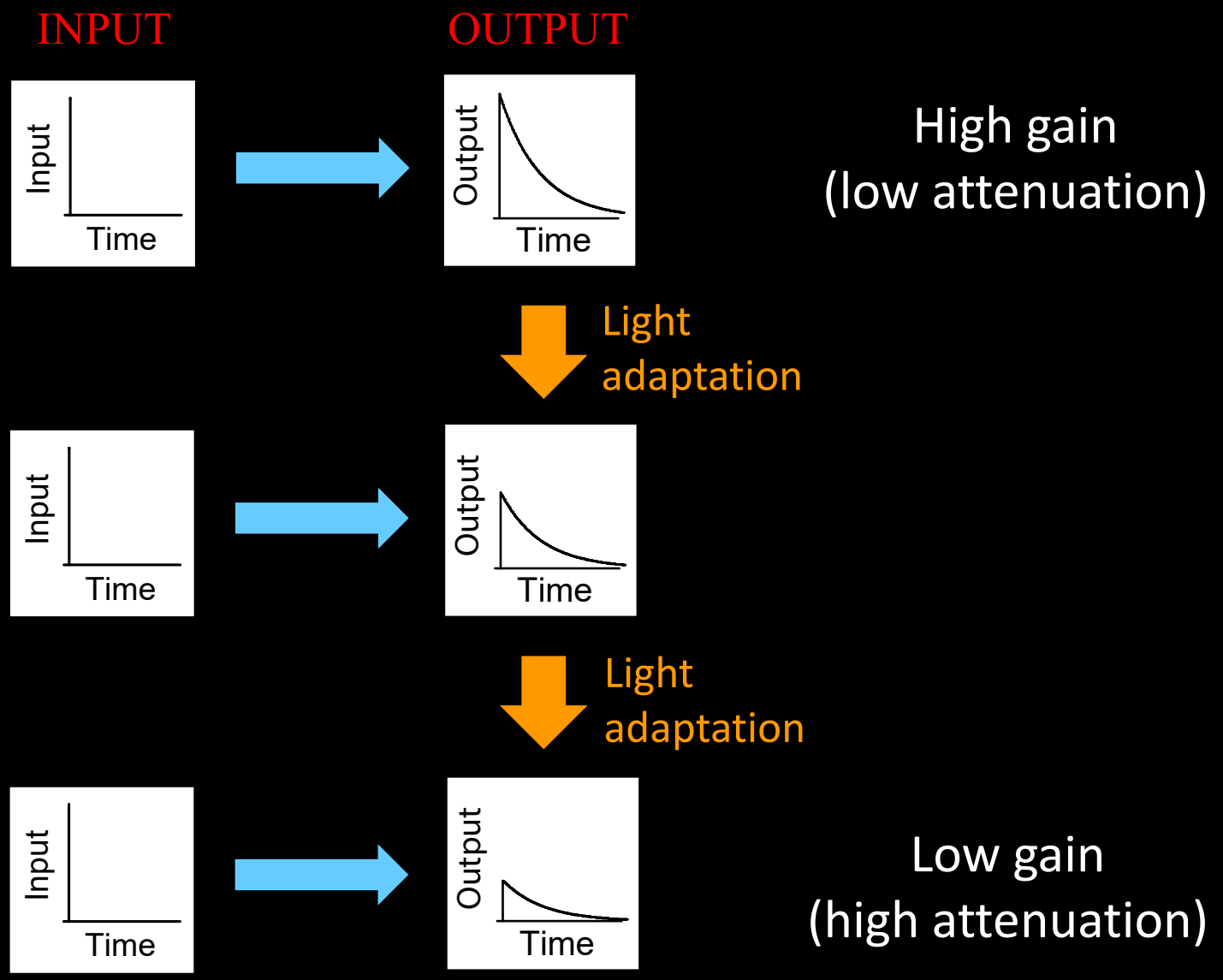


Photopigment bleaching (less photopigment available at high light levels)



Reduction in the number of open CNG-gated channels

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



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

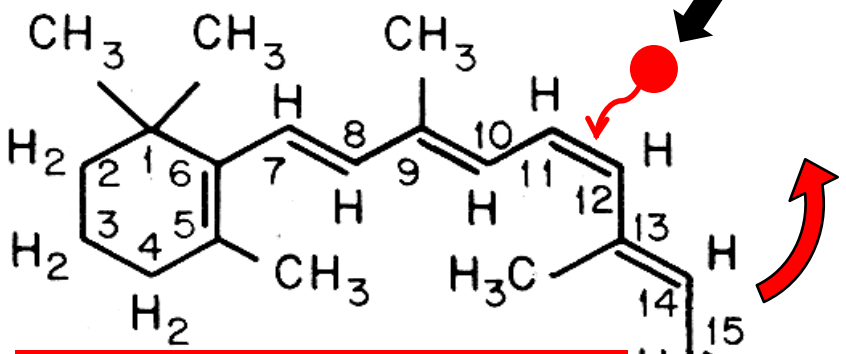
- Cones are 25 - 100 times less sensitive to single photons.
- They catch fewer photons (less visual pigment).
- They respond with faster kinetics (isoforms of transduction cascade).
- They have a much greater ability to adapt to background light.
- They do not saturate at normal environmental light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

TRANSDUCTION AND UNIVARIANCE

Chromophore

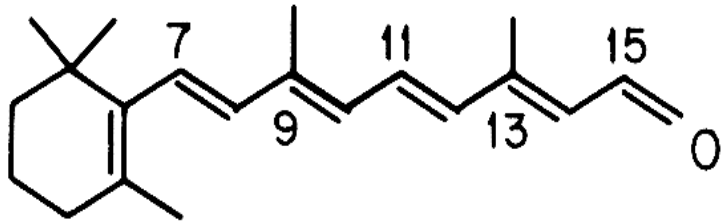
(*chromo-* colour, + *-phore,* producer)
Light-catching portion of any molecule

11-*cis* retinal



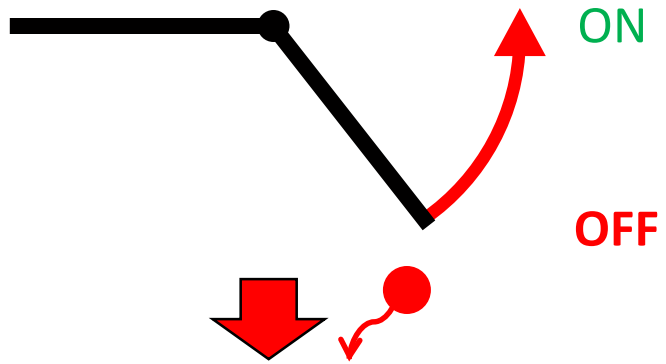
A photon is absorbed

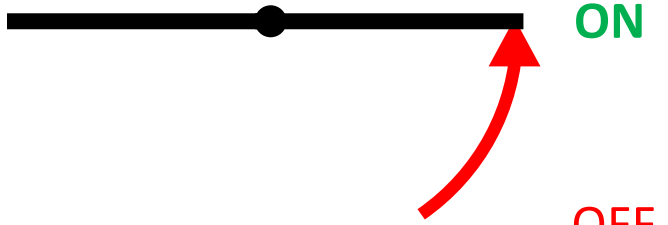
the energy of which initiates a conformational change to…



all-*trans* retinal

Think of the molecule as a photo-sensitive switch!



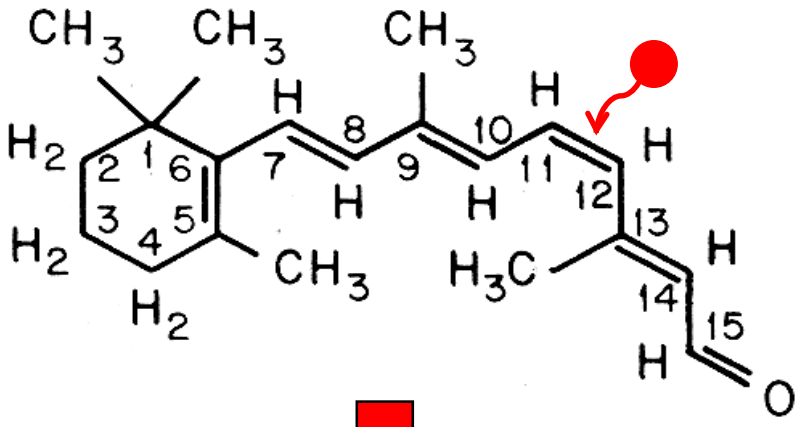


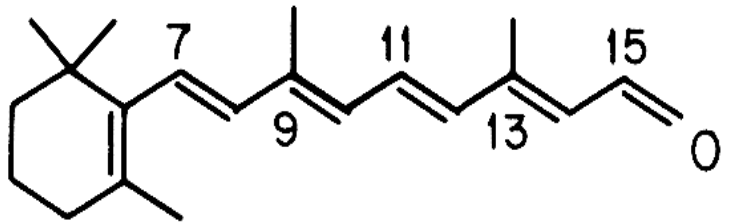
Chromophore

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

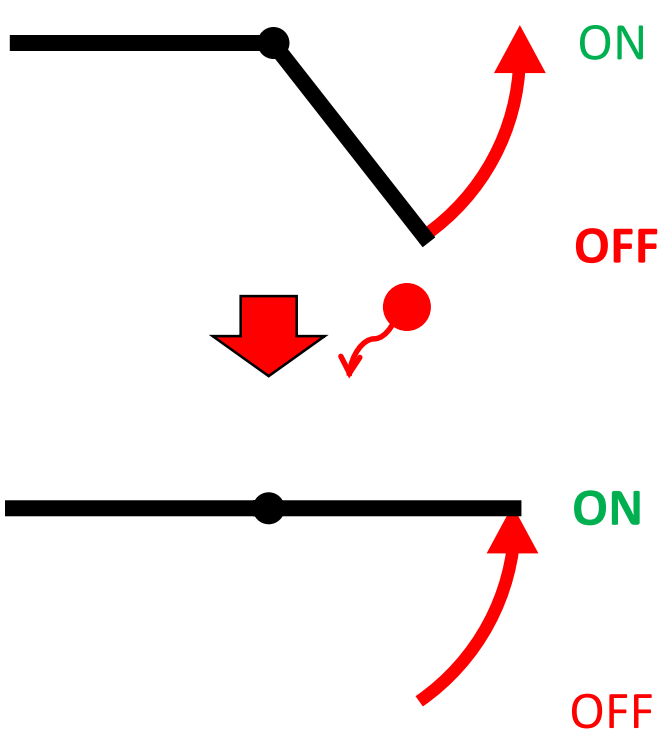
If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.

11-*cis* retinal





all-*trans* retinal



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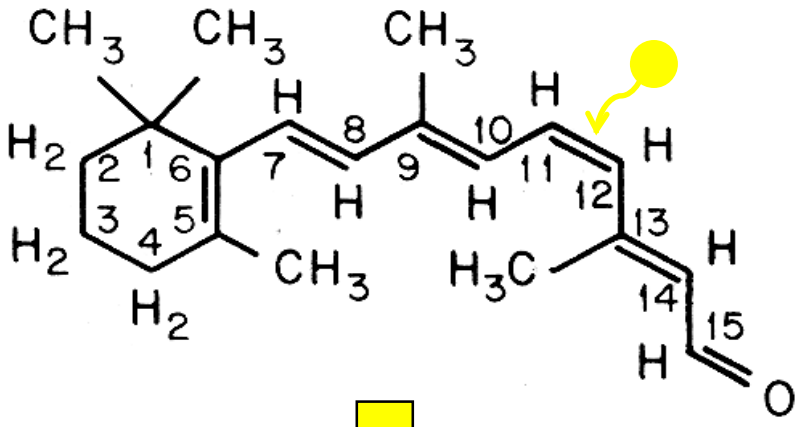
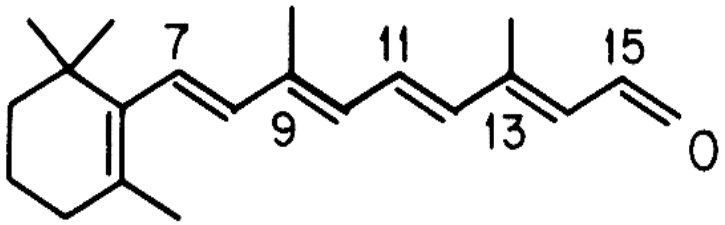
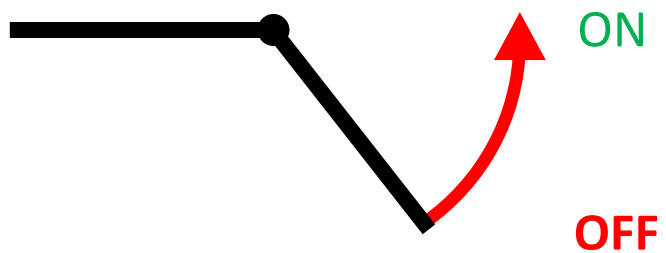
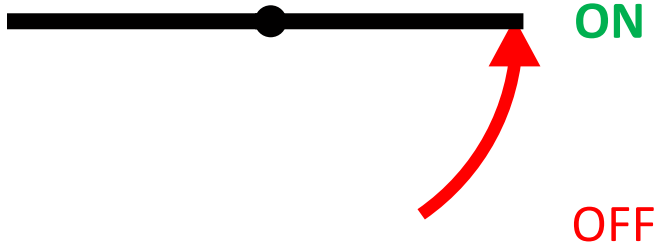




all-*trans* retinal

SAME EFFECT





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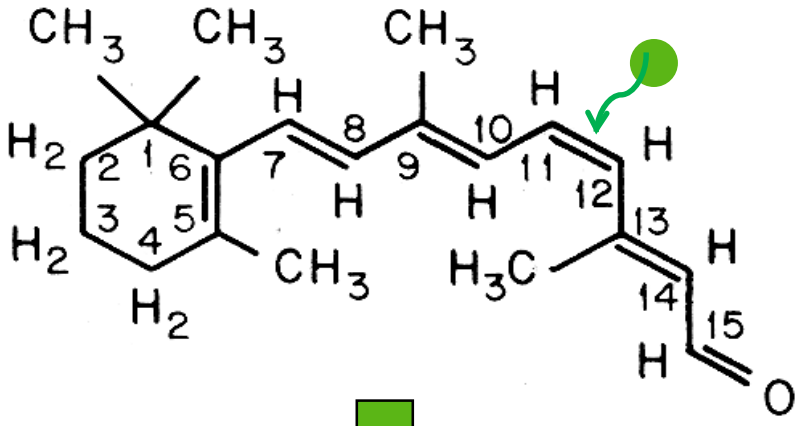
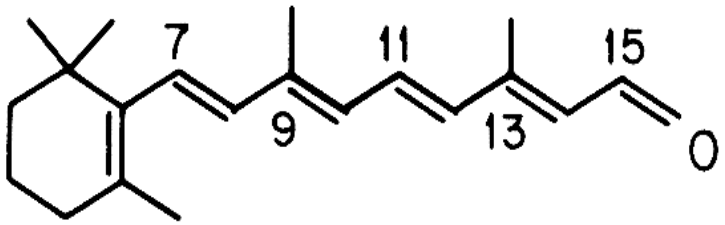
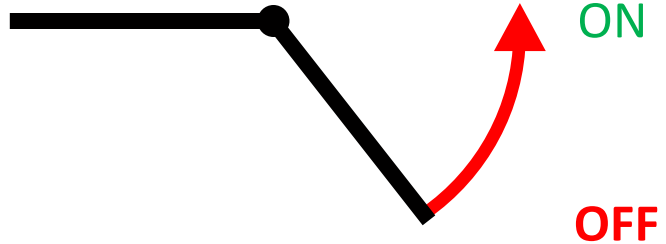
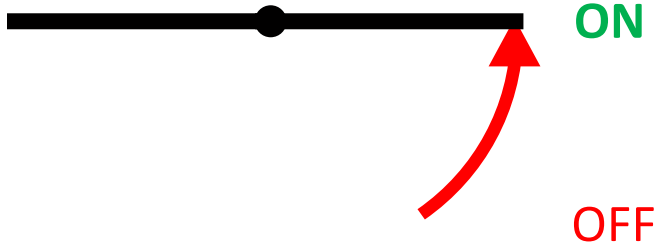




all-*trans* retinal

SAME EFFECT





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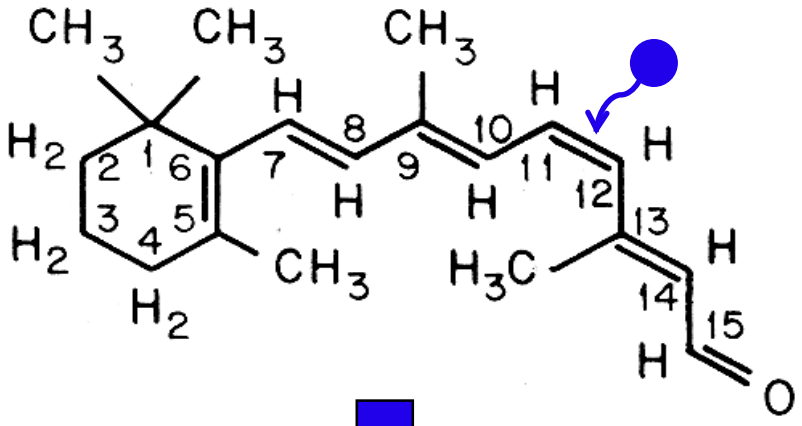
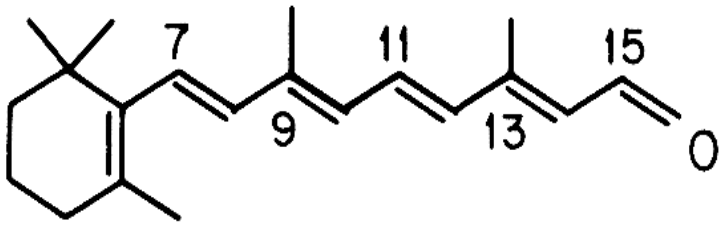
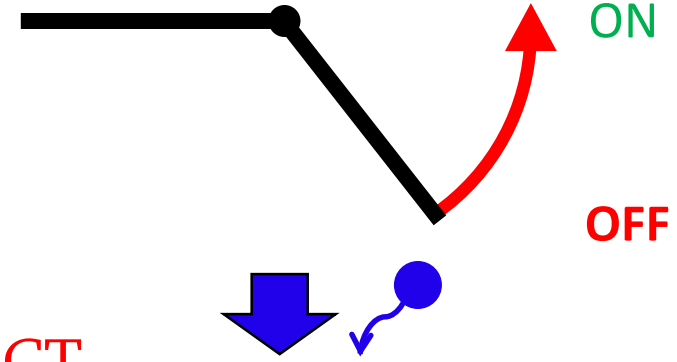
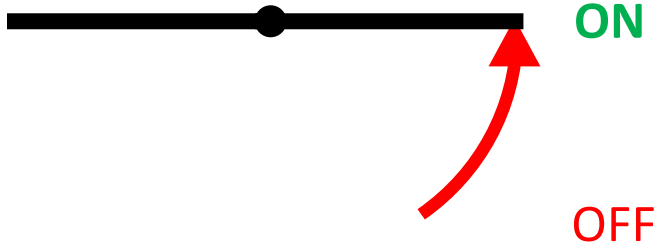




all-*trans* retinal

SAME EFFECT





Chromophore

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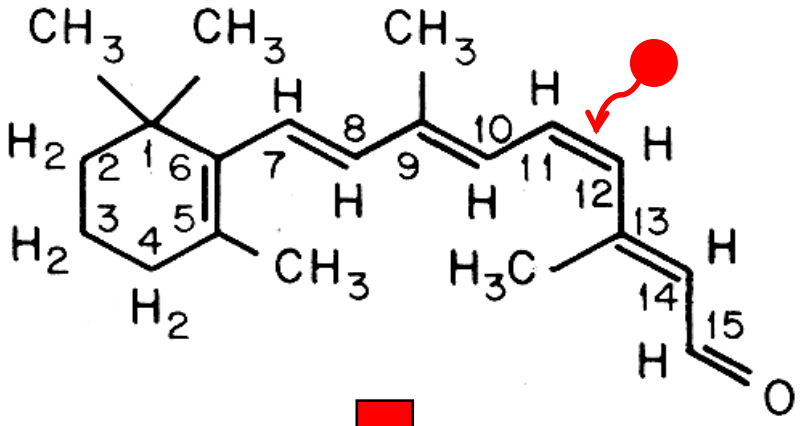
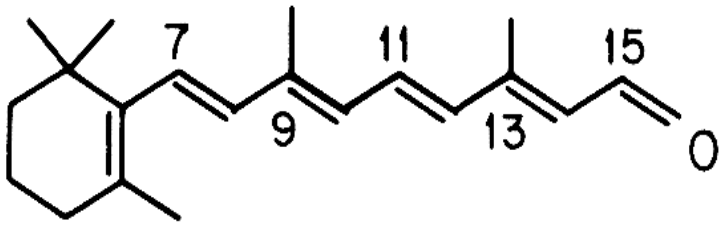
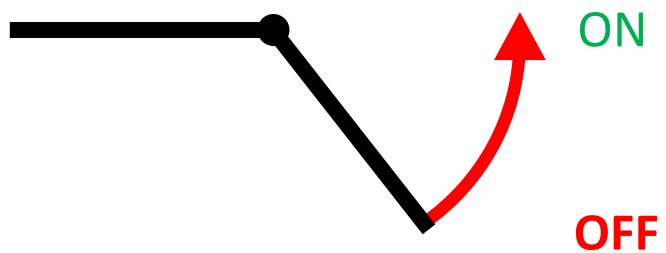
11-*cis* retinal

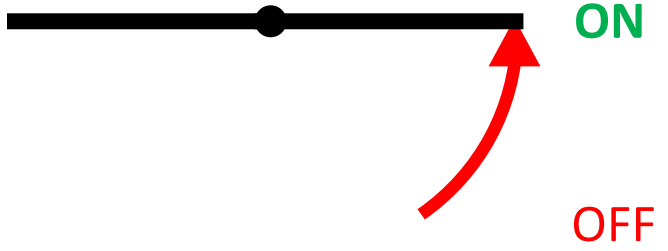




all-*trans* retinal

SAME EFFECT

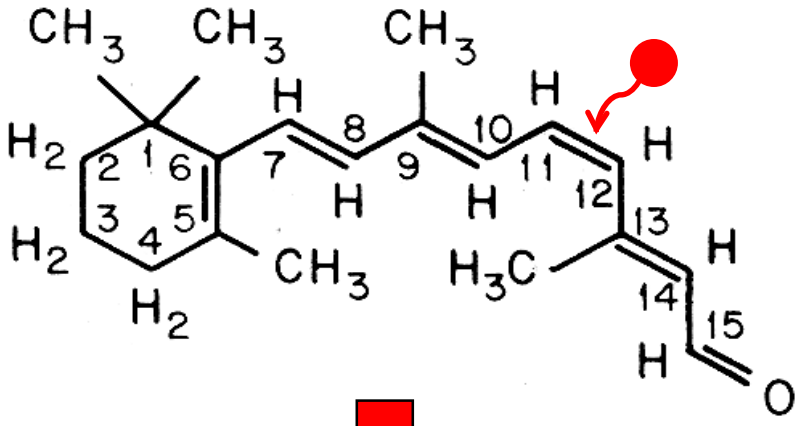


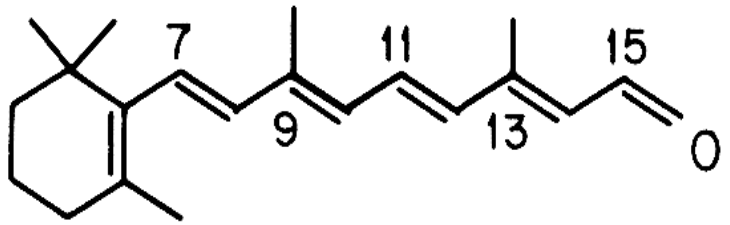


Chromophore

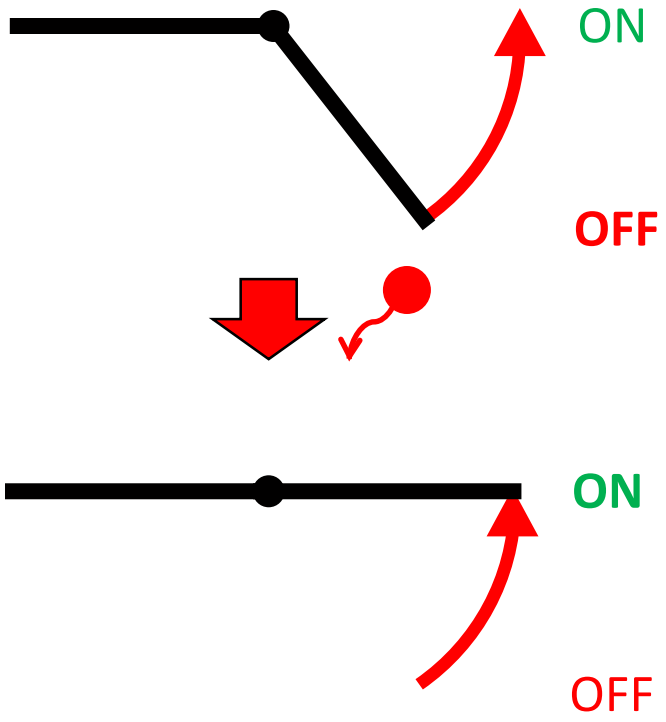
Can this process encode wavelength (colour)?

11-*cis* retinal





all-*trans* retinal

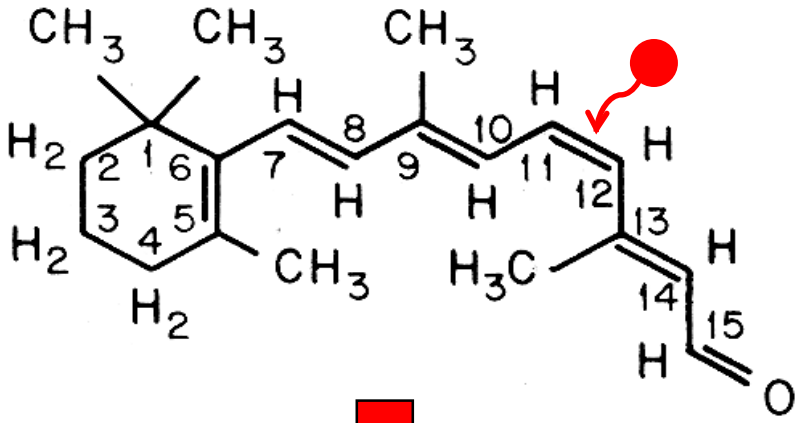


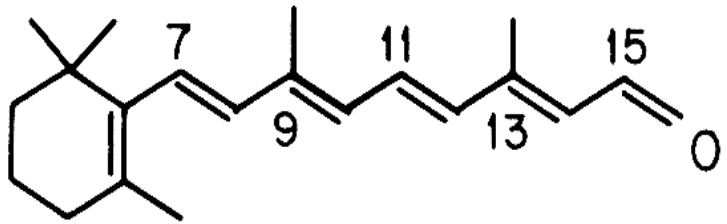
Chromophore

No, it cannot encode wavelength (colour)!

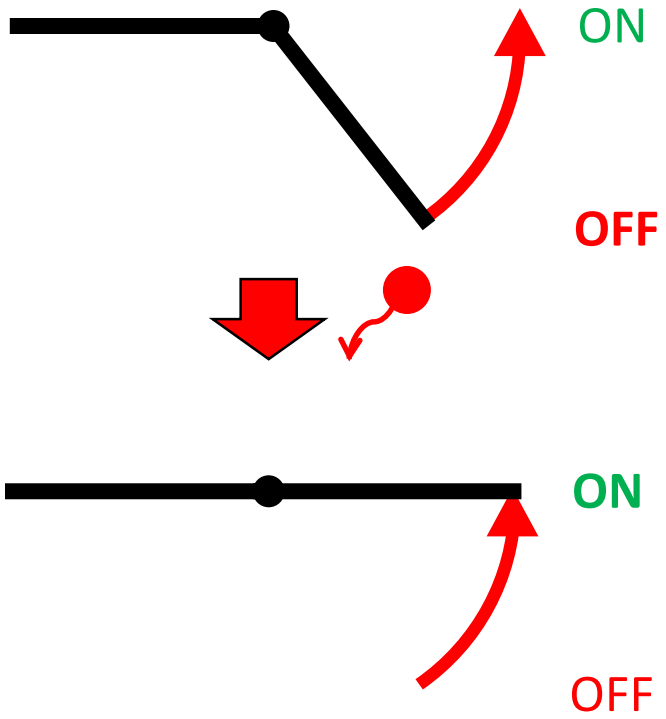
It is "UNIVARIANT"

11-*cis* retinal





all-*trans* retinal



Vision at the photoreceptor stage is relatively simple
because the output of each photoreceptor is:

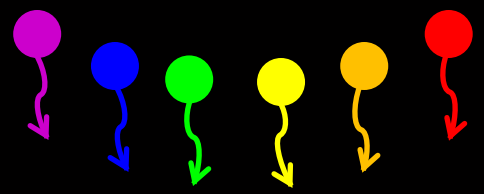
“UNIVARIANT”

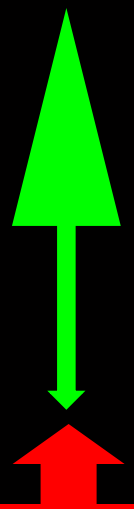
What does univariance mean in practice?

Use Middle-wavelength-sensitive (M) cones as an example…

UNIVARIANCE

Crucially, the effect of any absorbed photon is *independent* of its wavelength.



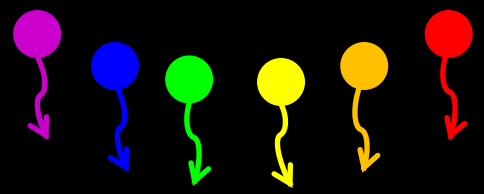


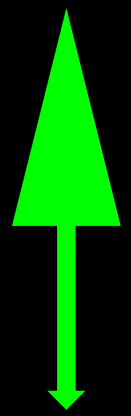
M-cone

So, if you monitor the cone output, you can't tell which wavelength (energy) of photon has been absorbed.

UNIVARIANCE

Crucially, the effect of any absorbed photon is *independent* of its wavelength.



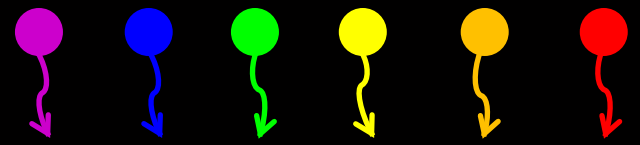


M-cone

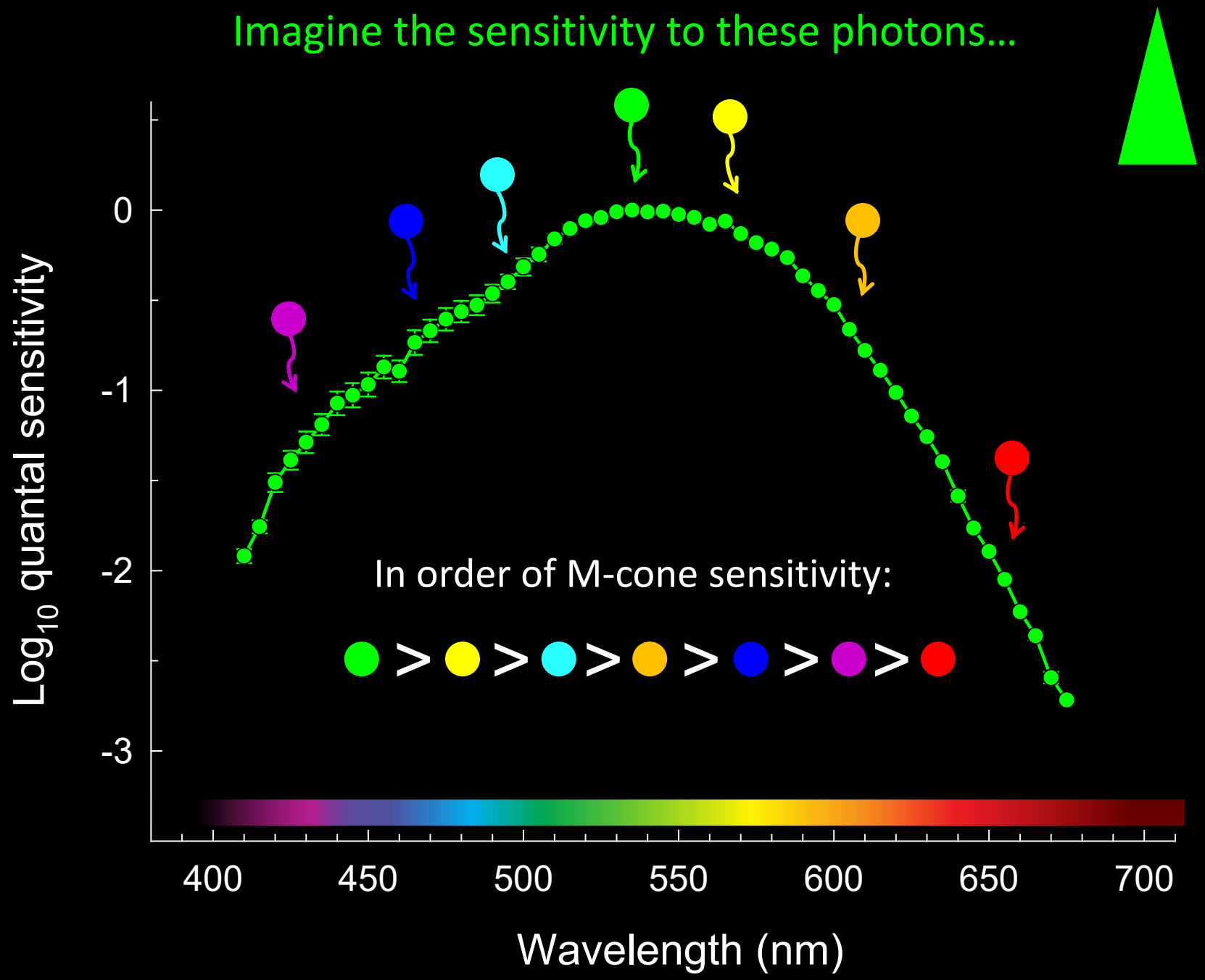
All the photoreceptor effectively does is to count photons.

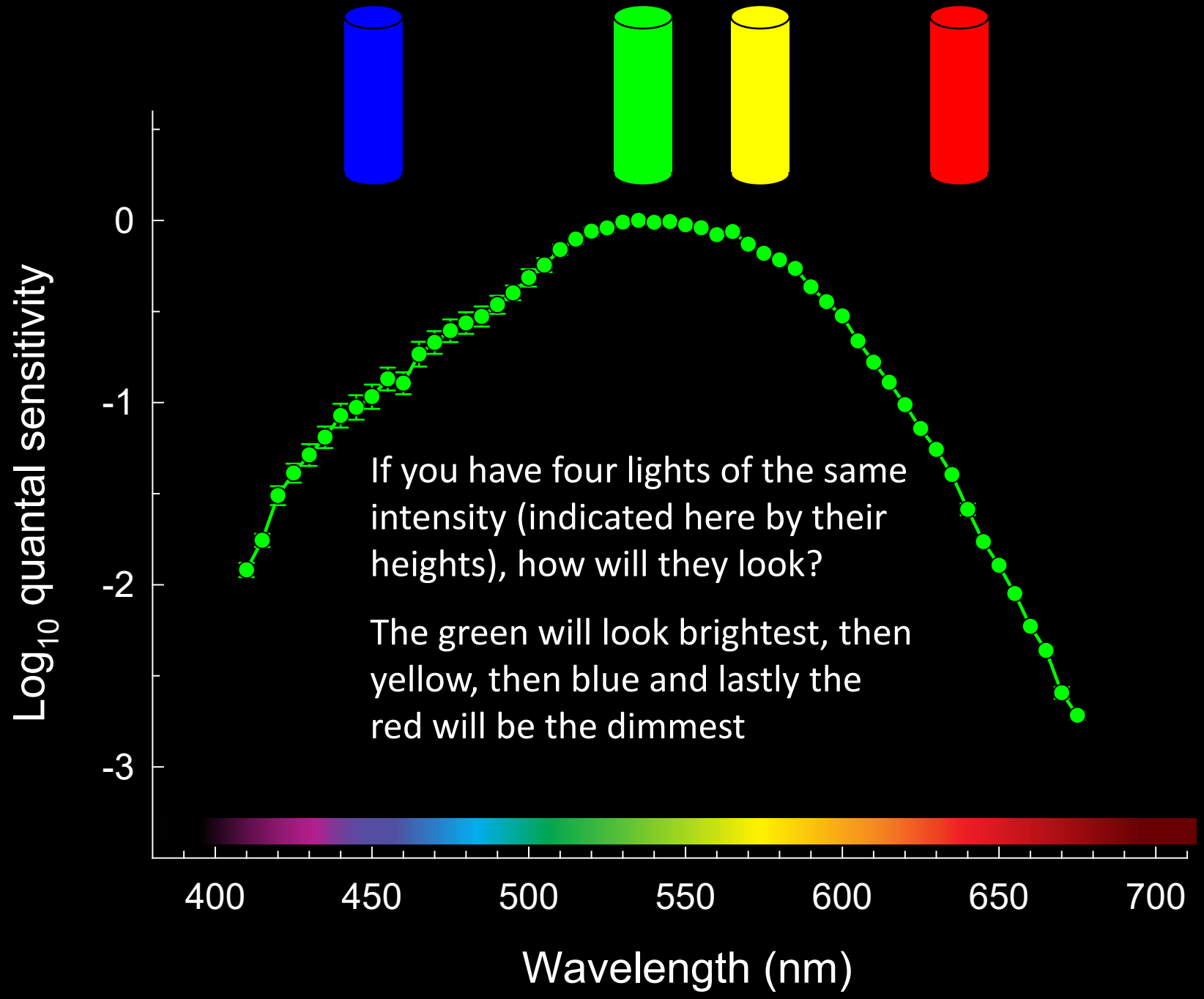
UNIVARIANCE

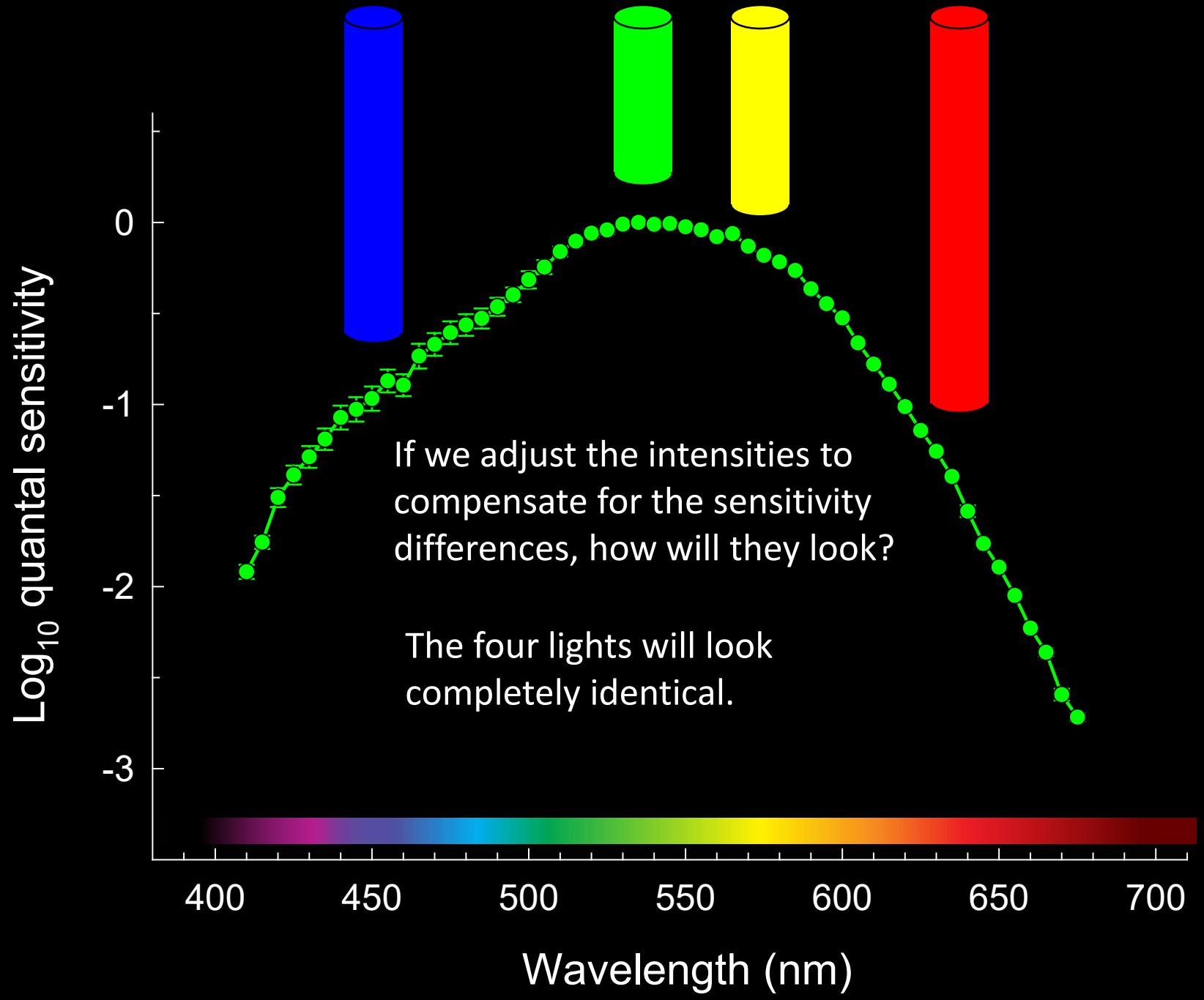
What does vary with wavelength is the
probability that a photon will be absorbed.

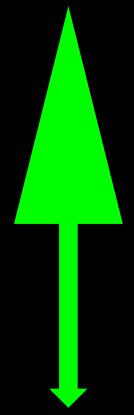


This is reflected in what is called a
“spectral sensitivity function”.


Imagine the sensitivity to these photons…
0
-1
-2
-3
Log10 quantal sensitivity
In order of M-cone sensitivity:
> > > > > >
400
450
500
550
600
650
700
Wavelength (nm)


0
-1
-2
-3
Log10 quantal sensitivity
400
450
500
550
600
650
700
Wavelength (nm)
If you have four lights of the same intensity (indicated here by their heights), how will they look?
The green will look brightest, then yellow, then blue and lastly the red will be the dimmest


0
-1
-2
-3
Log10 quantal sensitivity
If we adjust the intensities to compensate for the sensitivity differences, how will they look?
The four lights will look completely identical.
400
450
500
550
600
650
700
Wavelength (nm)



M-cone

Changes in light intensity are confounded with changes in colour (wavelength)

Vision at the photoreceptor stage is relatively simple because the output of each photoreceptor is:

UNIVARIANT

If we had only one photoreceptor type in our eyes, what colours would we see?

We would be colour-blind…

Examples: night vision, blue cone monochromats

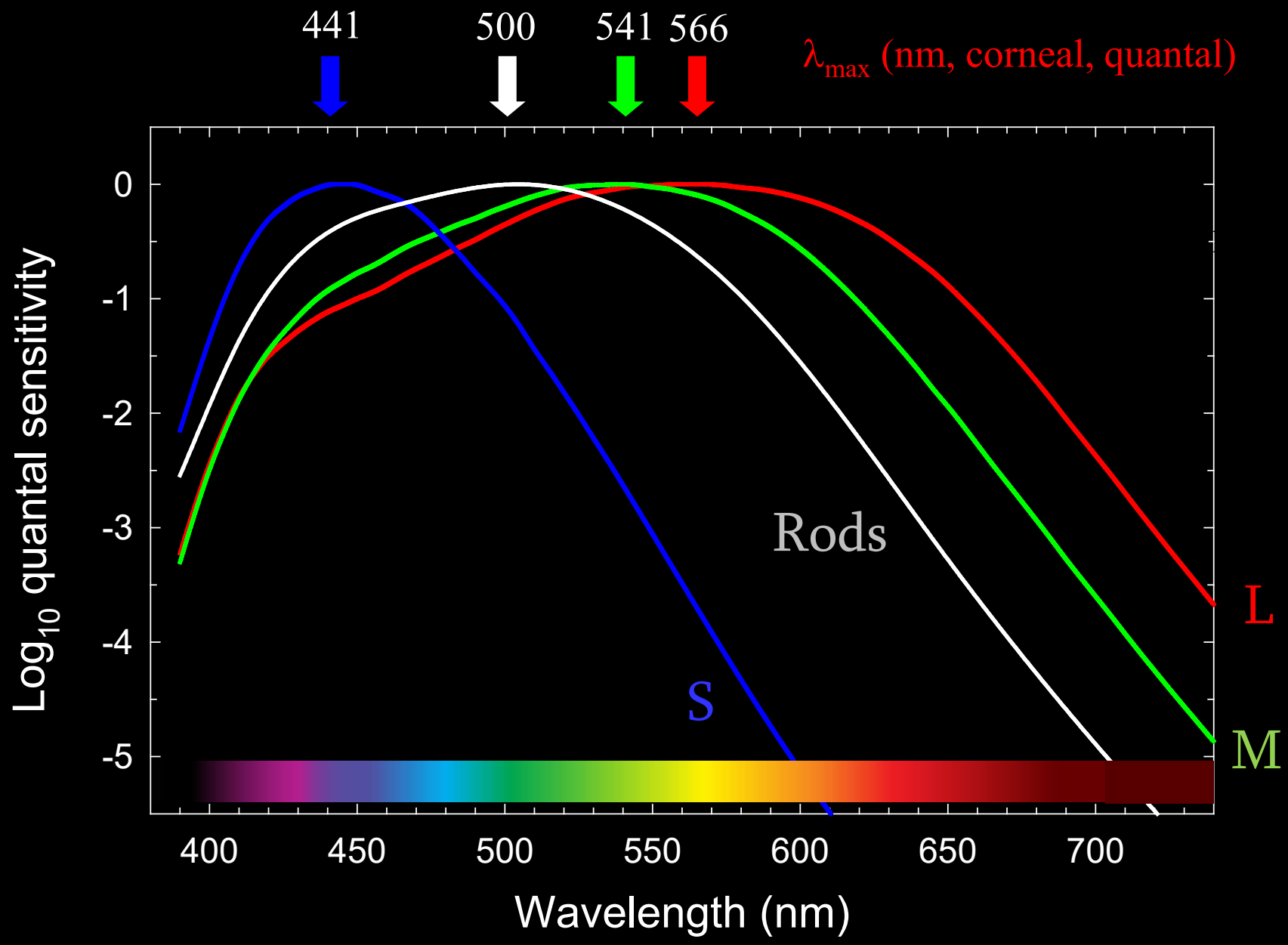
Univariance

If a cone is *n* times less sensitive to light A than to light B, then if A is set to be *n* times brighter than B, the two lights will appear identical whatever their wavelengths.

What does vary with wavelength is the *probability* that a photon will be absorbed, and this relationship is different for the four different photoreceptors.

This is reflected in the photoreceptor spectral sensitivity functions…

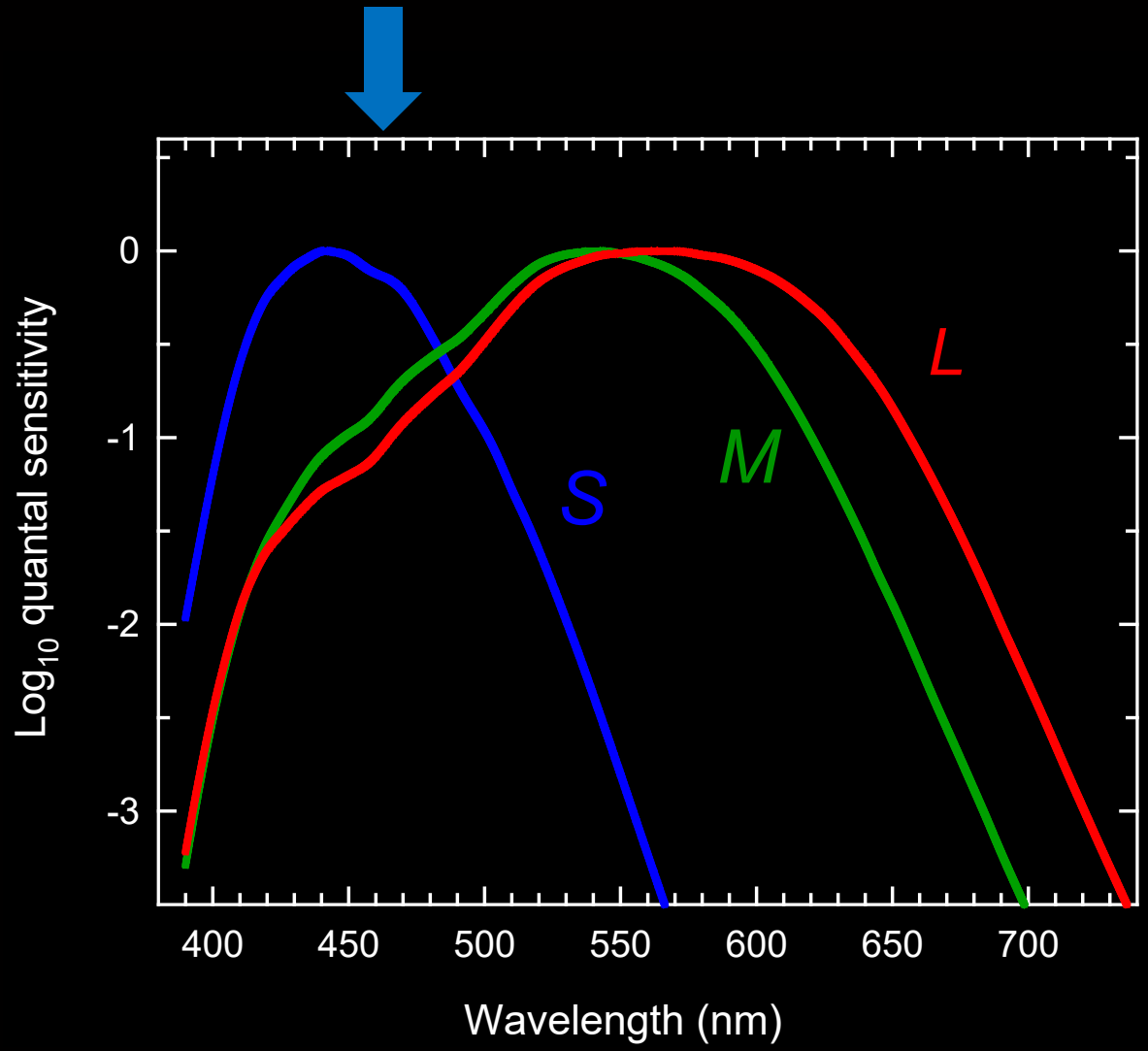
Four human photoreceptors have different spectral sensitivities



So, if each photoreceptor is colour-blind, how do we see colour?

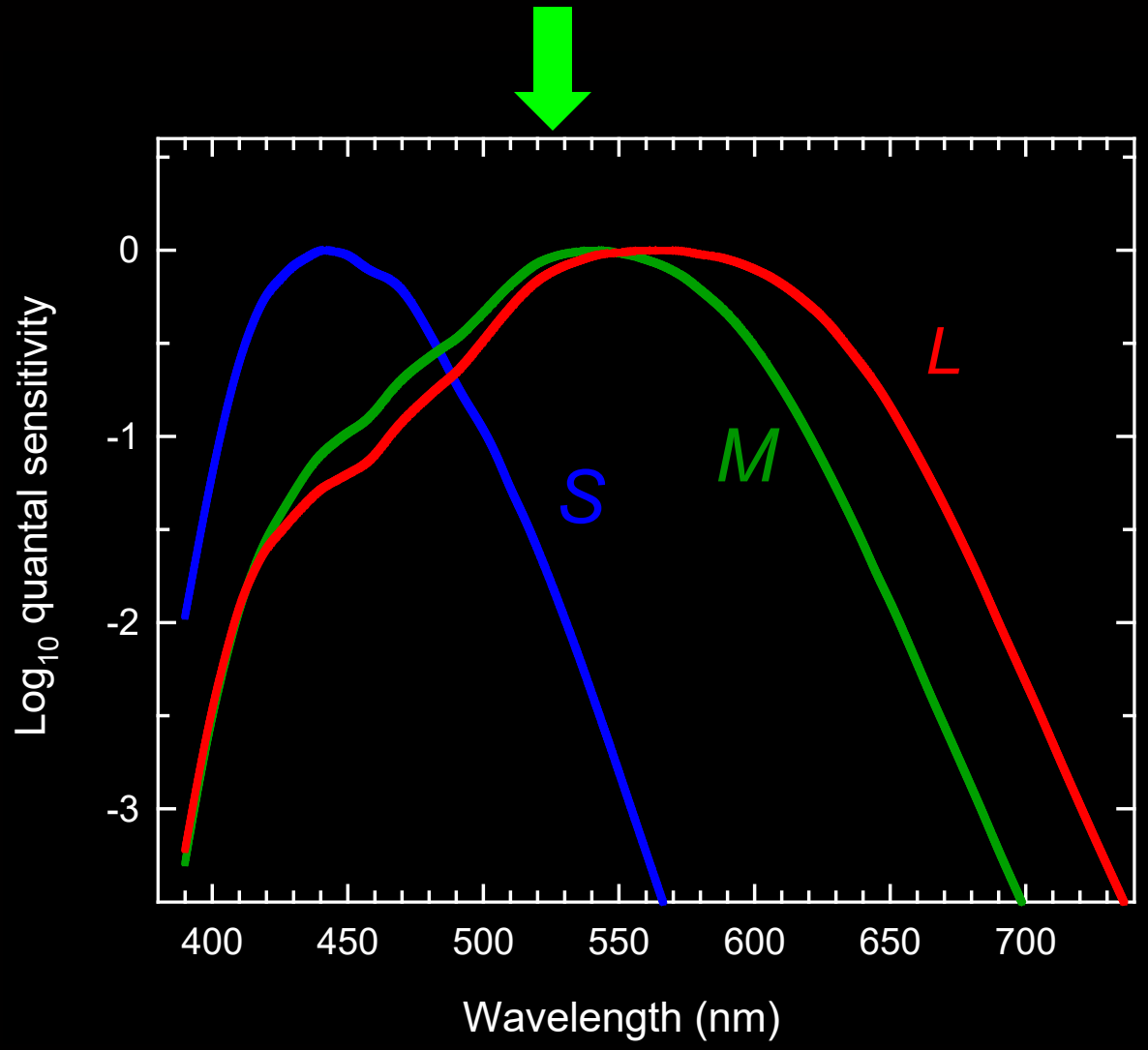
Or to put it another way: How is colour encoded at the input to the visual system?

Colour is encoded by the relative cone outputs



Blue light

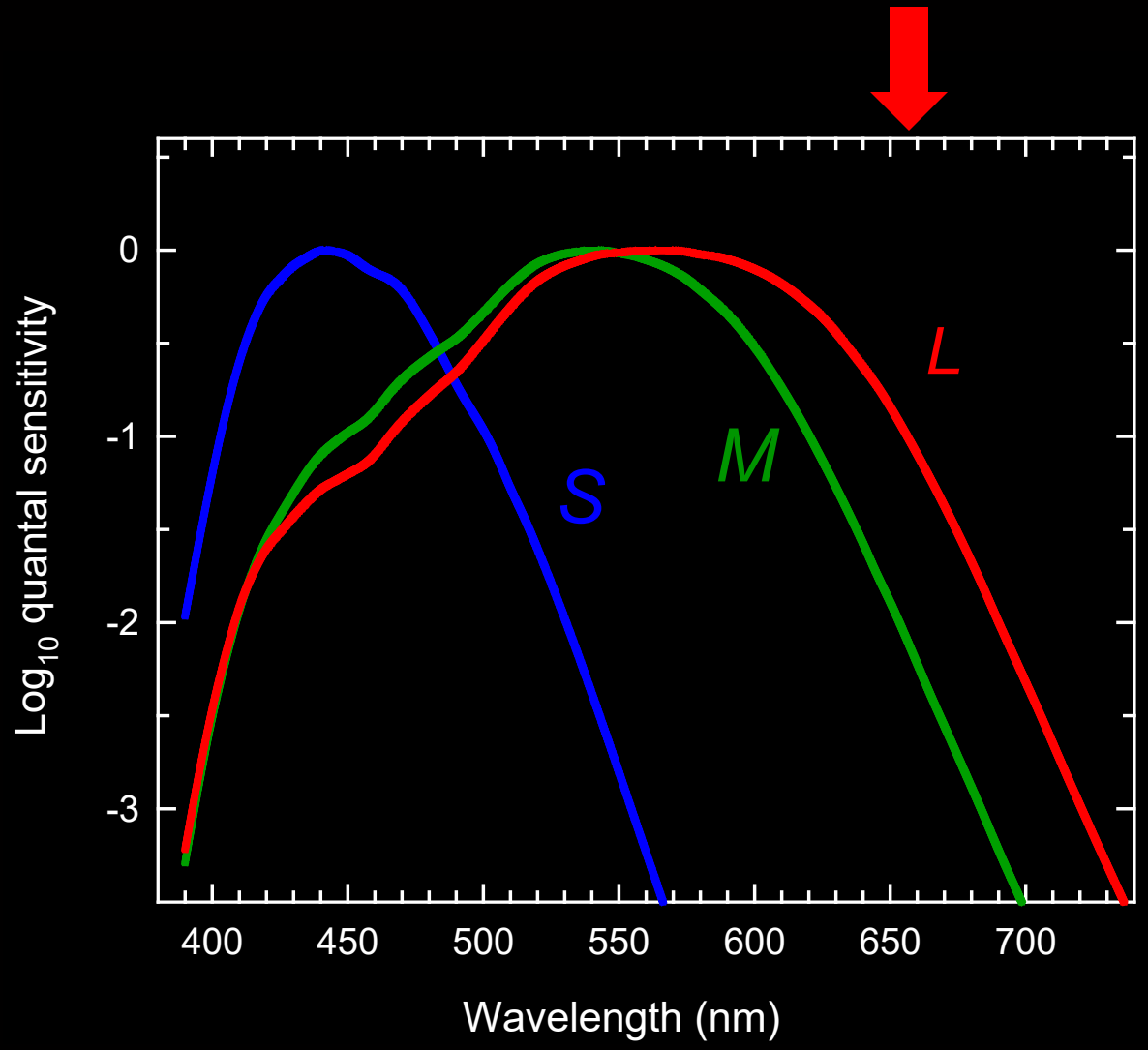
Colour is encoded by the relative cone outputs



Blue light

Green light

Colour is encoded by the relative cone outputs



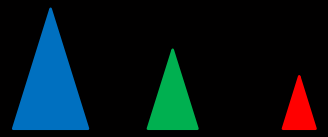
Blue light

Green light

Red light

Colour is encoded by the relative cone outputs

Blue light



Red light

Green light

Purple light

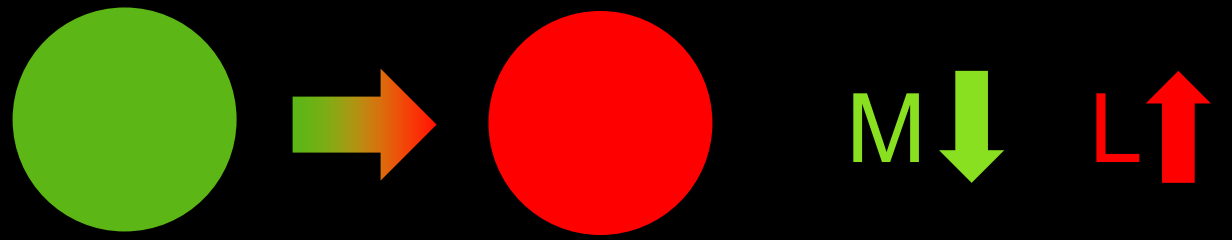
Yellow light

White light

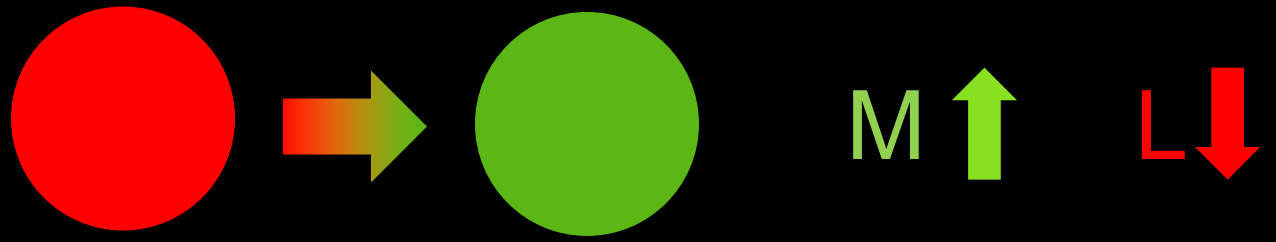
Because there are three univariant cones in the eye, human colour vision is a three-variable “trichromatic” system that depends on the relative outputs of the three cones.

TRICHROMACY

A change in colour from green to red causes a relative increase in the L-cone output but causes a decrease in the M-cone output.

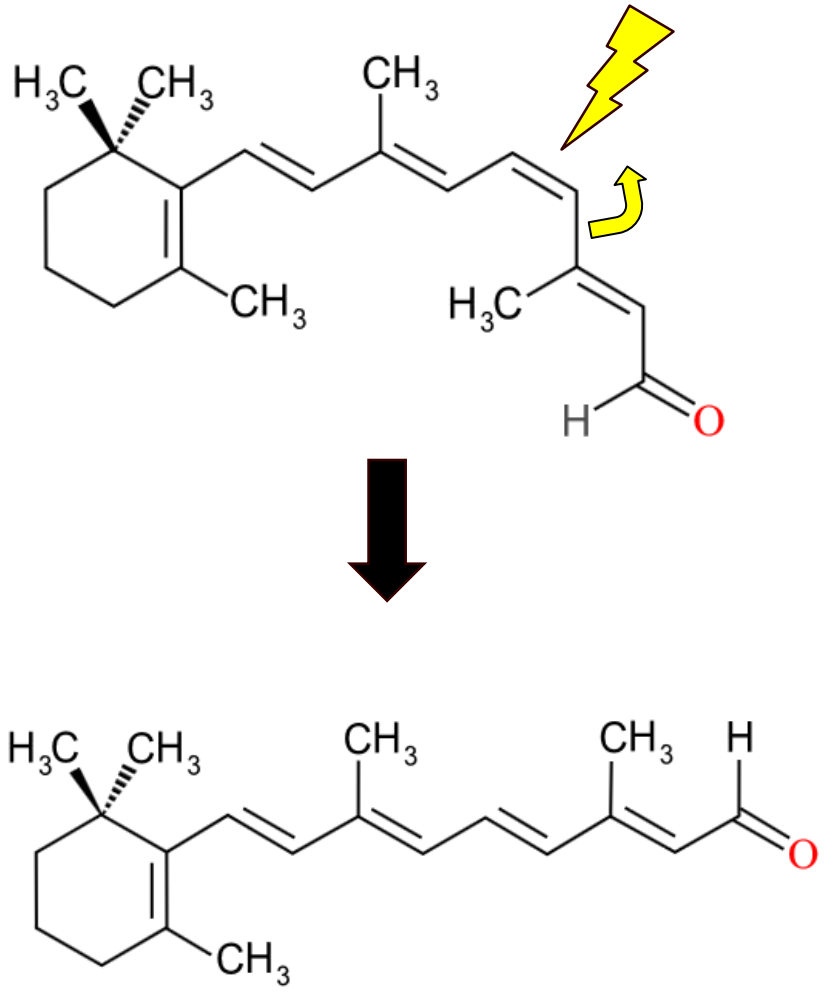


A change in colour from red to green causes a relative increase in the M-cone output but causes a decrease in the L-cone output.



Thus, colour can be encoded by *comparing* the outputs of different cone types…

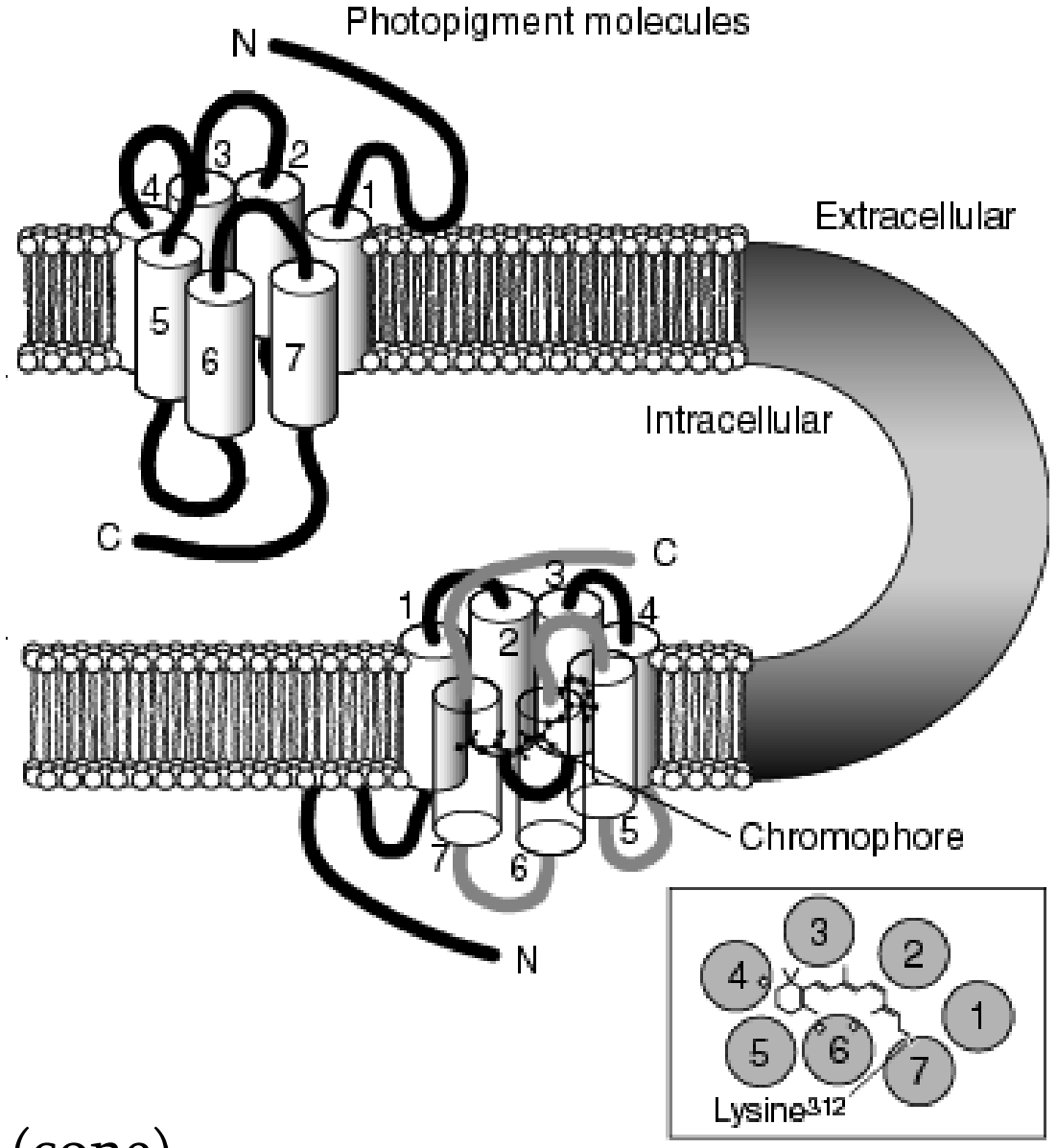
Photopigments and spectral tuning



The spectral sensitivities of the photopigments depend on the energy required to initiate the rotation of the chromophore from its 11-*cis* form to its all-*trans* form.

But the same chromophore is used in all four human photo-pigments.

So how is the initiation energy modified?

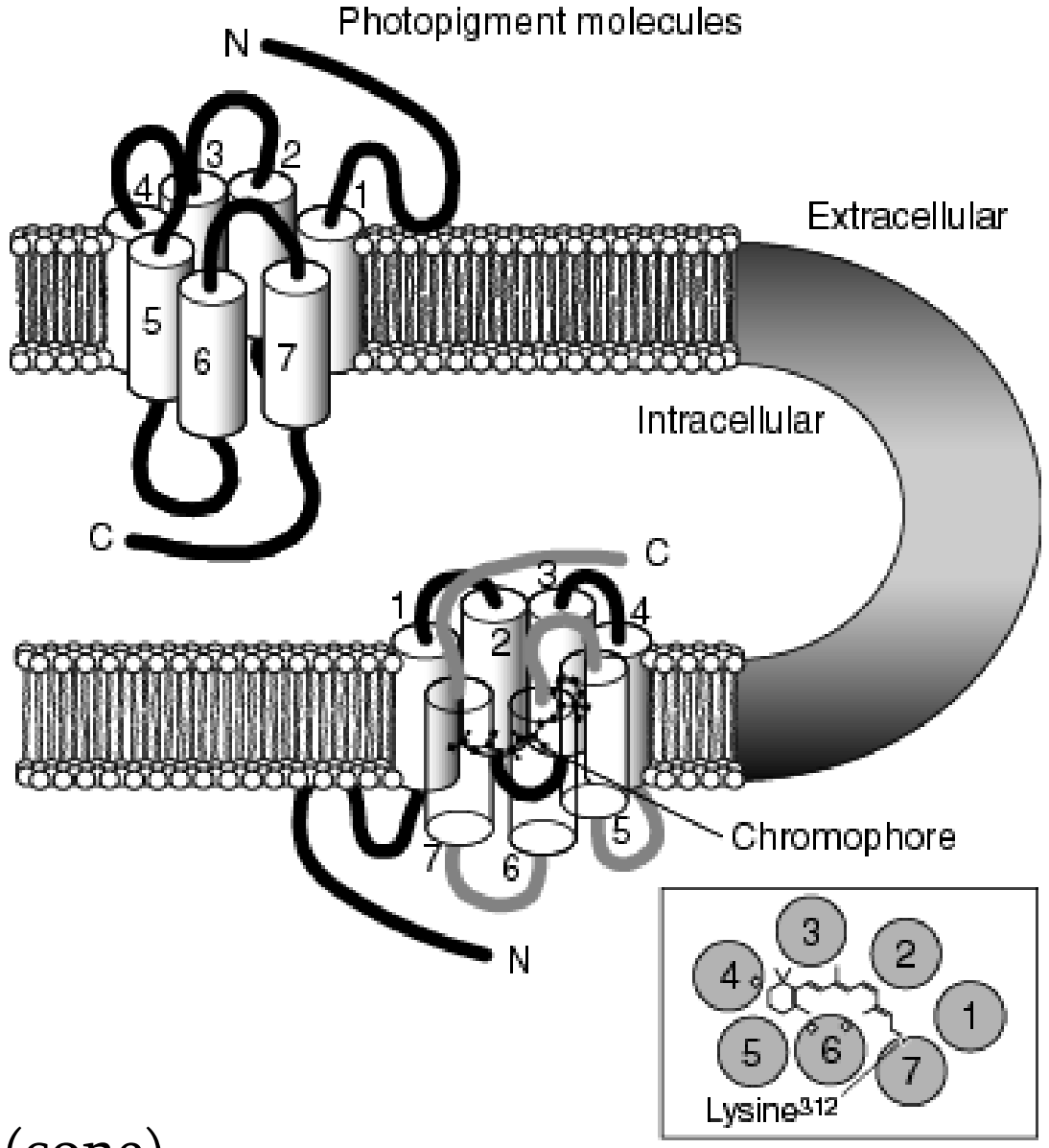


Photopigment molecule (cone)

From Sharpe, Stockman, Jägle & Nathans, 1999

But the same chromophore is used in all four human photo-pigments.

The initiation energy is altered by changing the amino acids in the surrounding opsin molecule.

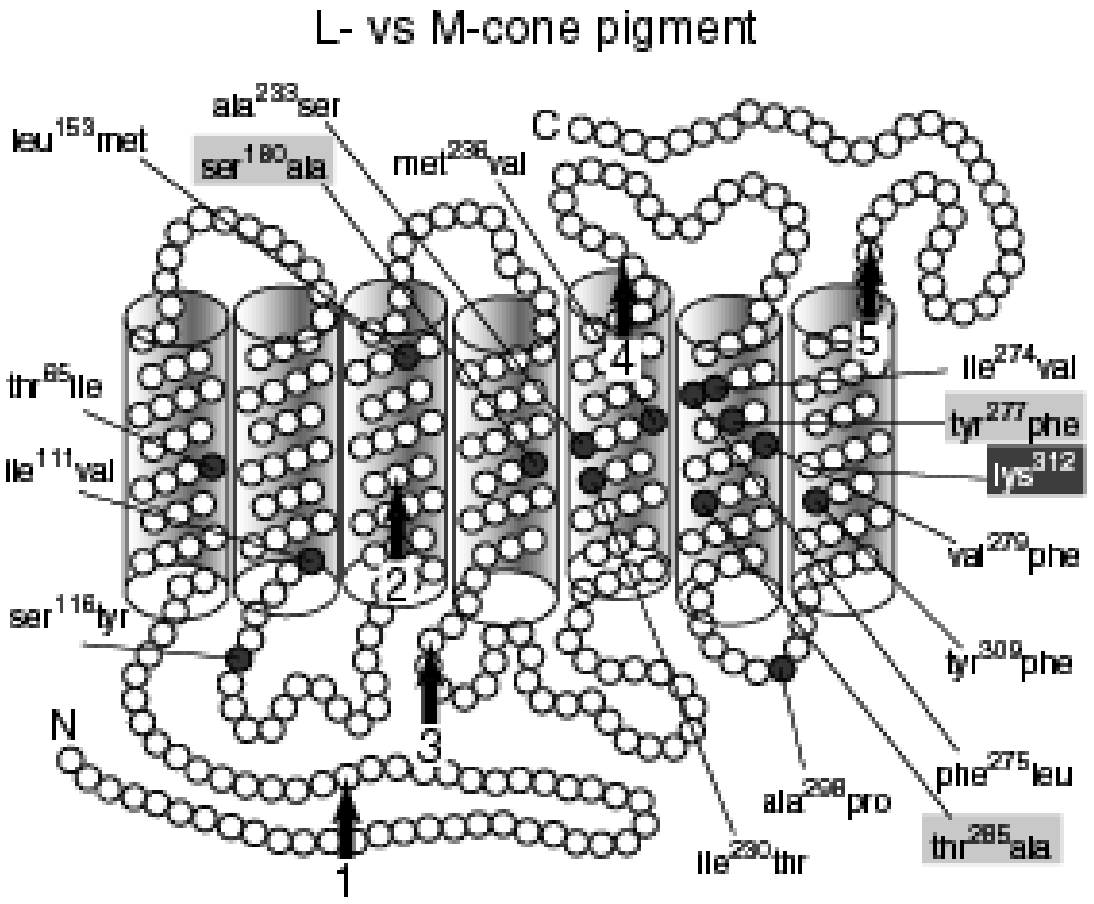


Photopigment molecule (cone)

From Sharpe, Stockman, Jägle & Nathans, 1999

Opsin differences

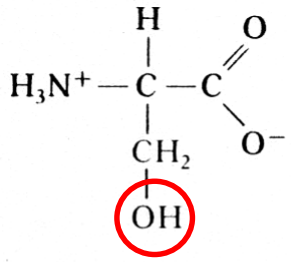
There are only 15 amino acid differences between L and M: 96% identical



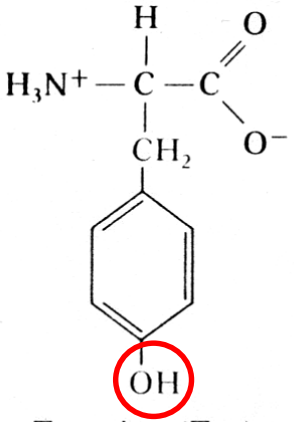
From Sharpe, Stockman, Jägle & Nathans, 1999

Three main amino acid differences are responsible for the spectral sensitivity difference between M and L.

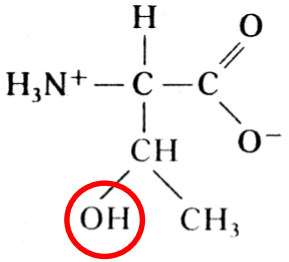
POLAR



Serine (Ser)



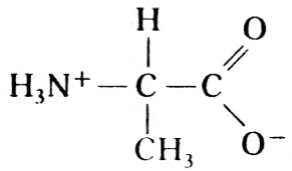
Tyrosine (Tyr)



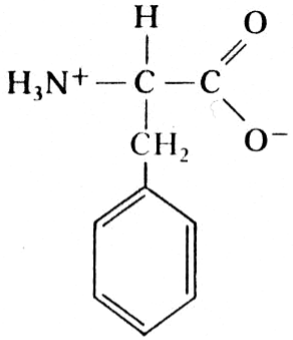
Threonine (Thr)

LWS
all with OH group

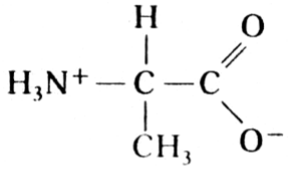
NON-POLAR



Alanine (Ala)



Phenylalanine (Phe)

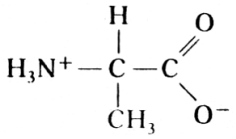
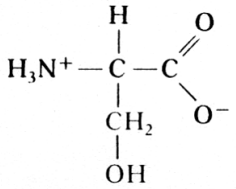
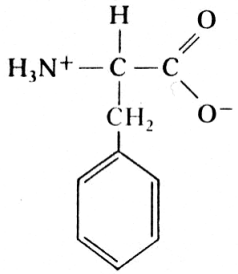
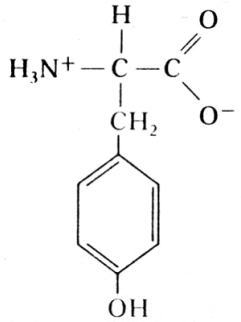
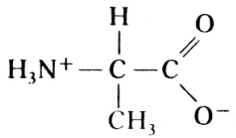
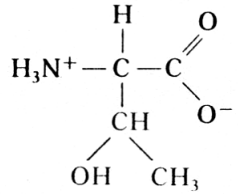


Alanine (Ala)

MWS

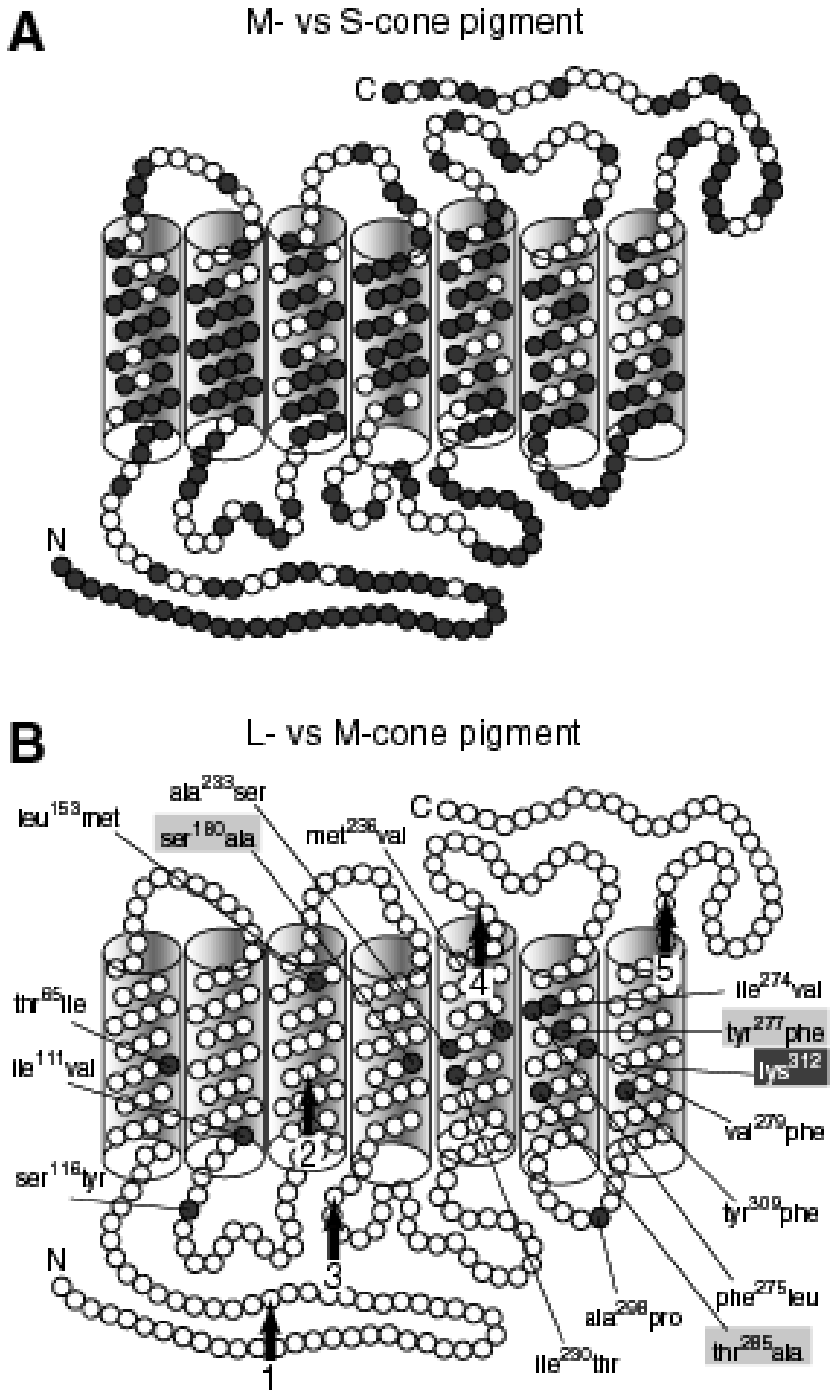
180 277 285

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

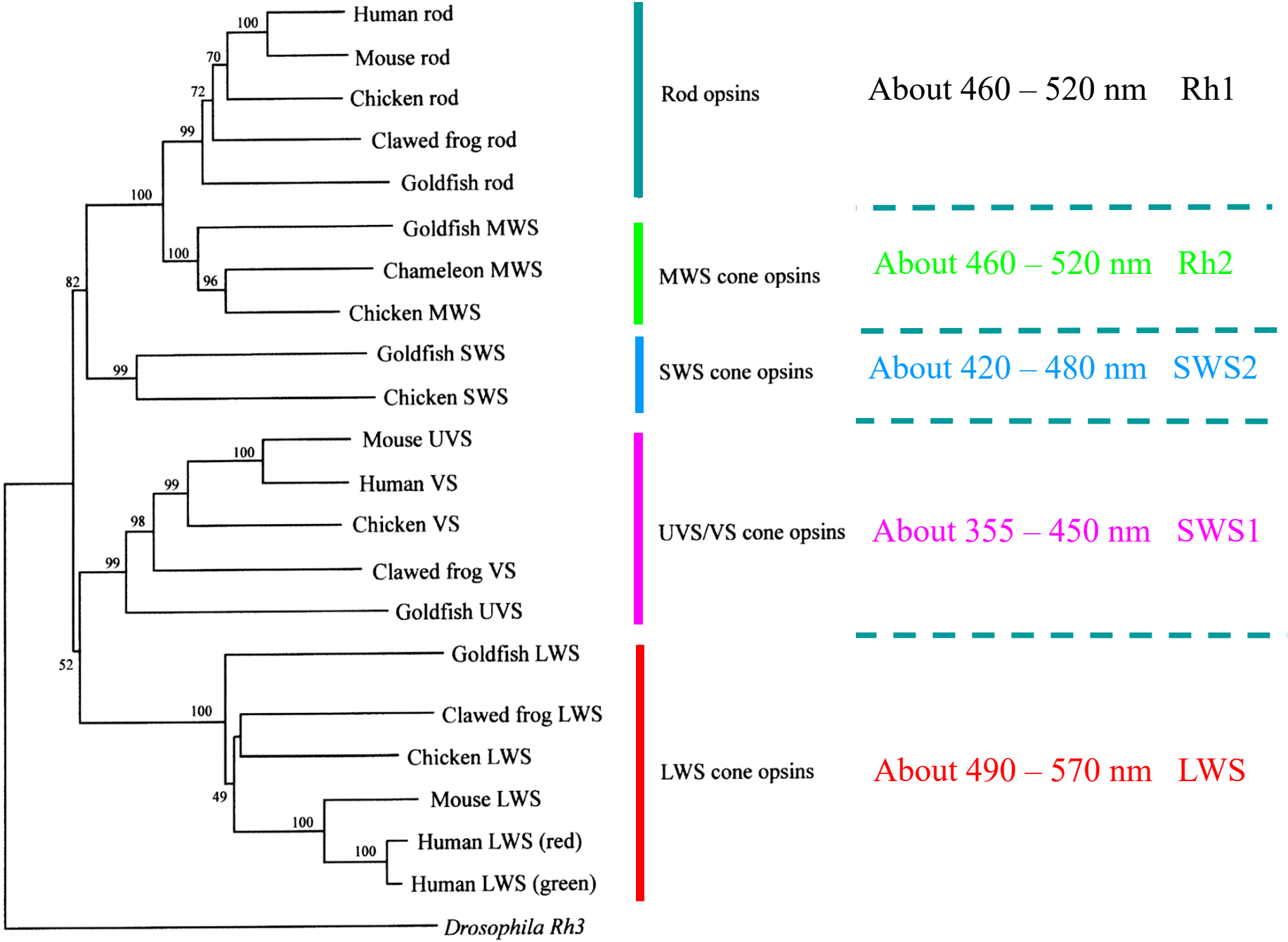
Amino acid differences

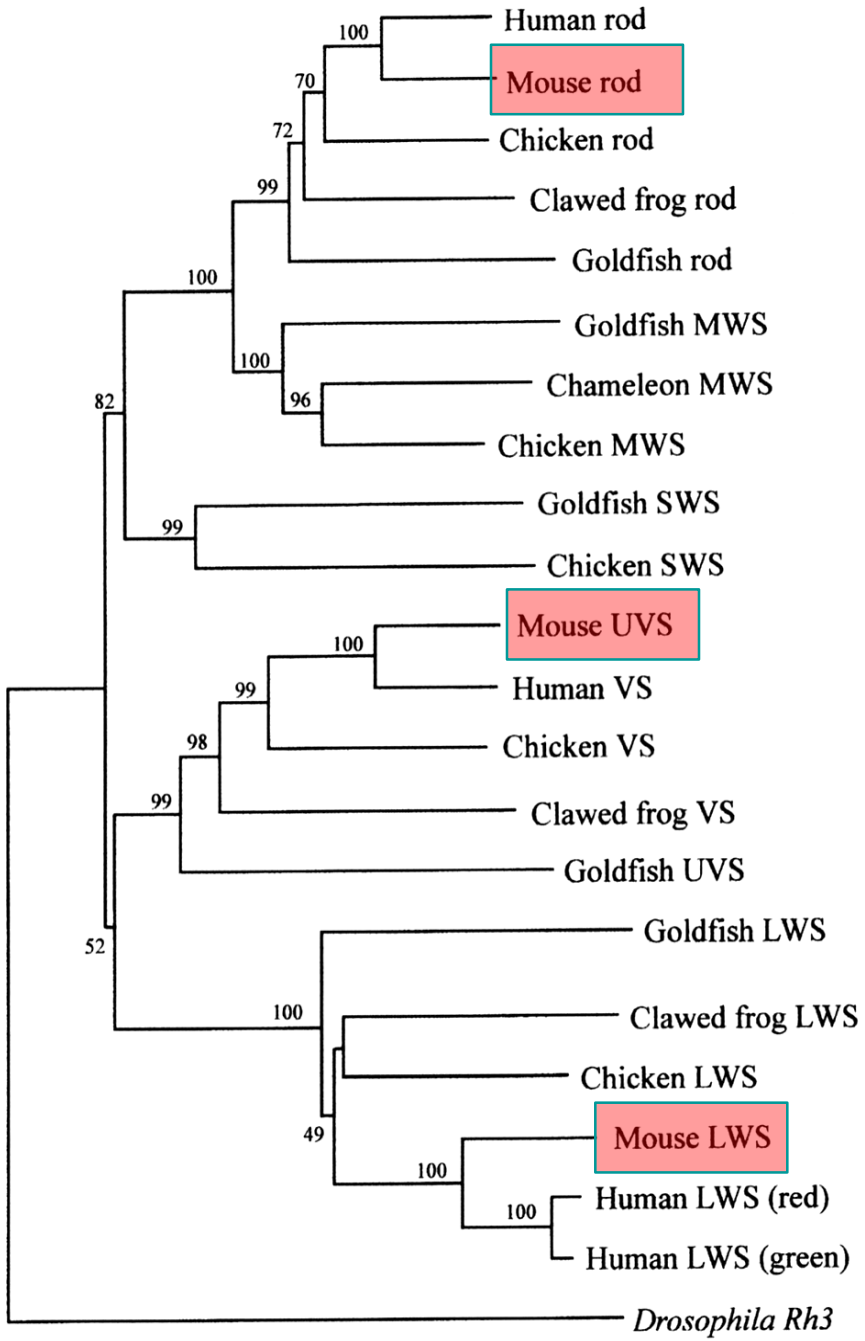
Why are the M- and L-cone opsins so similar?



From Sharpe, Stockman, Jägle & Nathans, 1999

Phylogenetic tree of visual pigments





Mammals

Rod opsins — 490 - 500 nm

MWS cone opsins — lost

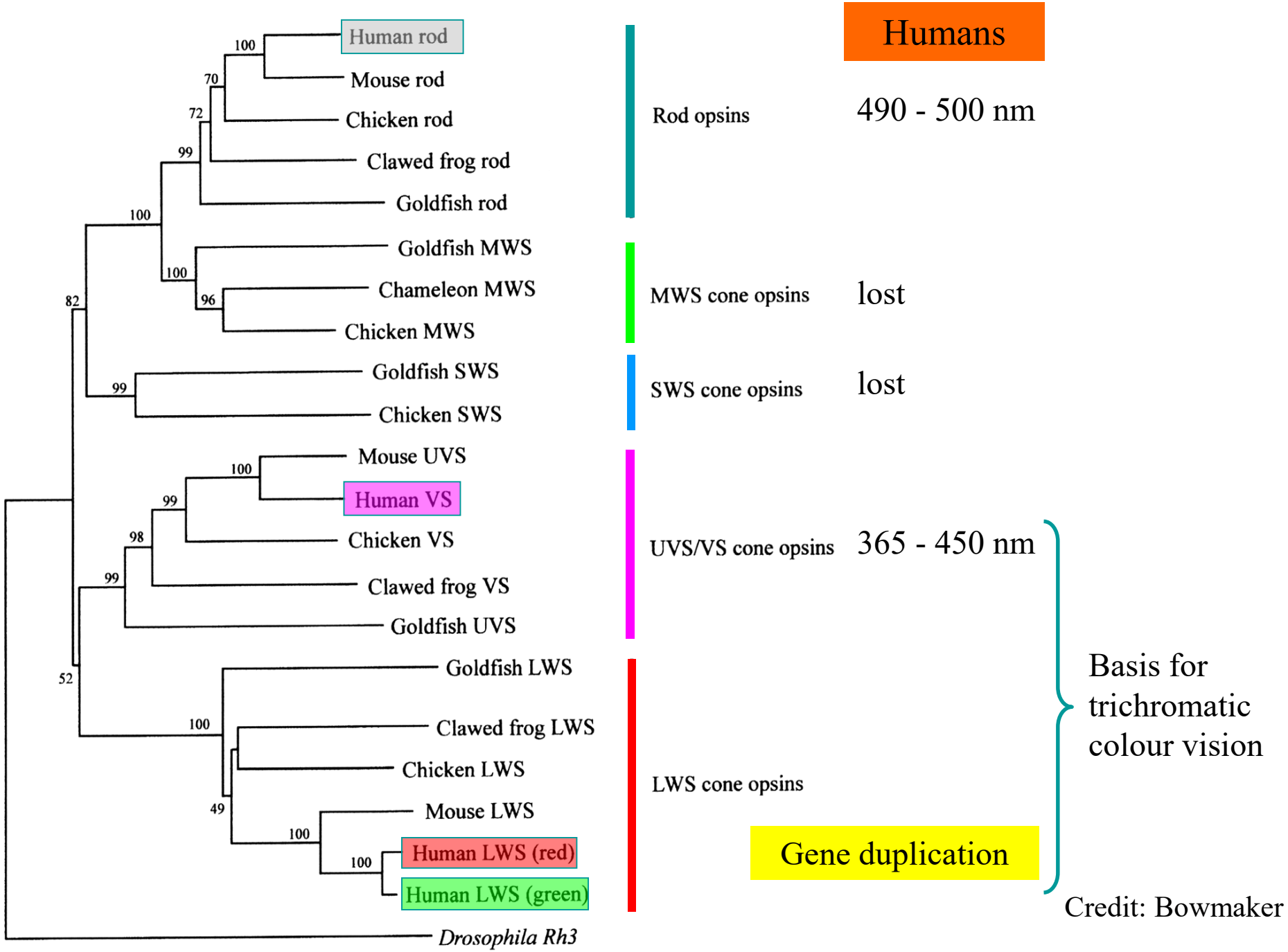
SWS cone opsins — lost

UVS/VS cone opsins — 365 - 450 nm

LWS cone opsins — 490 - 565 nm

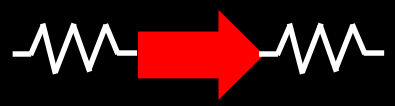
Basis for dichromatic colour vision

Credit: Bowmaker

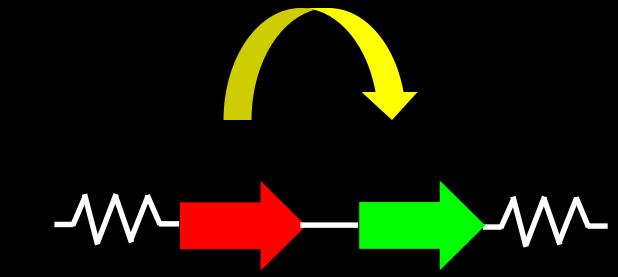


Credit: Bowmaker

Gene duplication on the X-chromosome



Mammal



L-cone photopigment opsin gene

M-cone photopigment opsin gene

Human/ Old world primate