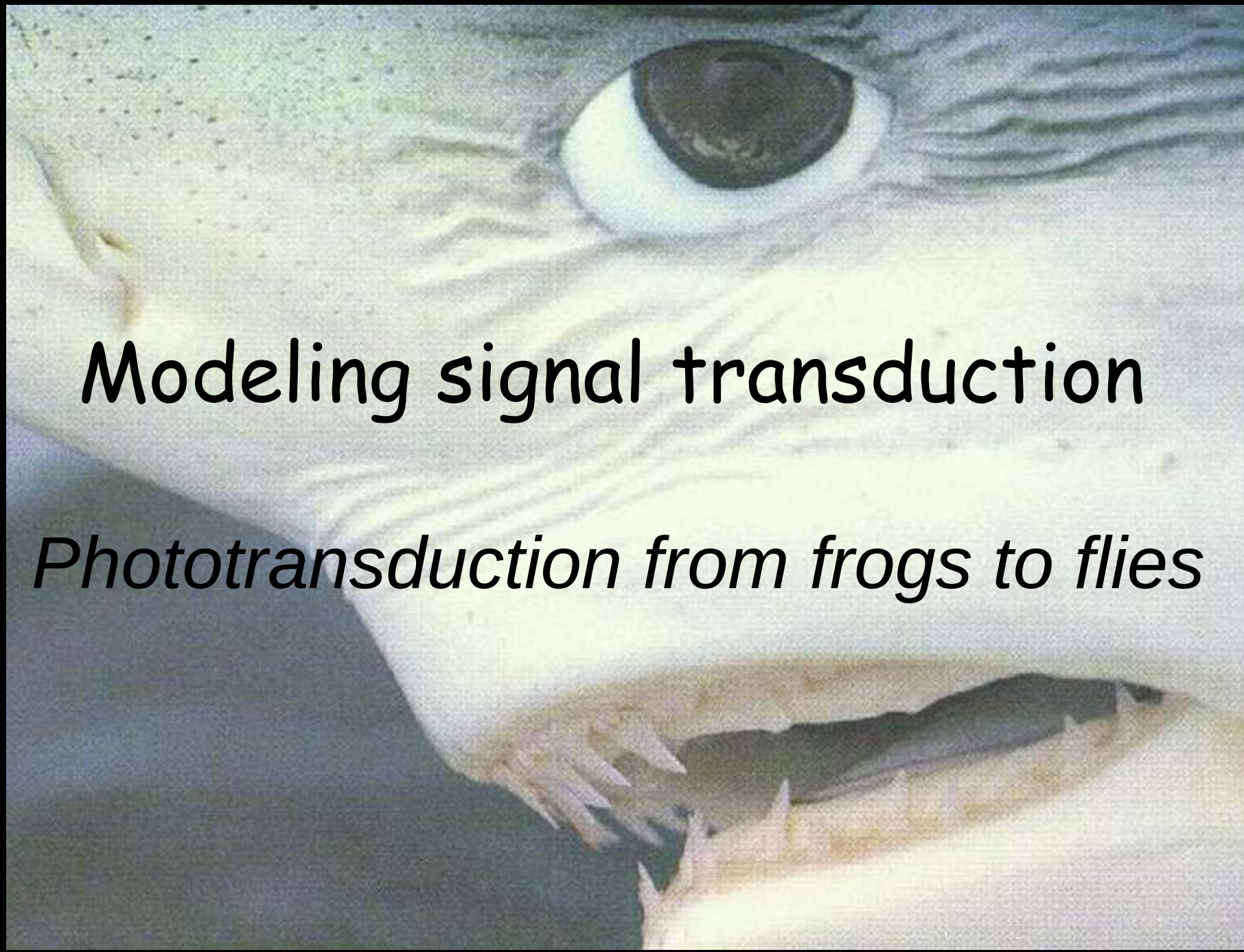


Modeling signal transduction

Phototransduction from frogs to flies

Eye as an evolutionary challenge

"To suppose that the eye, with all its inimitable contrivances …, could have been formed by natural selection, seems, I freely confess, absurd in the highest possible degree. Yet reason tells me, … "

C. Darwin, The Origin of Species:

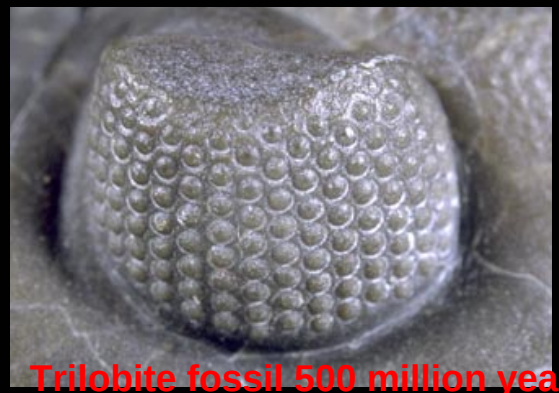
Darwin goes on to describe how eye may have evolved through accumulation of gradual improvements

A number of different designs exist:
e.g. vertebrate, molluskan or jellyfish "camera" eyes or insect "compound eye"

The eye may have evolved independently 20 times!!

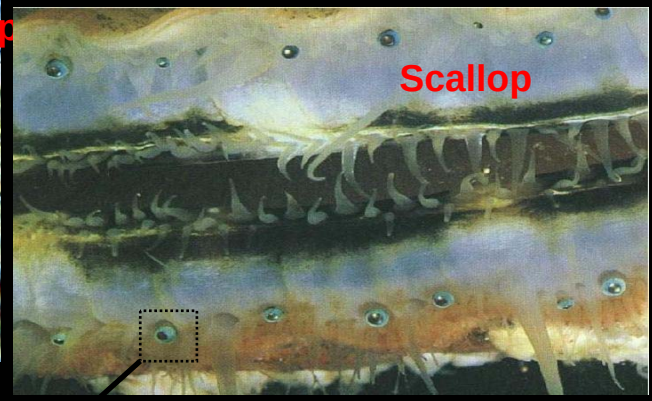
(read "Cells, Embryos and Evolution" by Gerhart&Kirschner)

Eyes are present in most metazoan phyla: how did they evolve?



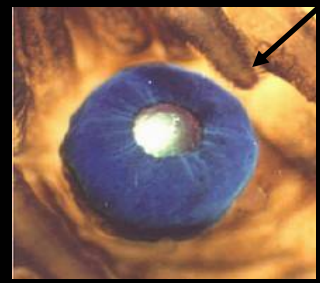
Trilobite fossil 500 million years

Copepod's 2 lens telescop



Scallop

House Fly



Black ant

Octopus

Cuttlefish

See Gerhart and Kirschener, “Cells, Embryos and Evolution”

The grand challenge:

To understand the evolutionary processes that underlie the appearance of a complex organ (an eye).

To what extent is the similarity between different eyes (e.g. vertebrate and mollusk) is due to common ancestry or is the result of evolutionary convergence due to physical constraints?

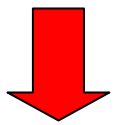
What can we learn from the similarities and differences in the architecture/anatomy, development and *molecular machinery of eyes (or light sensitive cells) in different species*?

BUT before we can address the question of evolution we need first to learn how it works…

More broadly:

Molecular pathway(s) of phototransduction are similar to many other signaling pathways:

olfaction, taste, etc



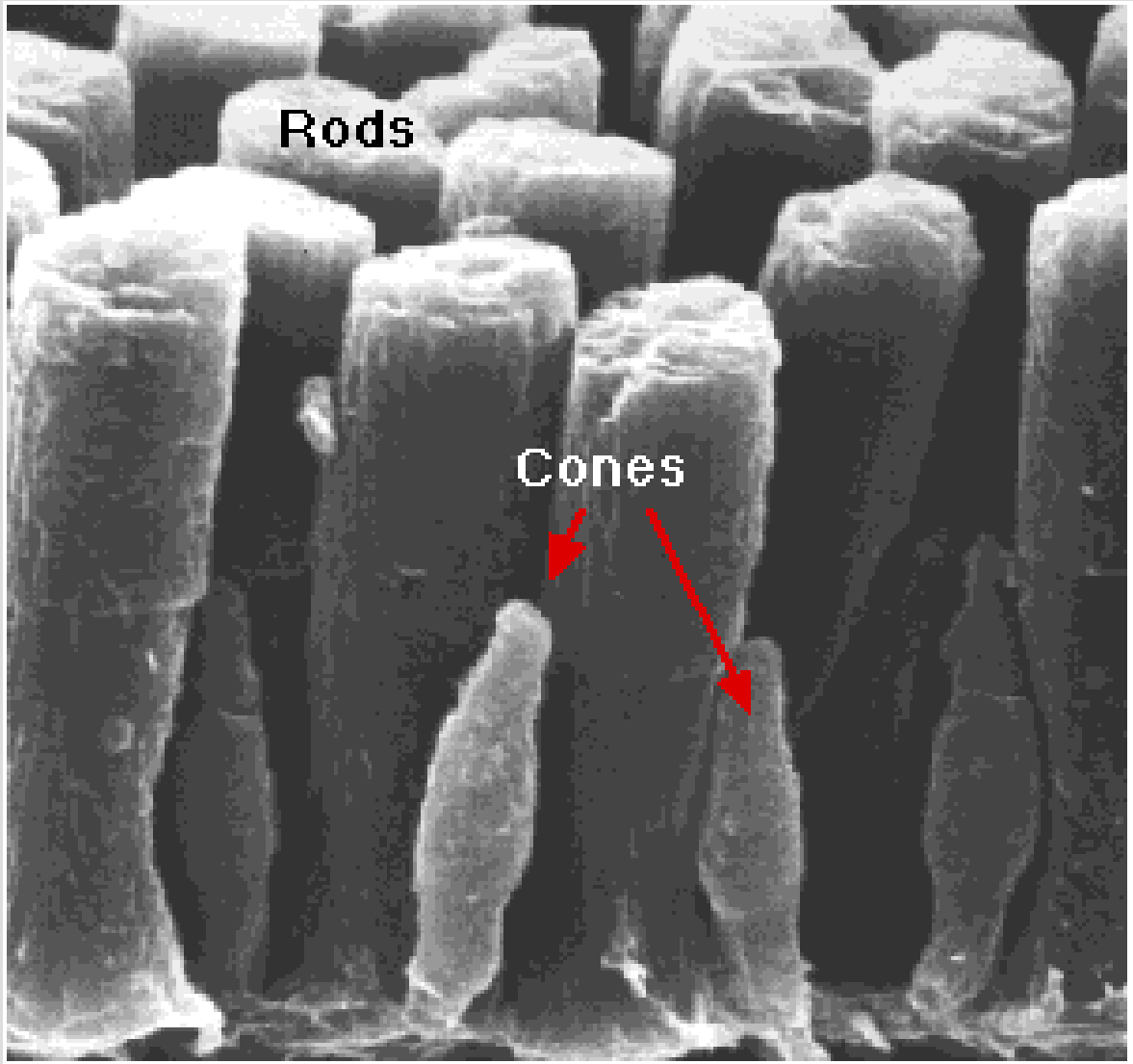
"Comparative Systems Biology"

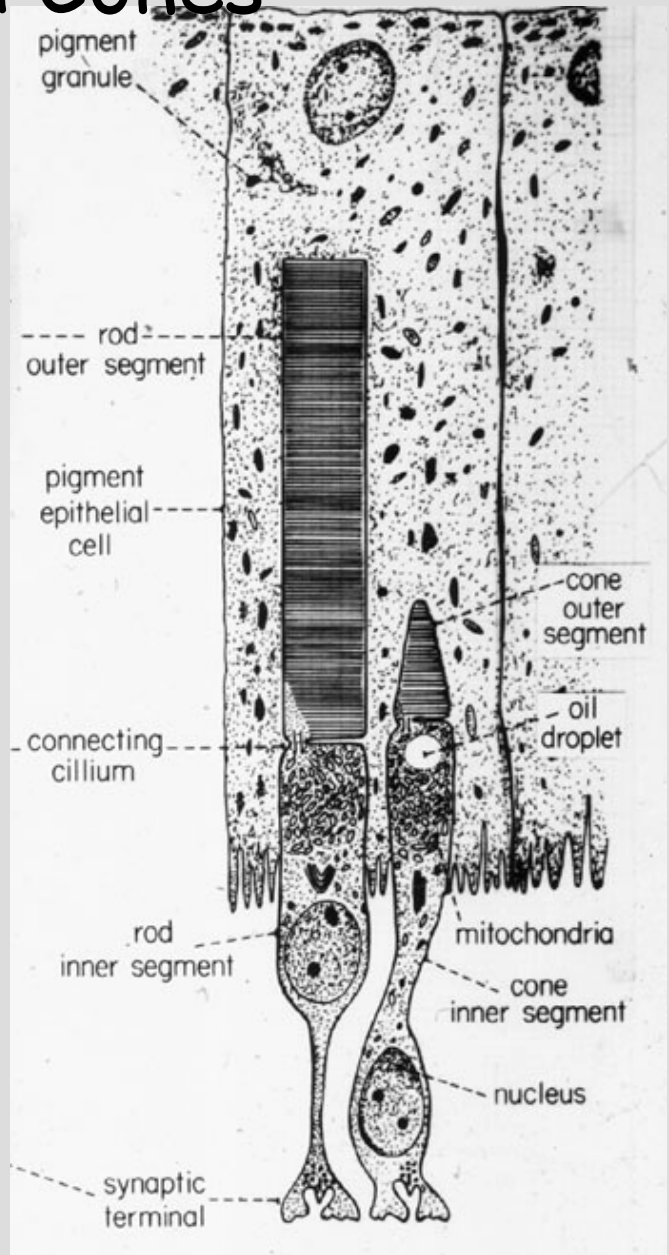
Modeling molecular mechanisms of photo-transduction

Case studies: 1) vertebrate and 2) insect

Phototransduction as a model signaling system

Vertebrate Rods and Cones

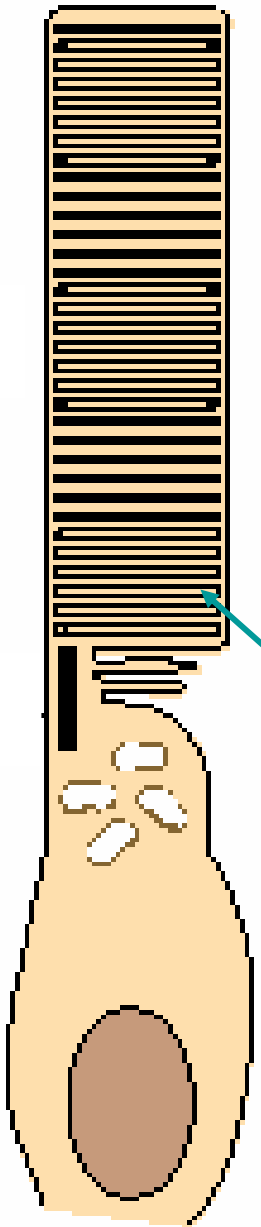




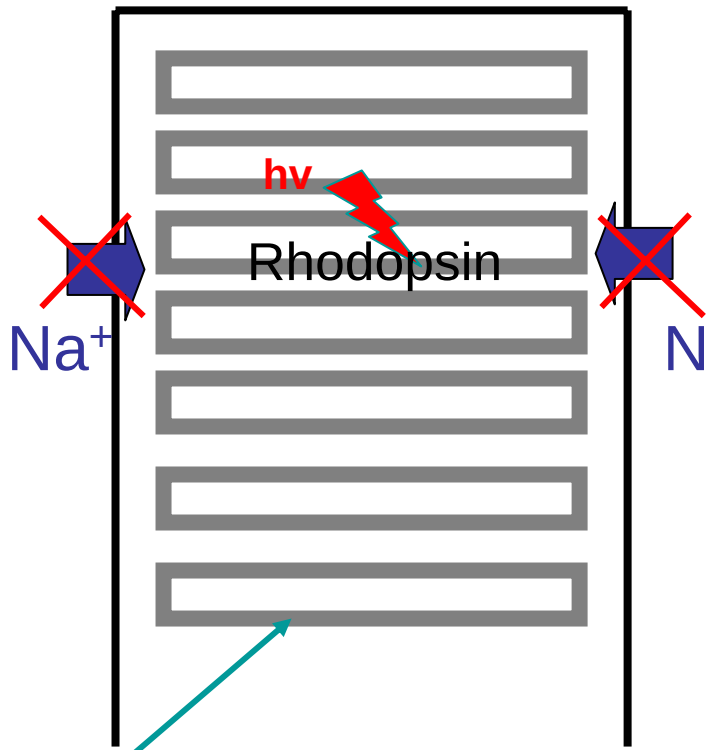
Vertebrate photoreceptor cell

Rod
Outer
Segment

(2000
Discs)



100μm



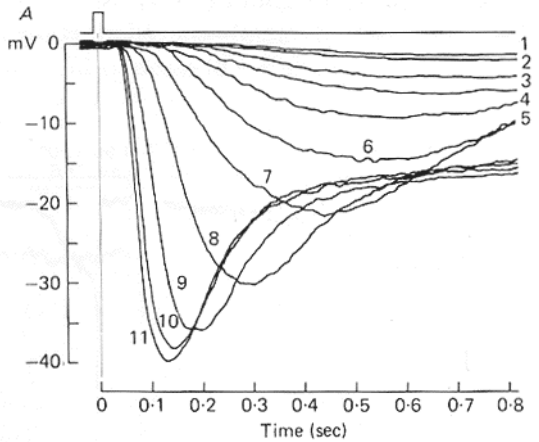
Discs

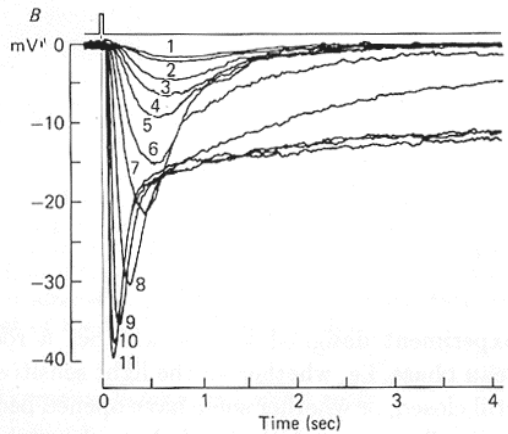
Light reduces the

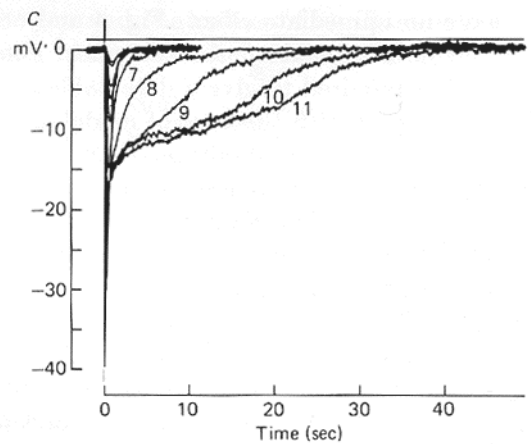
"dark current" into the cell

Intracellular Voltage in Rods and Cones

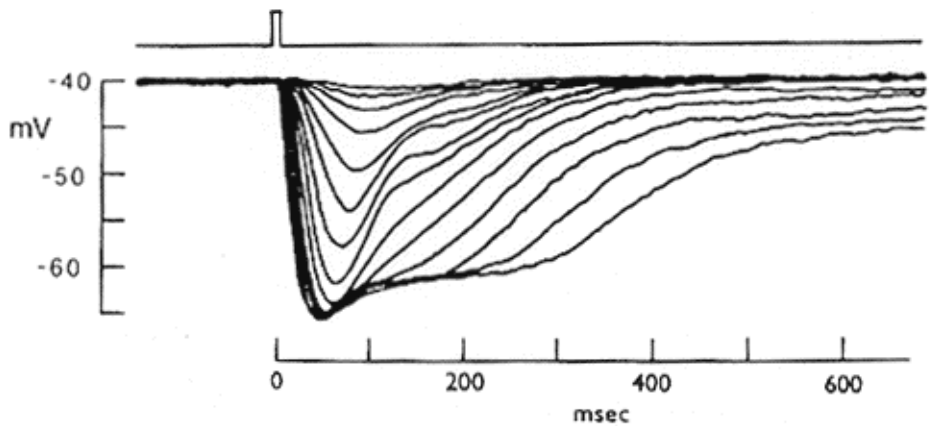
Turtle Rod: a flash response family shown on different time scales



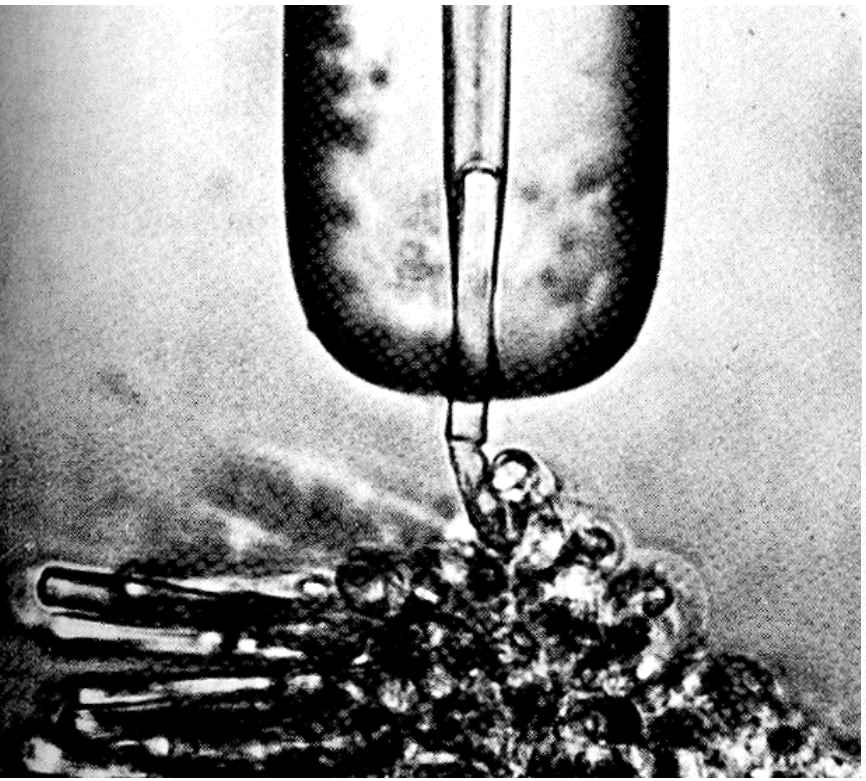


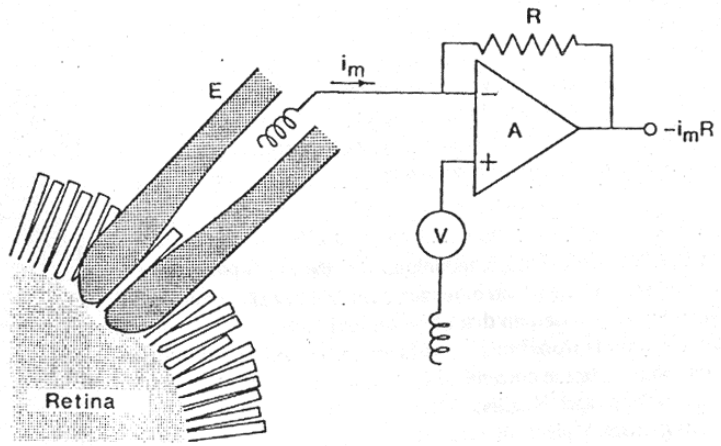


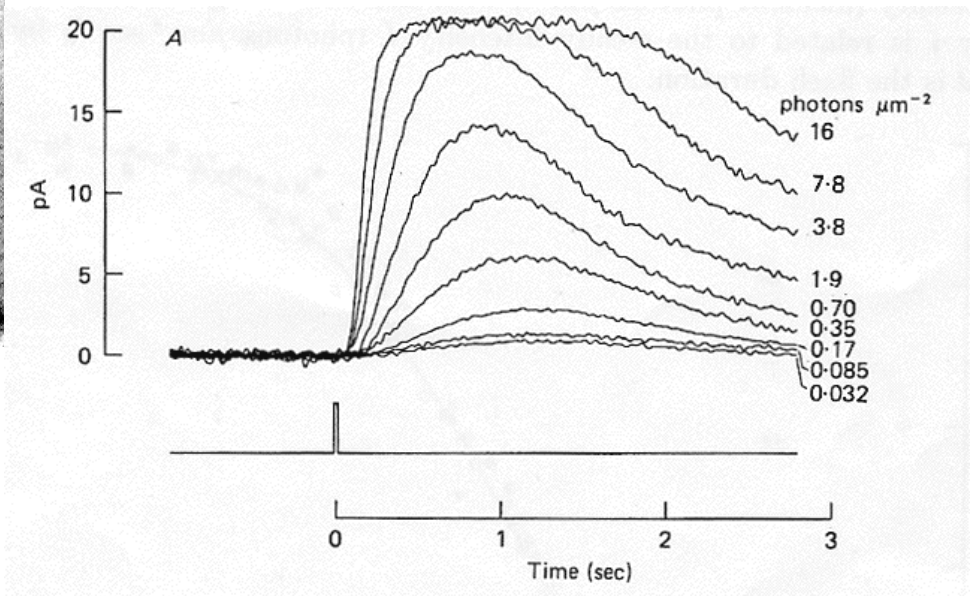
Turtle Cone



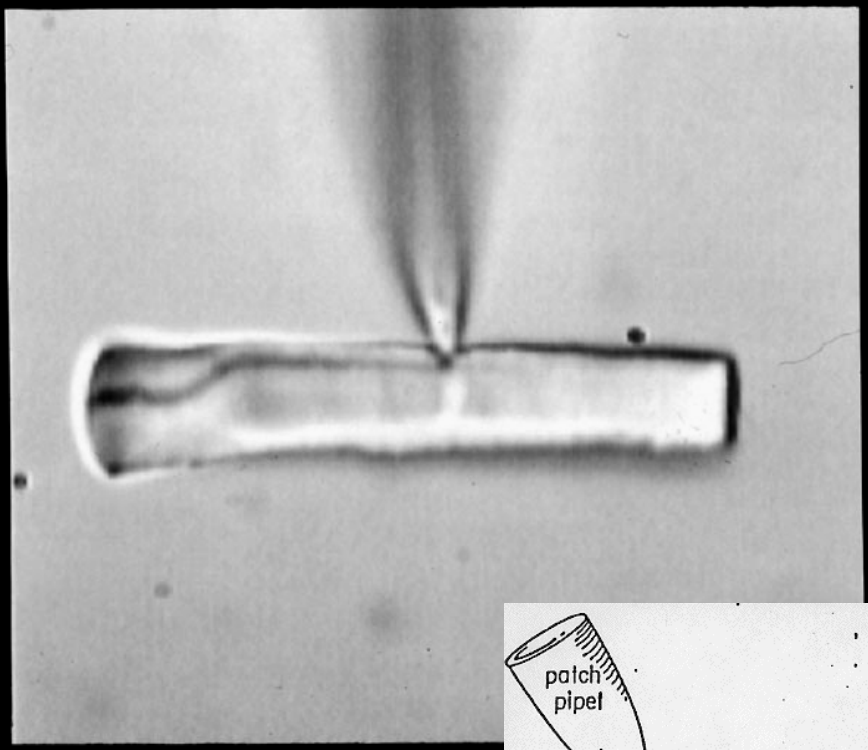
Measuring currents

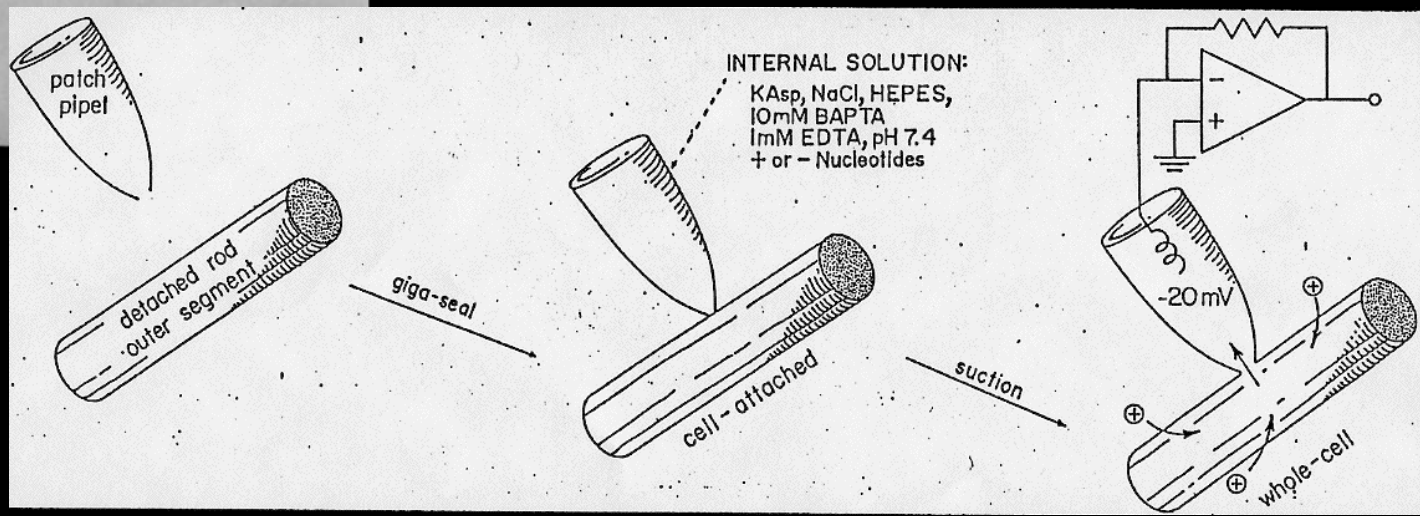






Patch clamp




patch pipet
detached rod outer segment
giga-seal
INTERNAL SOLUTION:
KAsp, NaCl, HEPES,
10mM BAPTA
1mM EDTA, pH 7.4
+ or – Nucleotides
cell-attached
suction
-20 mV
whole-cell

Single Photon Response

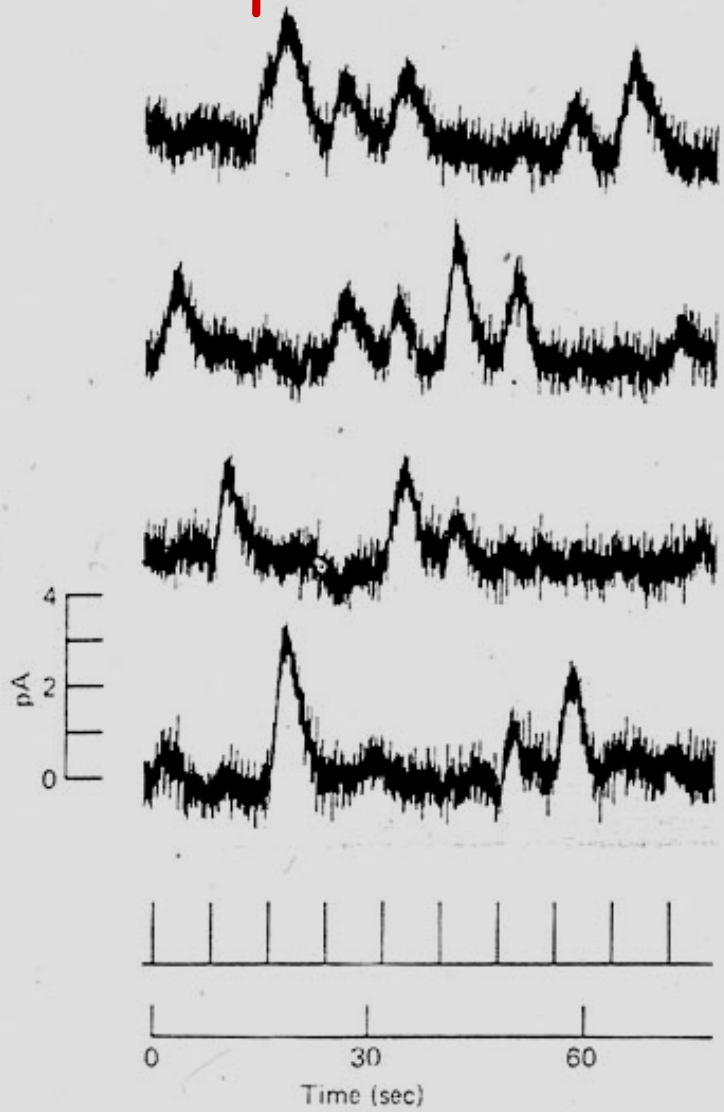
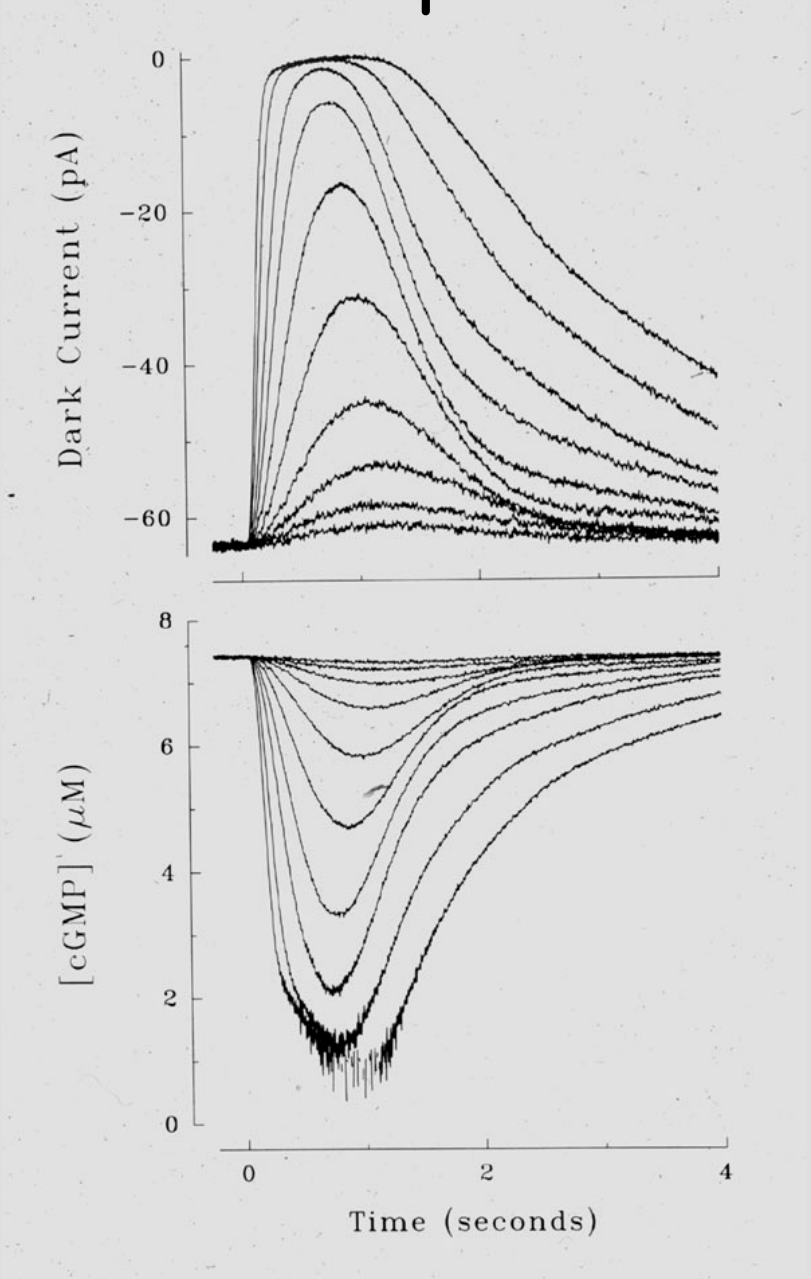


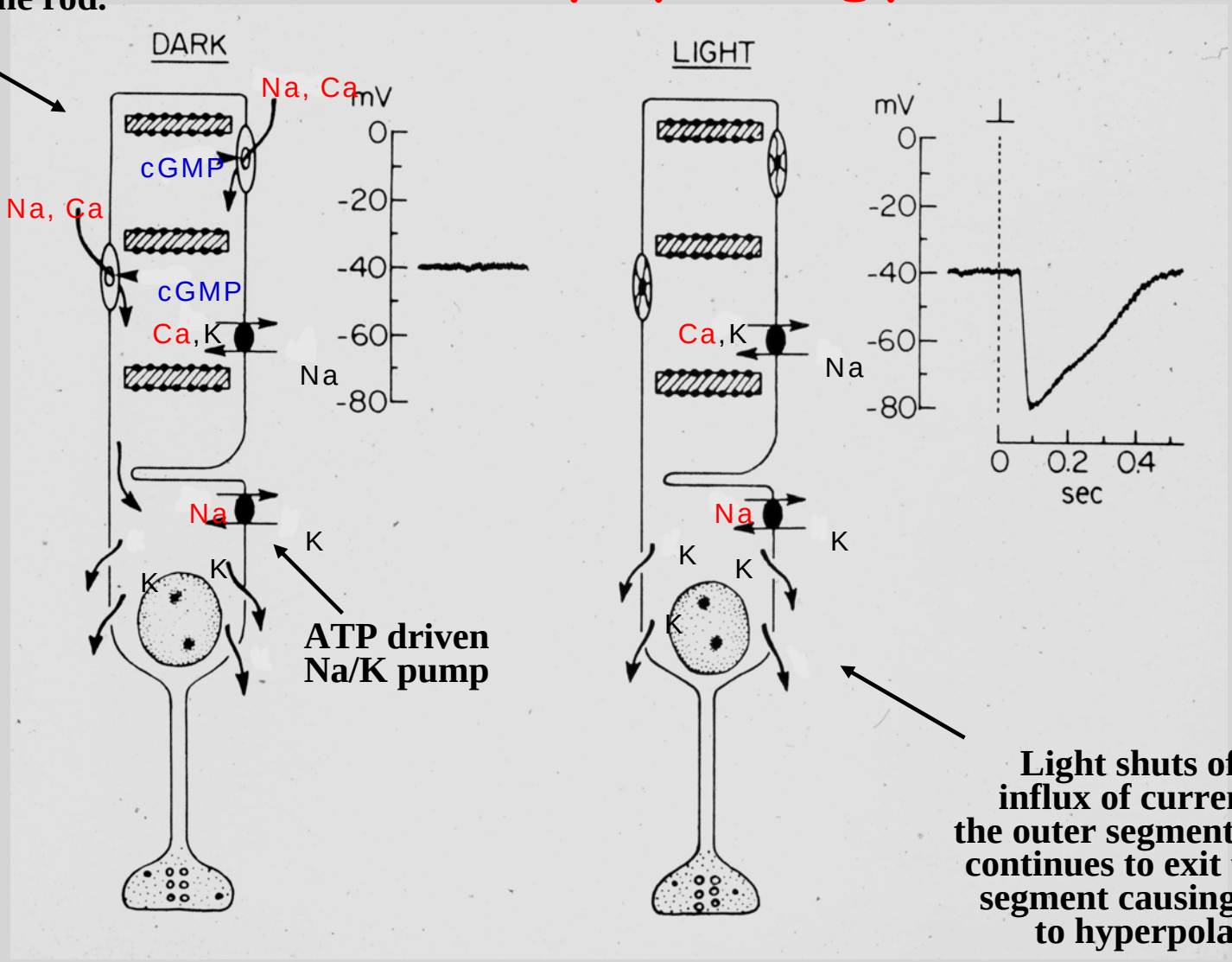
Fig. 2. Response of outer segment to a series of forty consecutive dim flashes. Local illumination; 20 msec flash delivering 0·029 photons μm^{-2} at 500 nm, flash timing monitored below; saturating response 12 pA. Same cell as Fig. 1. Low pass filtered at 30 Hz.

Patch clamp recordings



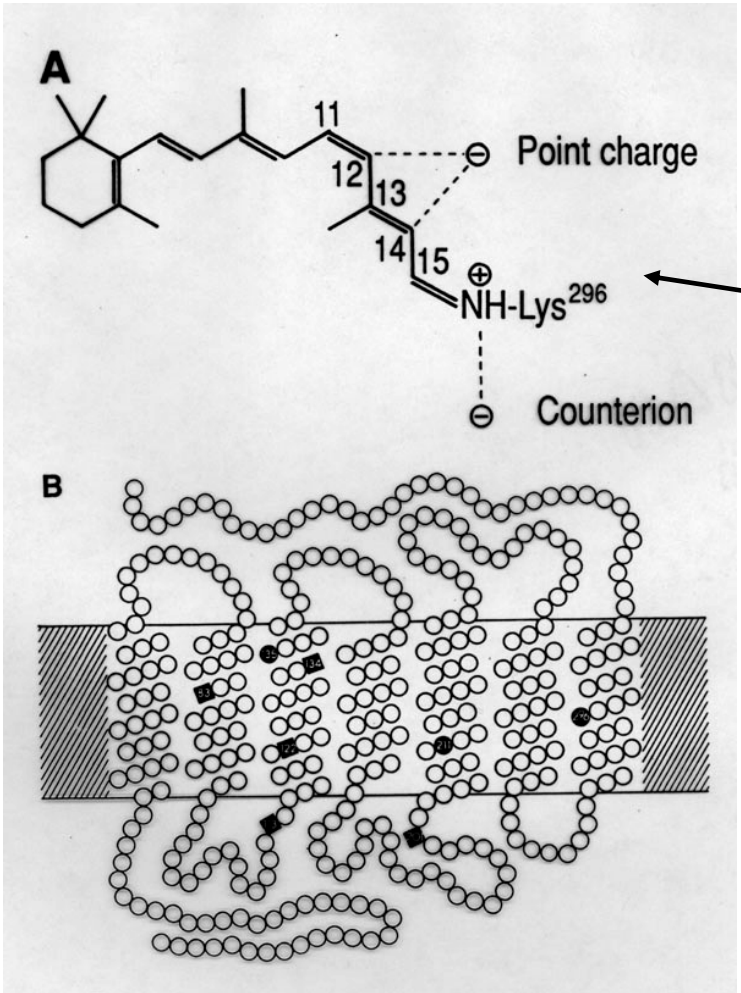
Electrophysiology of Rods

In the dark a standing current circulates through the rod.



Na,Ca channels of outer segment need cGMP to stay open

Light detector protein: Rhodopsin



Rhodopsin

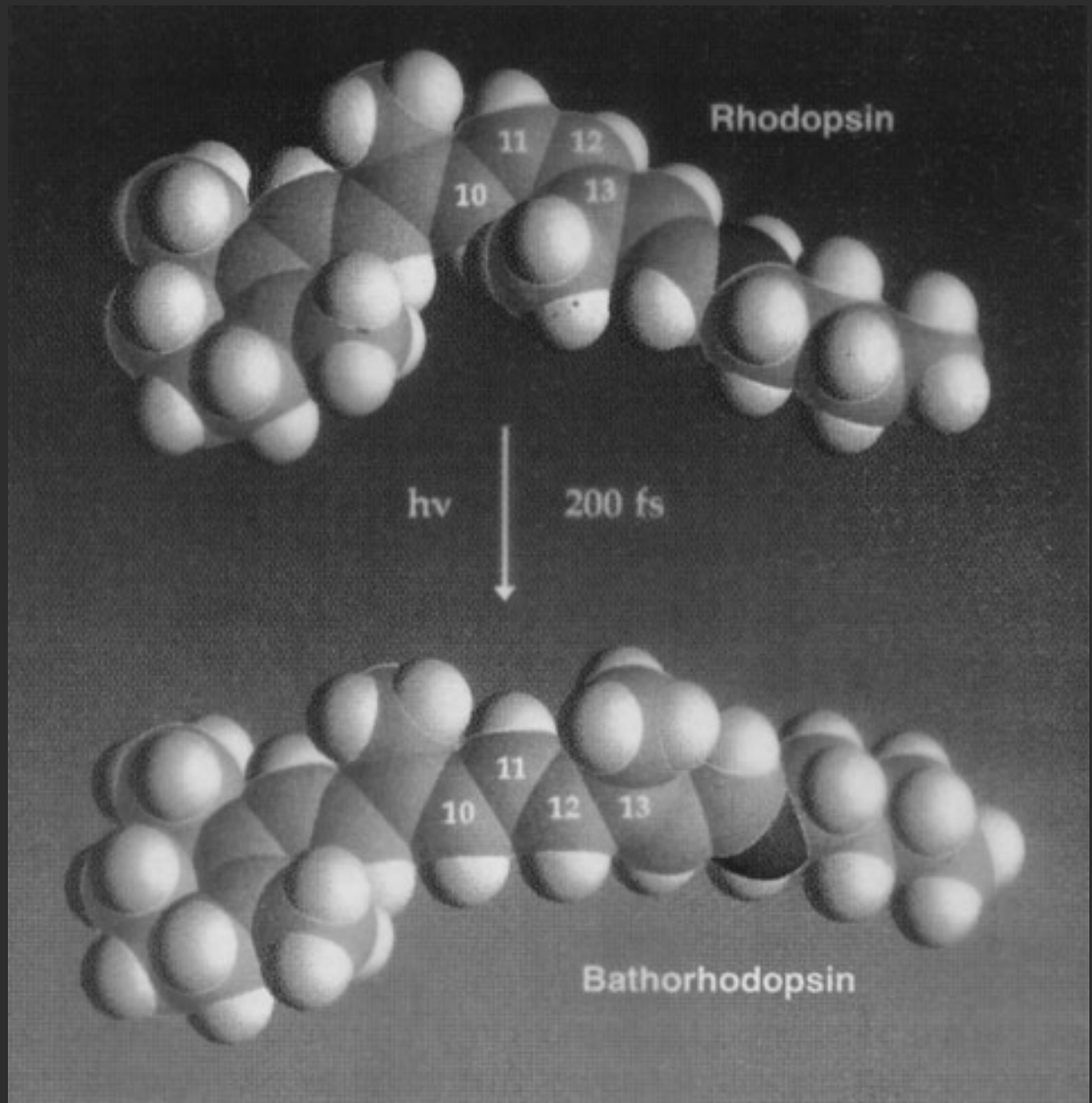
undergoes light-induced conformational change

chromophore, 11-cis retinal

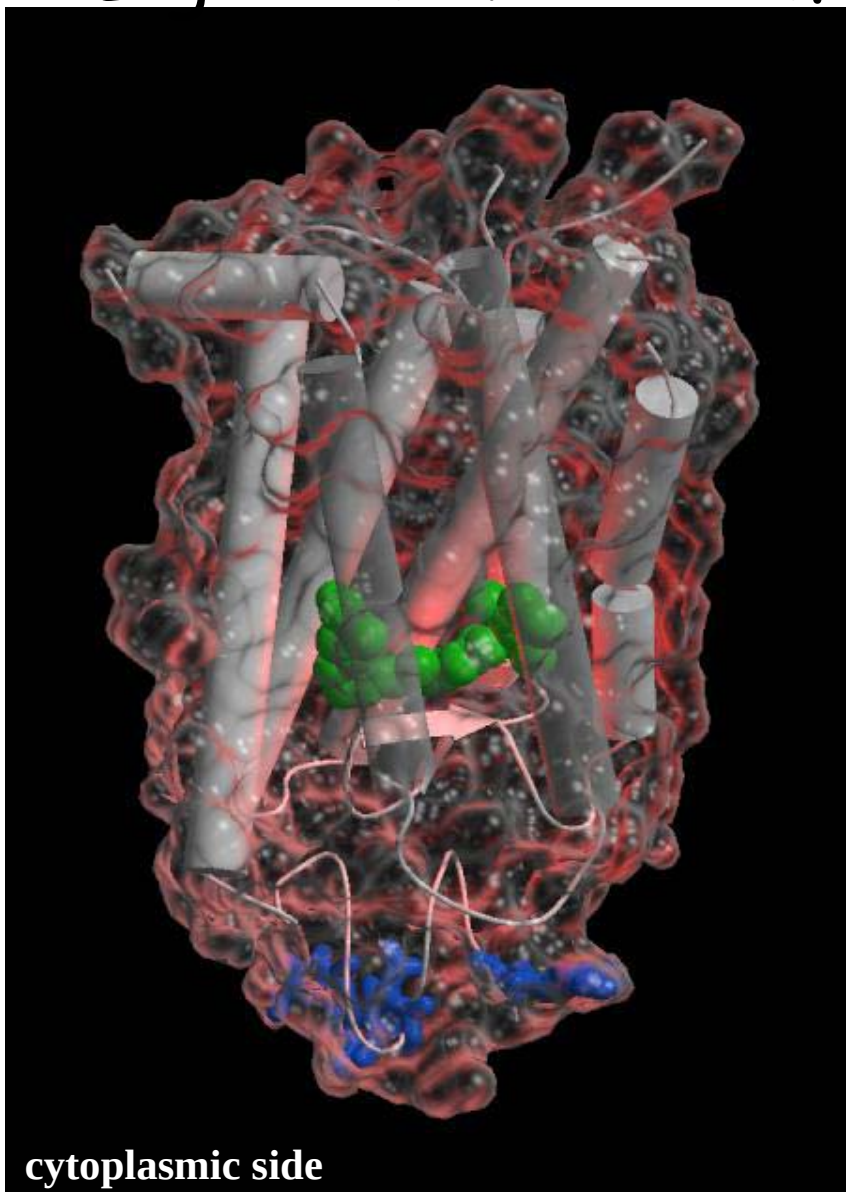
+

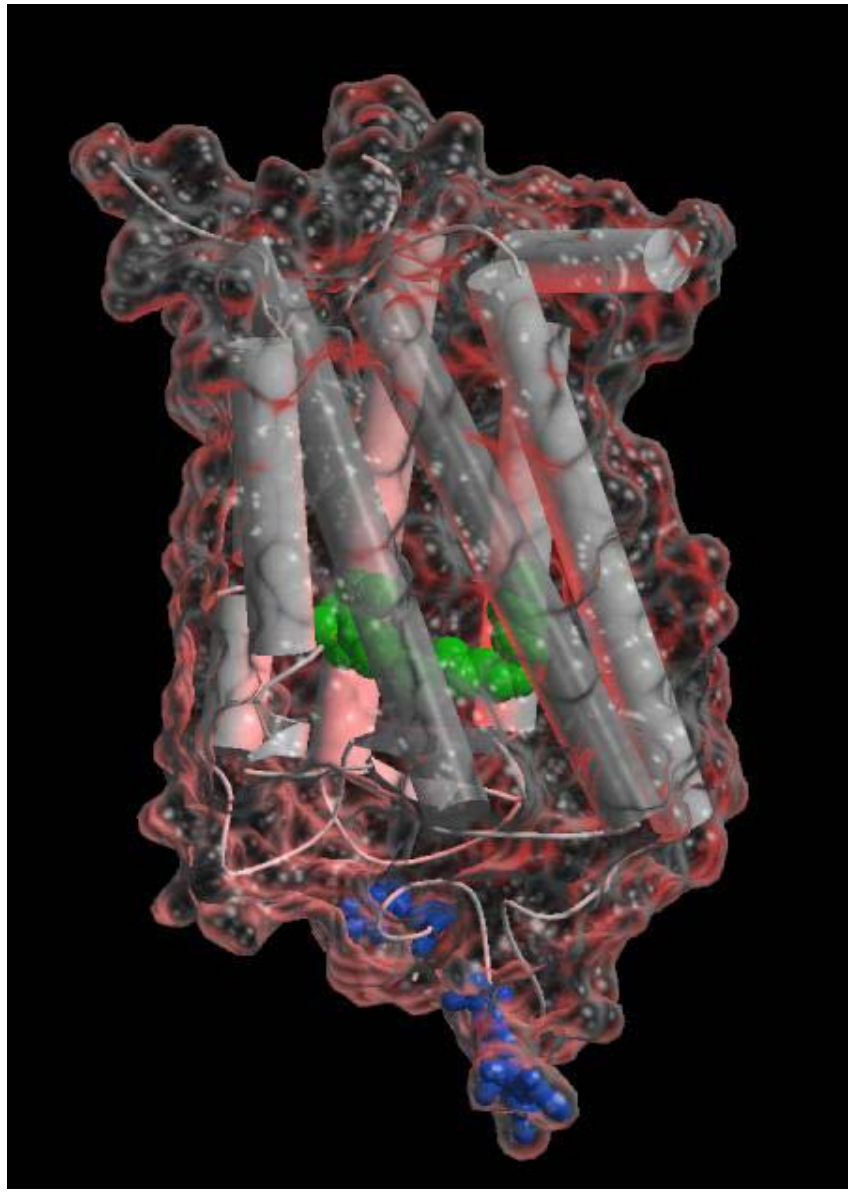
opsin, a membrane protein with 7 transmembrane segments

cis/trans -retinal transition

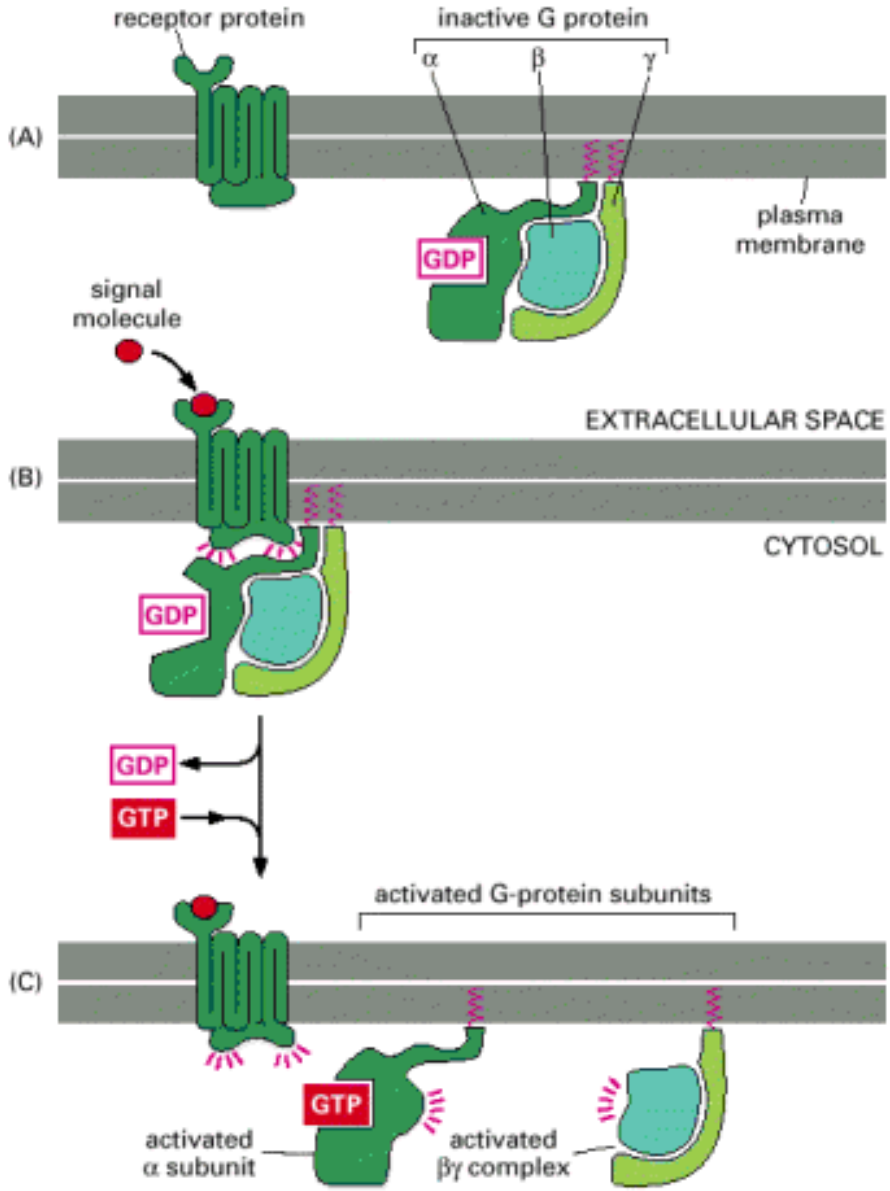


Light-induced conformational transition



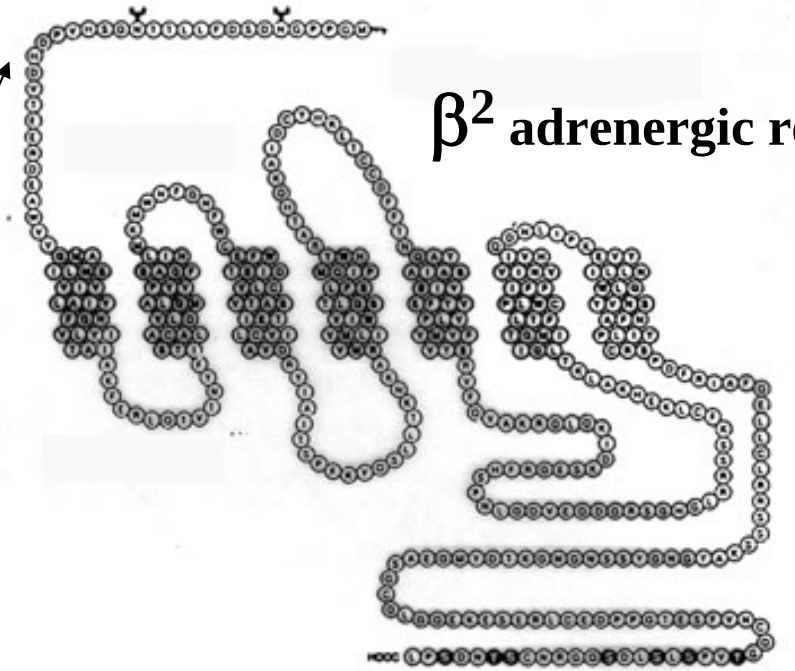


Rhodopsin is a G-Protein coupled receptor

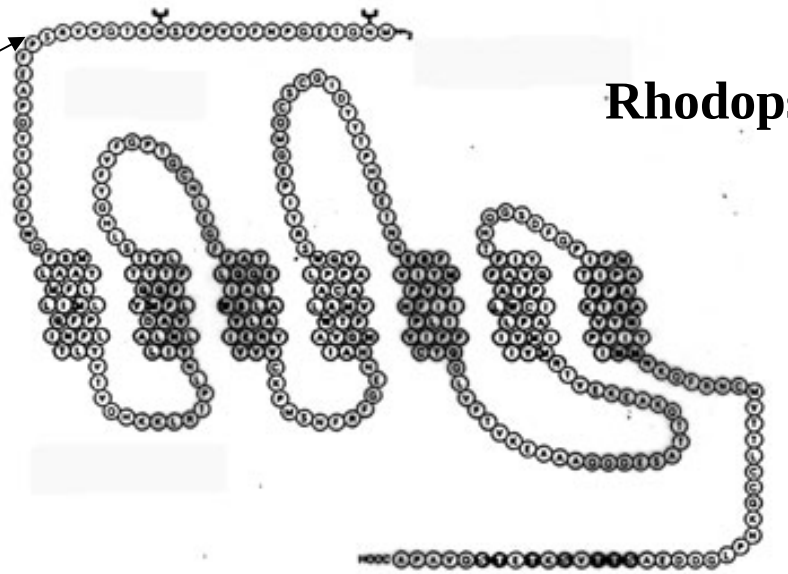


Seven-Helix G-protein-Coupled Receptors form a large class

(5% C. elegans Genome !)



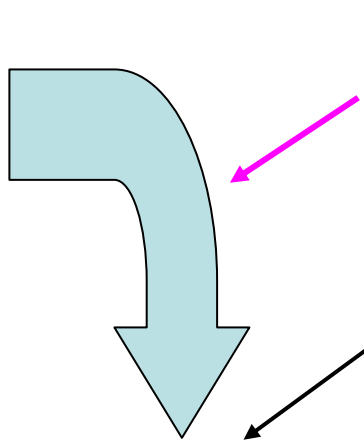
β^2 adrenergic receptor



Rhodopsin

cytoplasmic end

Rhodopsin is a G-Protein coupled receptor

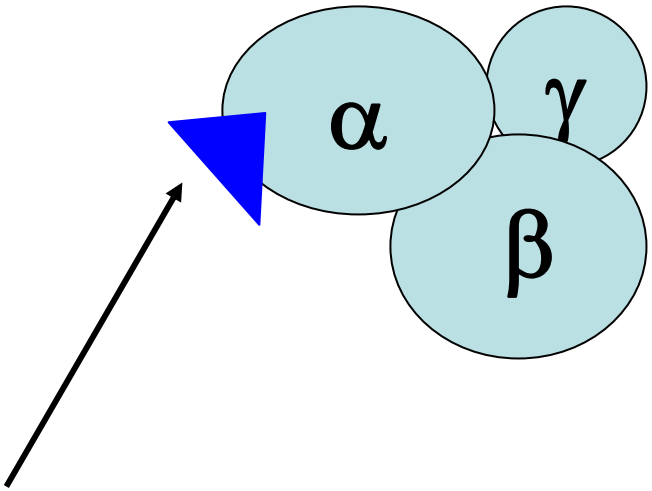


about 10^9 rhodopsin molecules and about 10^8 G-protein molecules per rod outer segment (ROS)

photoactivated rhodopsin (Rh*) forms in about 1 ms and serially activates 100 to 1000 Transducin molecules per second

Transducin is a heterotrimeric G protein specific to vision

G- proteins



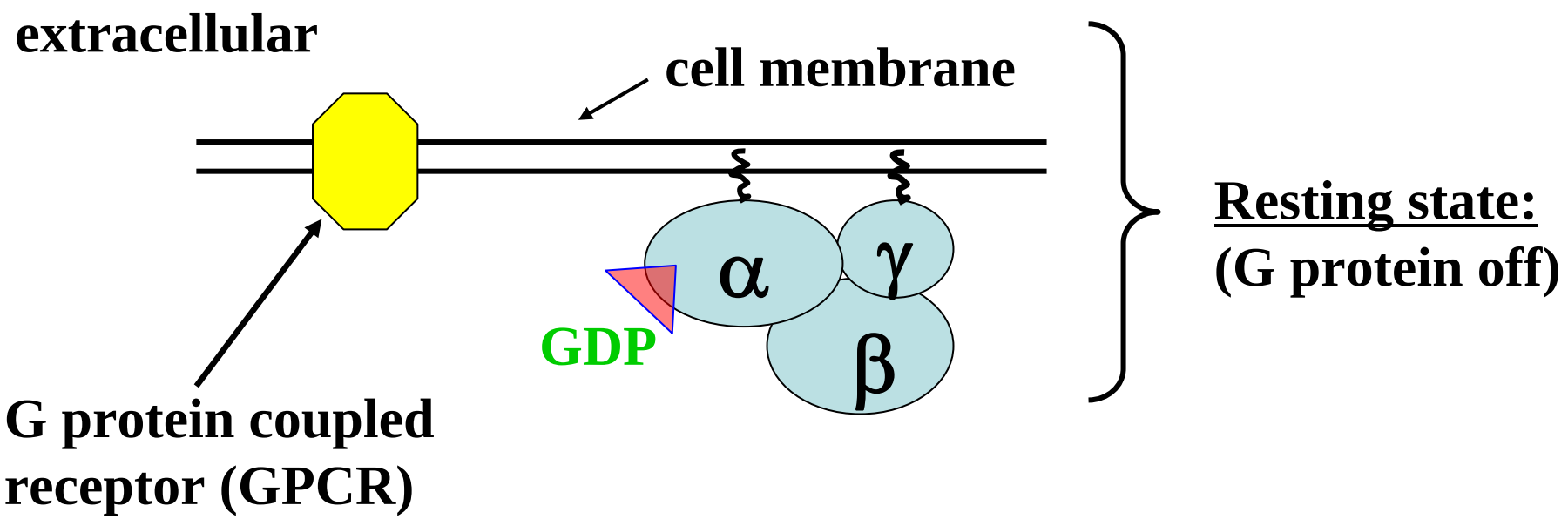
guanine nucleotide binding site on α subunit

α subunit: 35-45 kD

β subunit: 35-40 kD

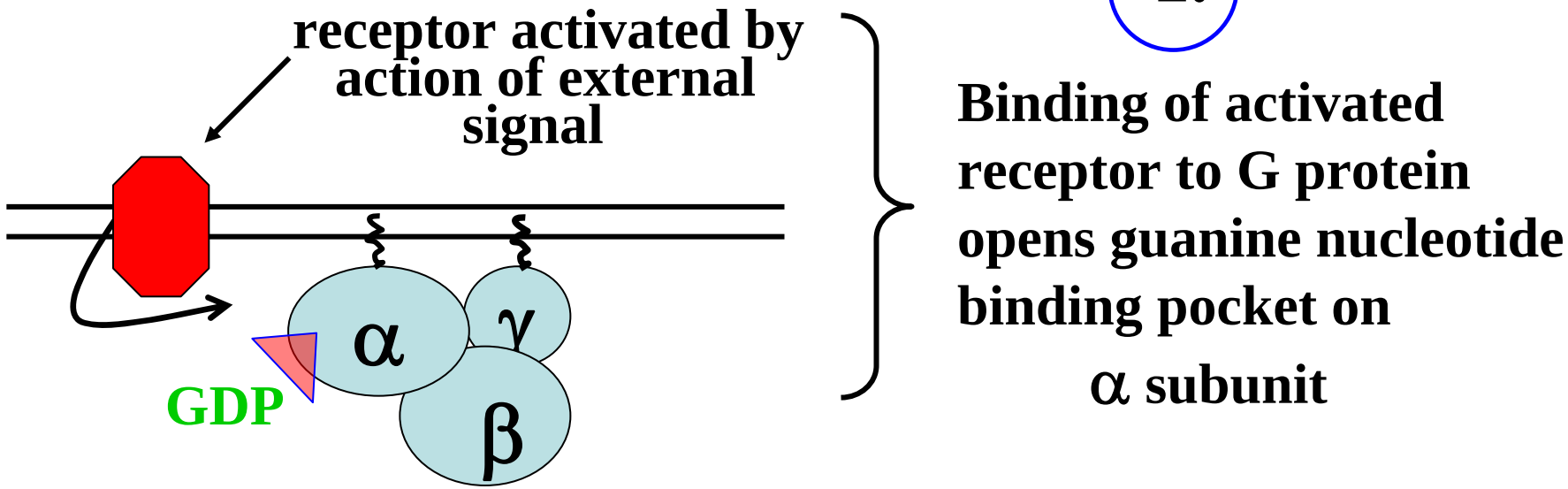
γ subunit: 6-12 kD

At least 15 different genes for α subunit and several different genes for β and γ subunits

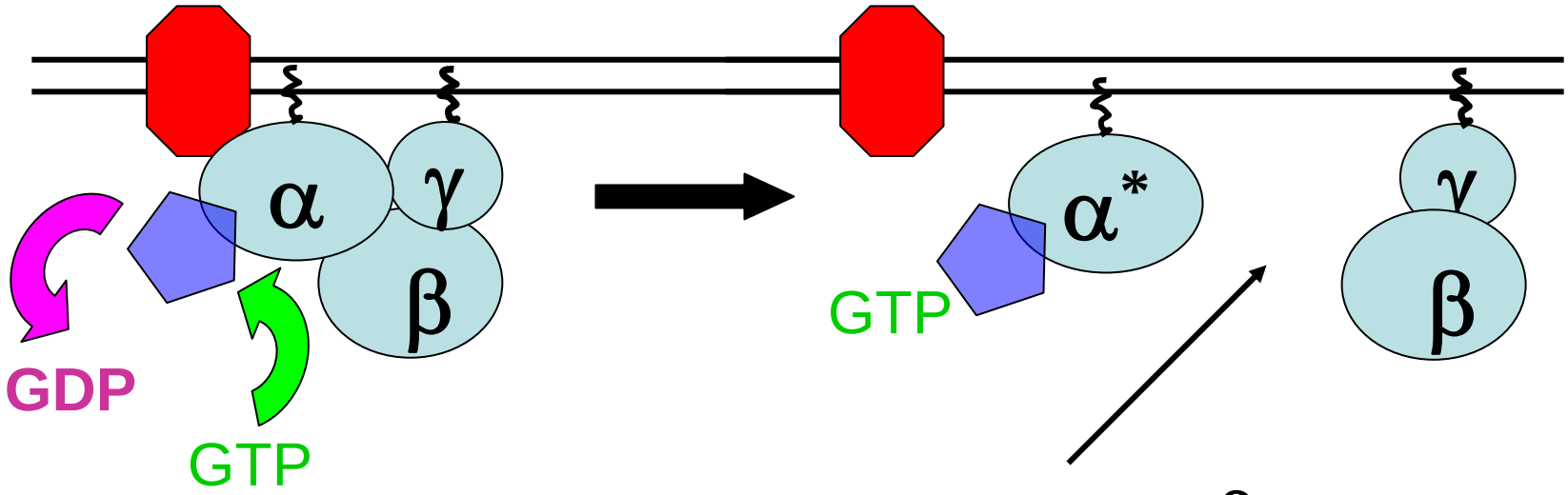


Steps in activation

1.

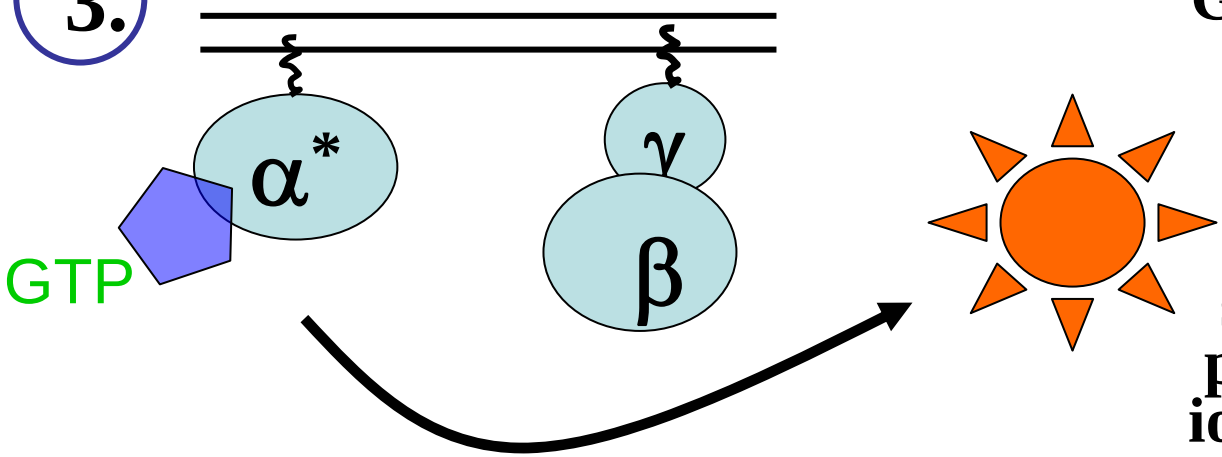


2. **GDP -- GTP exchange at nucleotide binding site activates the G protein**

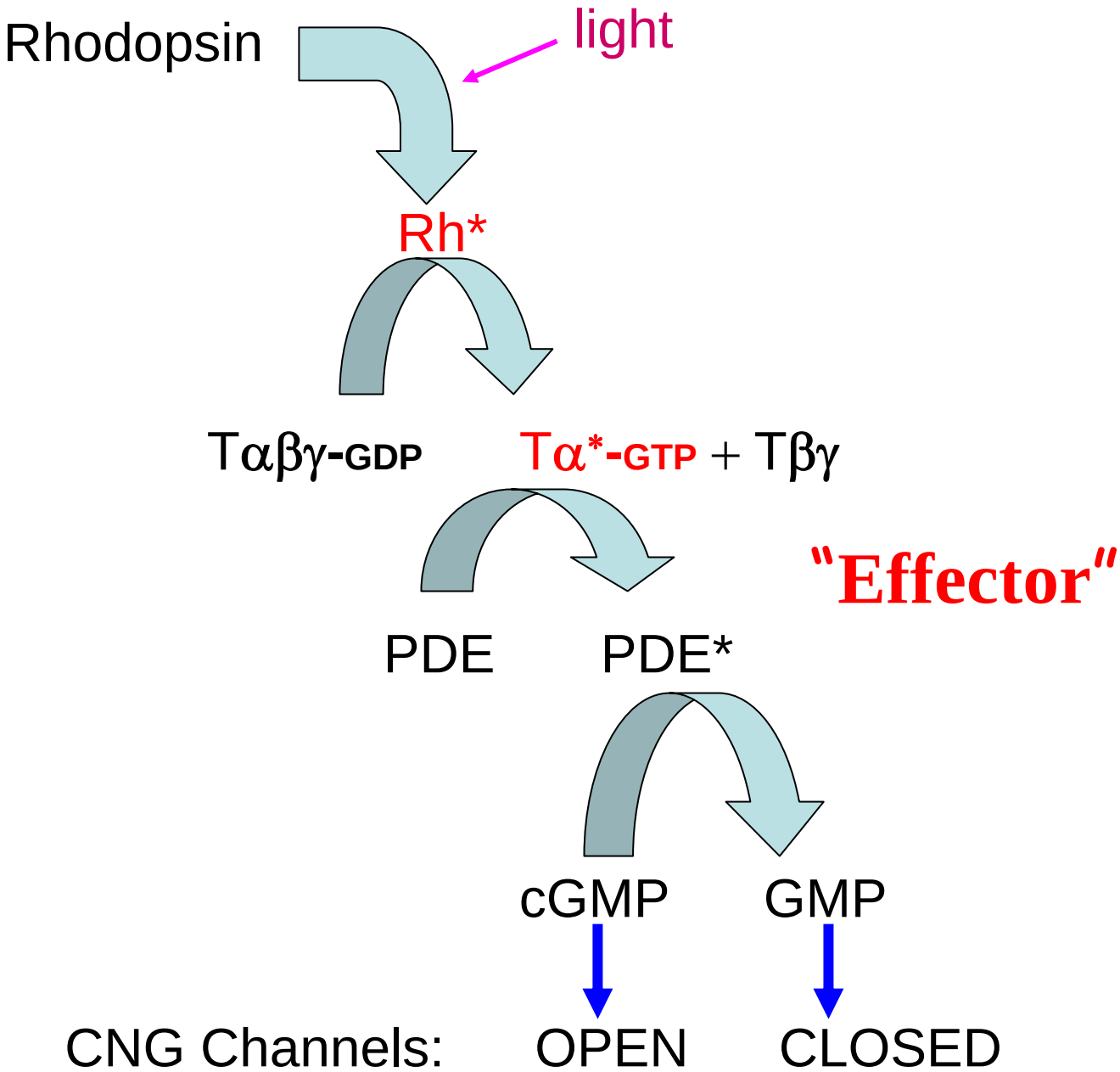


The α and βγ subunits dissociate from activated GPCR

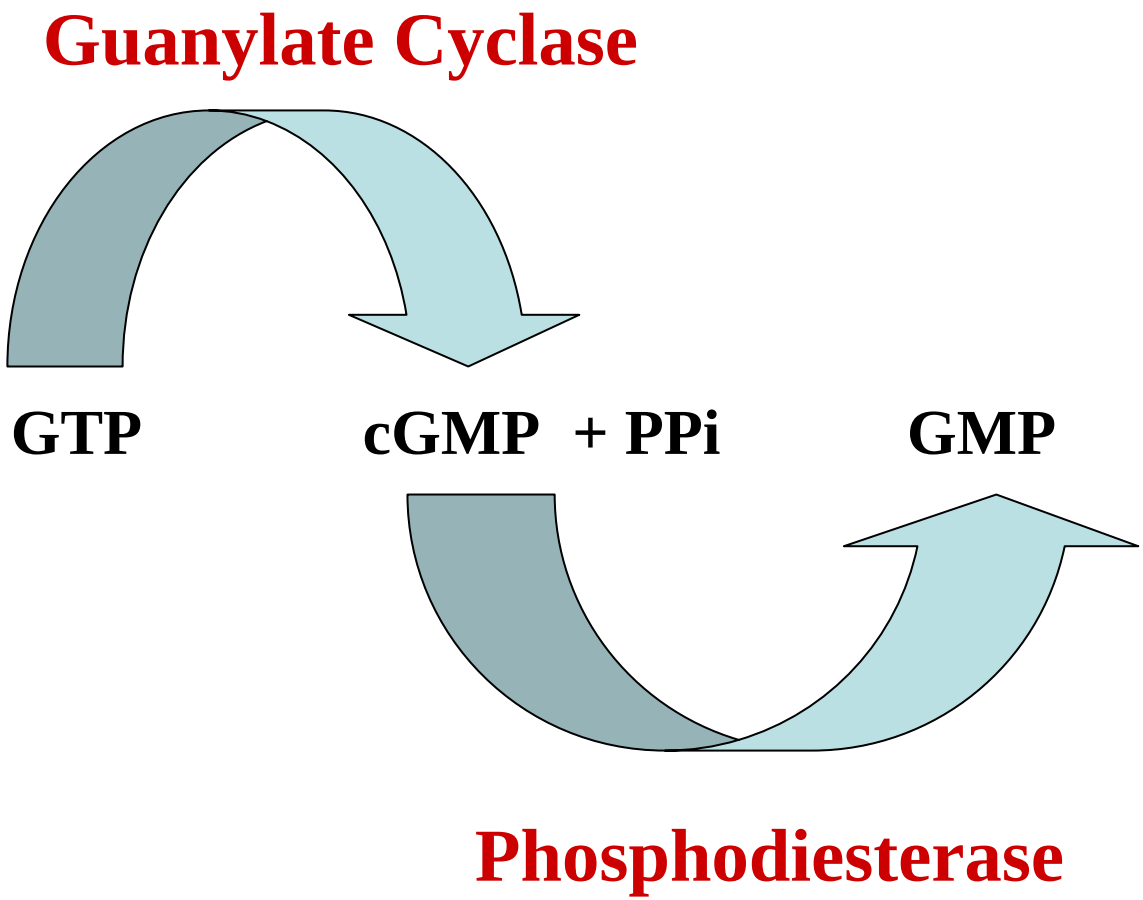
3.



Activated α subunit stimulates effector proteins: enzymes or ion channels


Rhodopsin
light
Rh*
Tαβγ-GDP
Tα*-GTP + Tβγ
"Effector"
PDE
PDE*
cGMP
GMP
CNG Channels:
OPEN
CLOSED

Where did cGMP come from?



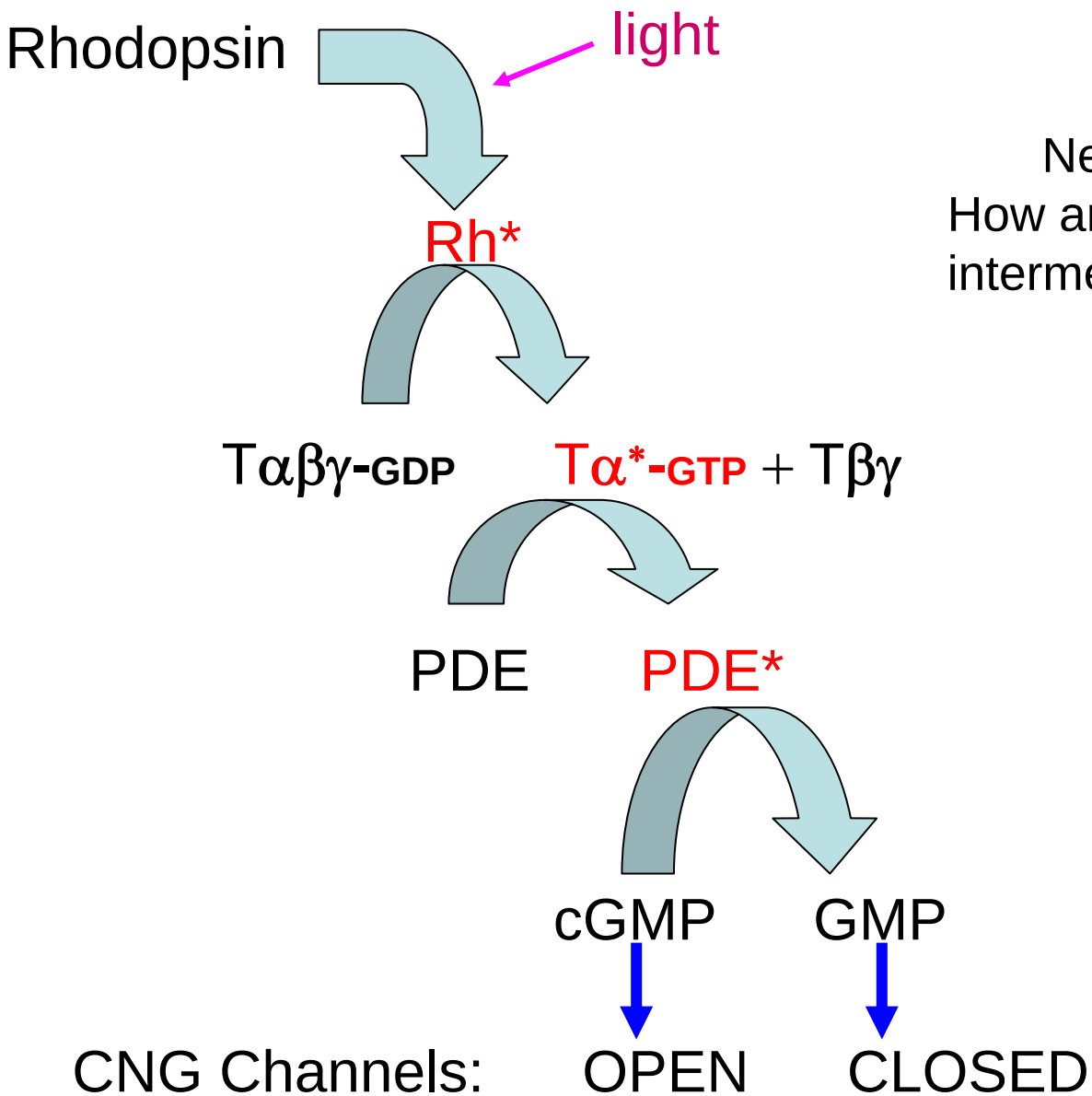
G Protein Effectors Include

Enzymes:

- **Phosphodiesterase (PDE)** — **hydrolyzes cyclic nucleotide; e.g. cGMP ⟶ GMP**
- **Adenylyl cyclase** — **synthesizes cAMP from ATP**
- **Phospholipase C** — **hydrolyzes PIP_2 to produce IP_3 and diacylglycerol (DAG)**

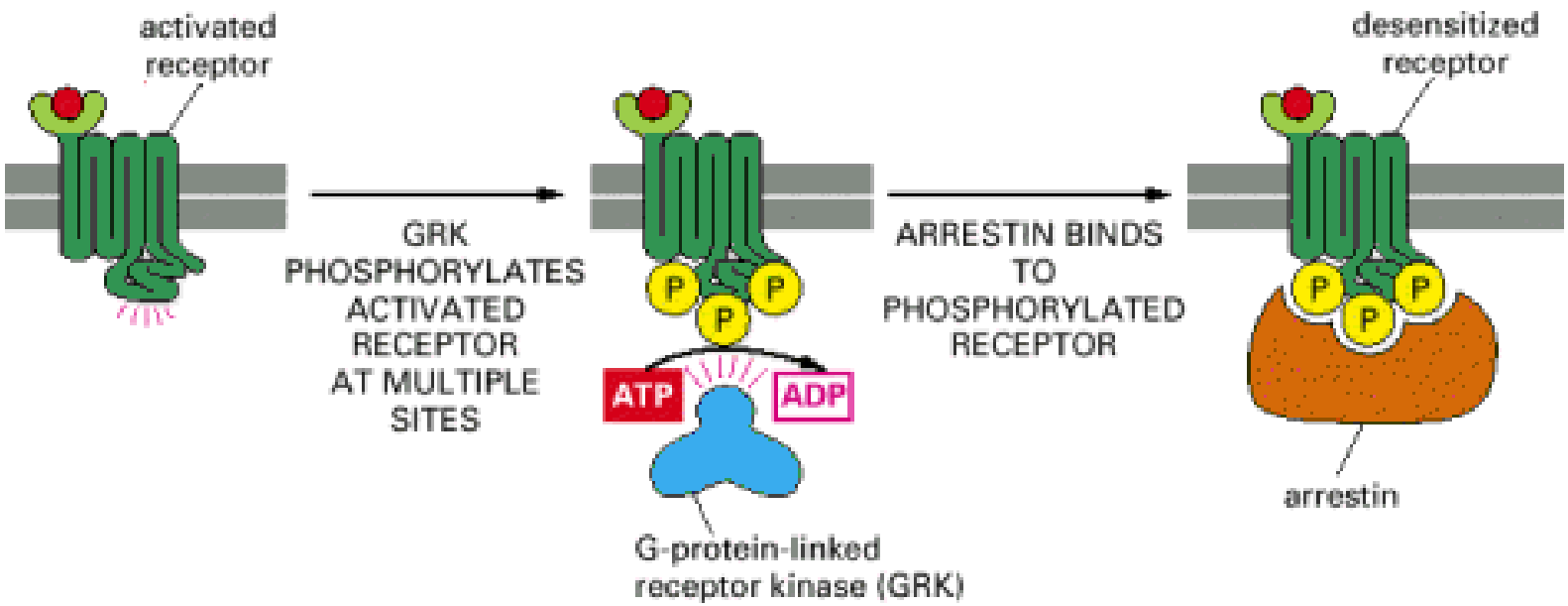
Ion channels: **e.g. K, Ca and Na channels**

Shut-off

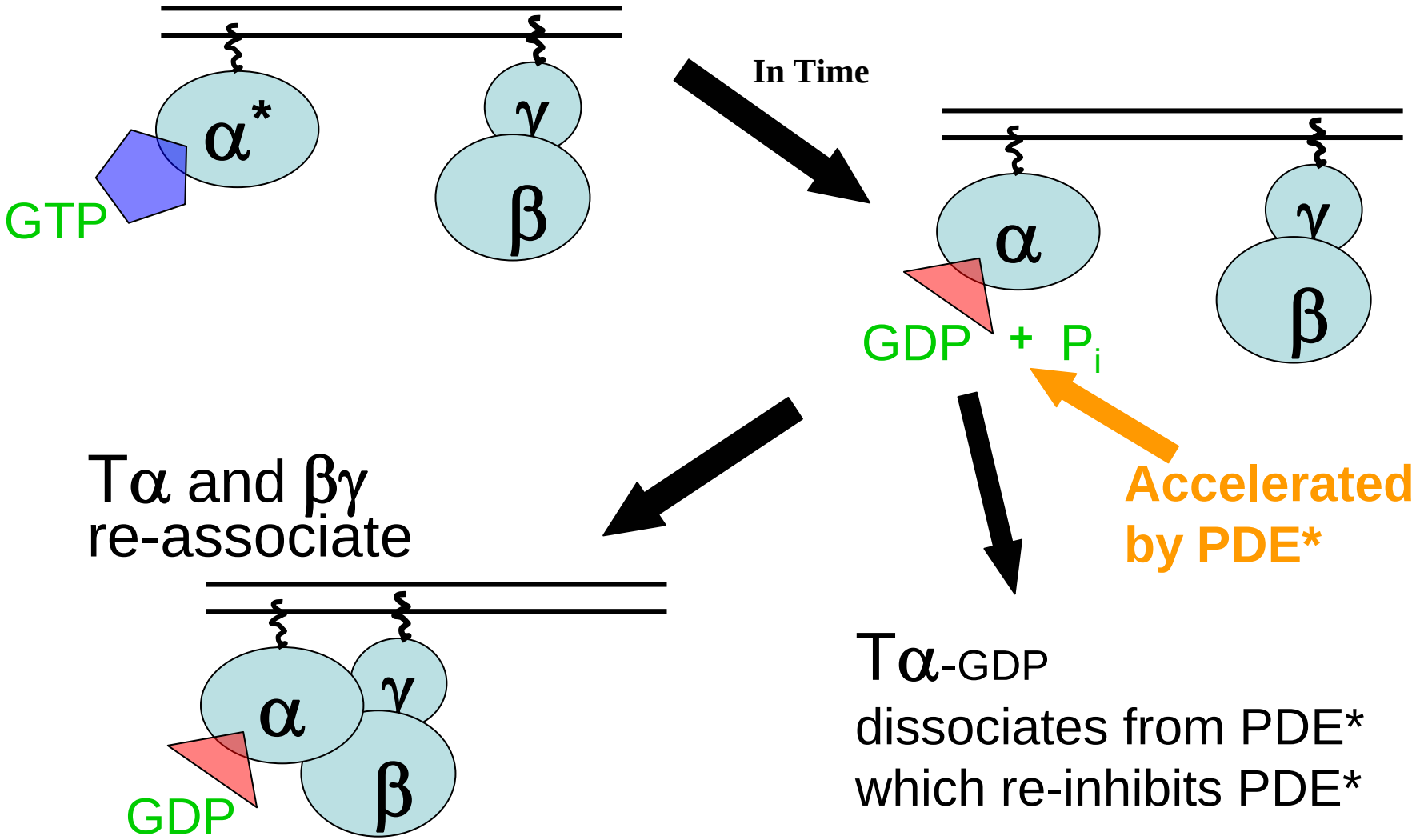


Next question:
How are the activated intermediates shut off?

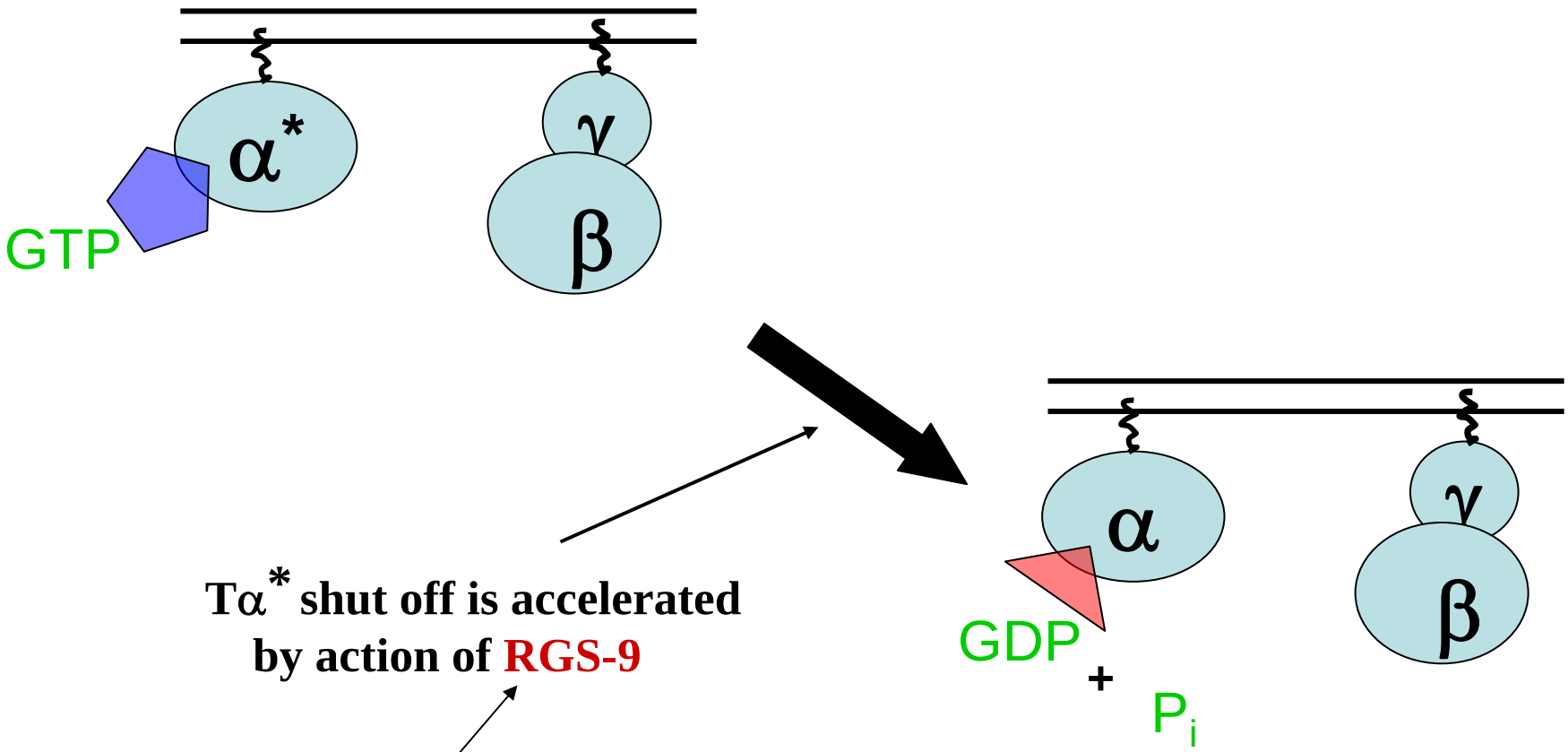
Rhodopsin inactivation



Transducin is inactivated by the α intrinsic GTPase activity which hydrolyzes GTP to GDP

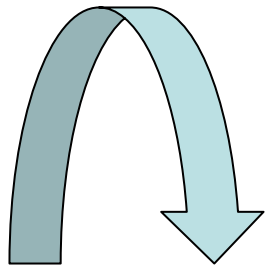


The intrinsic GTPase activity of the α subunit is regulated by a GAP (GTPase accelerating protein) RGS-9



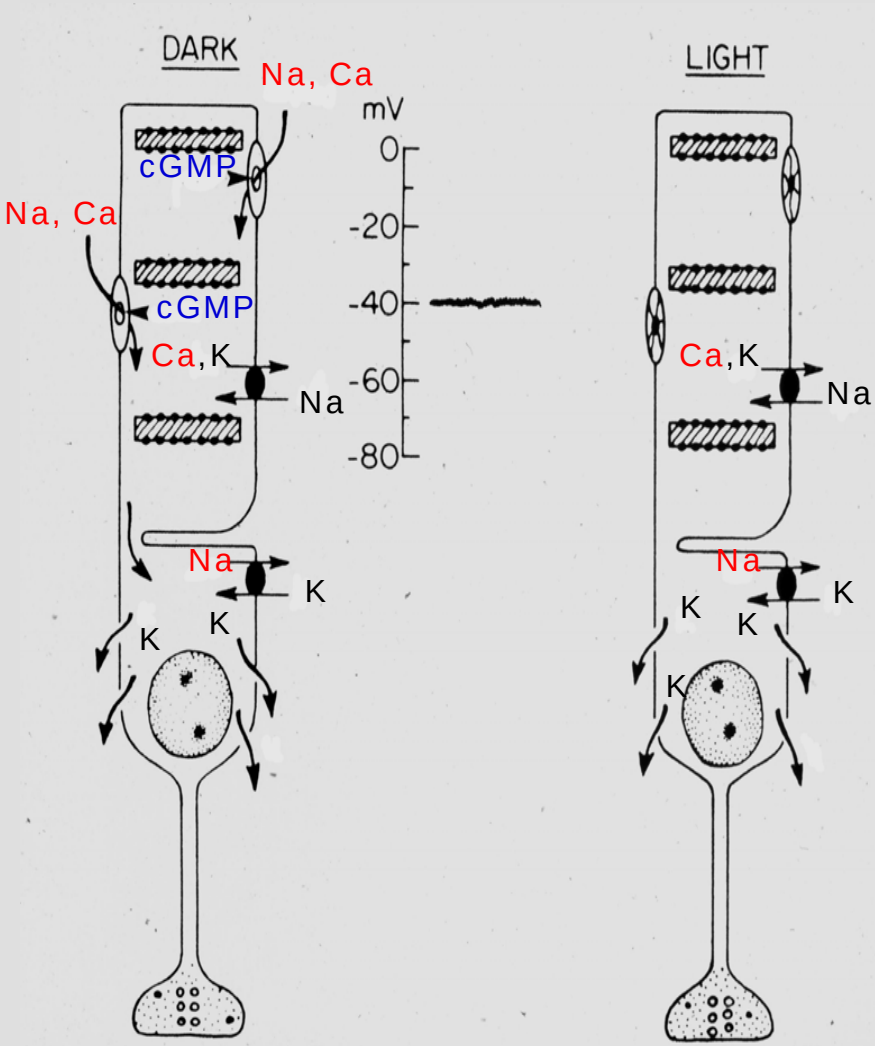
Recovery to the resting state requires resynthesis of the cGMP that had been lost to hydrolysis.

This is accelerated by low Ca (feedback signal!) that stimulates Guanylate Cyclase



GTP **cGMP + PPi**

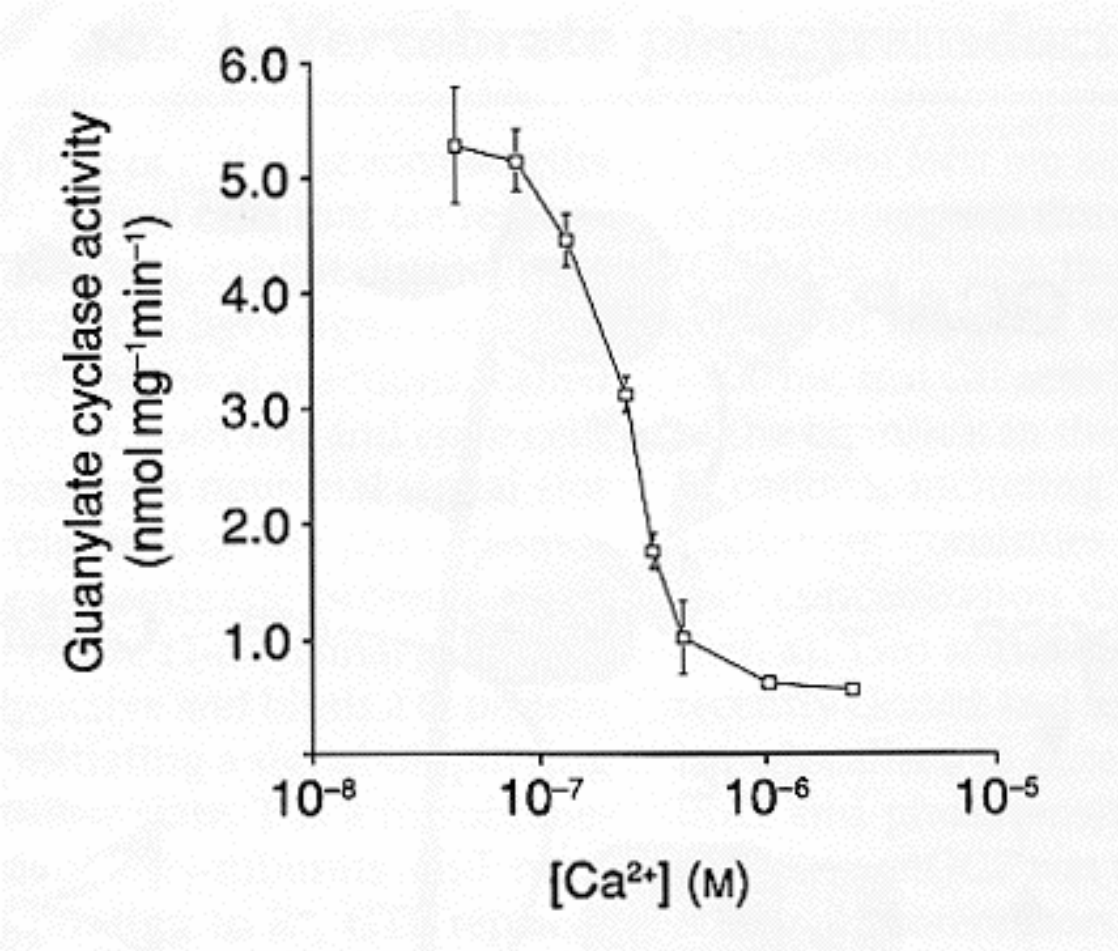
Ca- the second "2nd messenger"

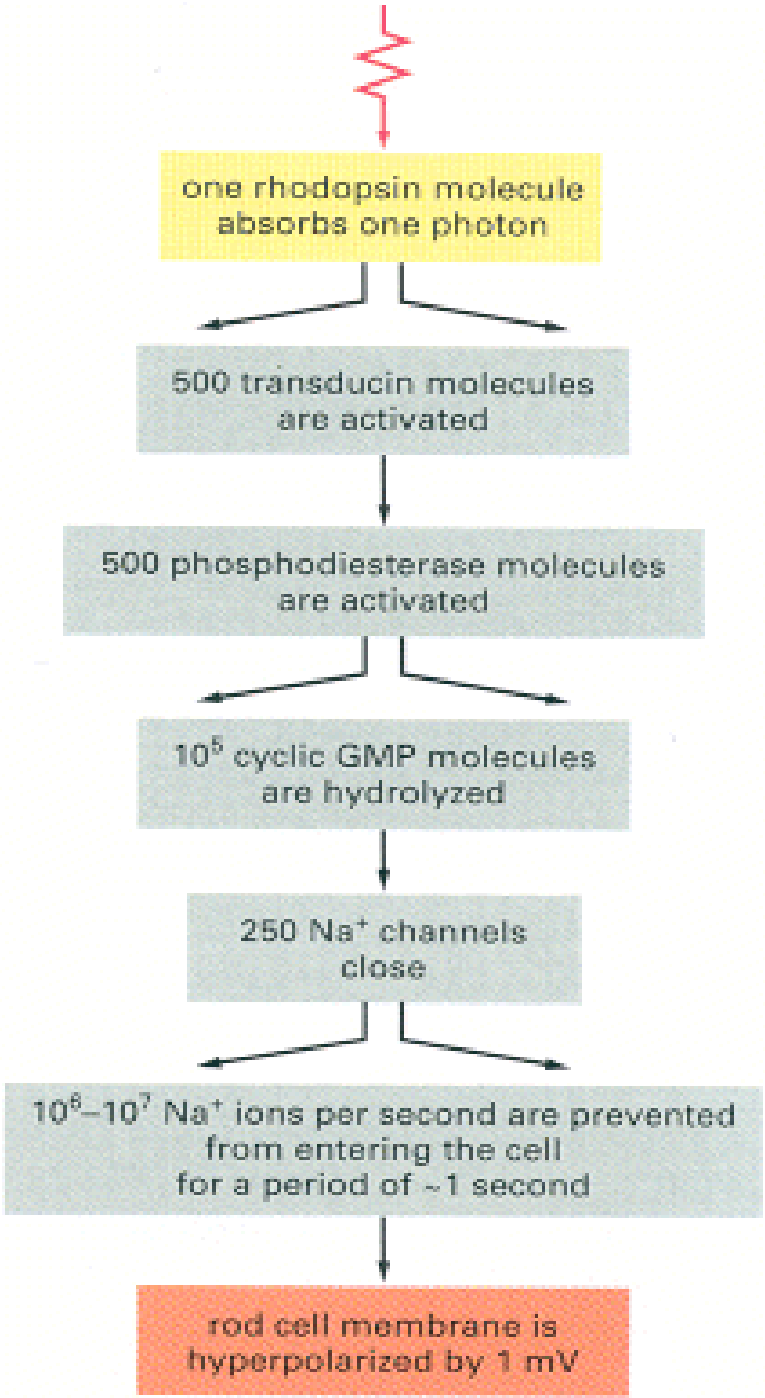


channel closure shuts off Ca influx, while efflux by Na:Ca,K exchange continues and internal Ca declines. The fall in [Ca] is the [Ca] feedback signal.

Negative feedback

Ca^{2+} Regulation of Guanylate Cyclase



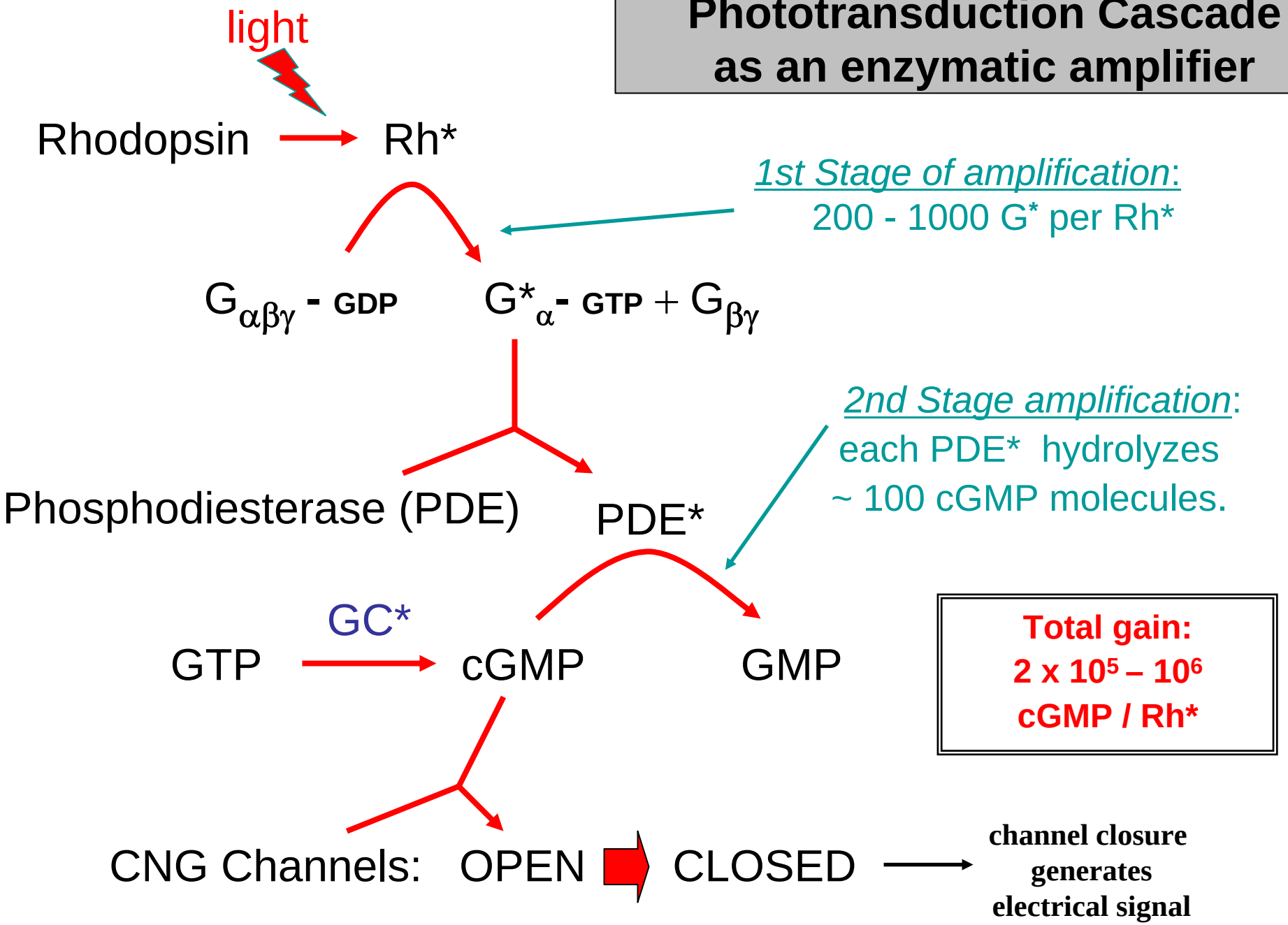


Signaling cascade

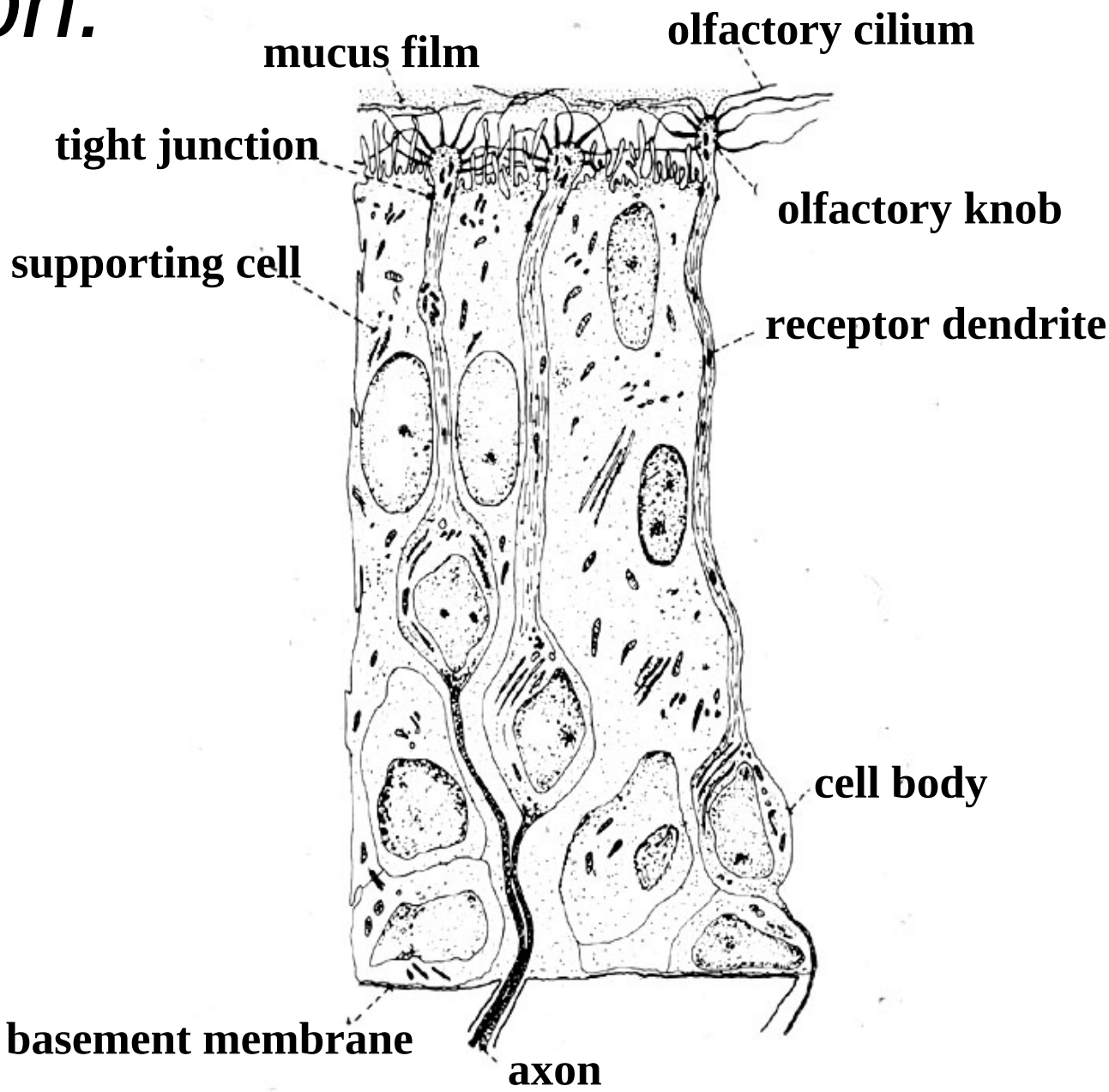
Converts a microscopic stimulus activation of a single molecule –
-into a macroscopic response

Alberts et al, "Mol Bio of the Cell"

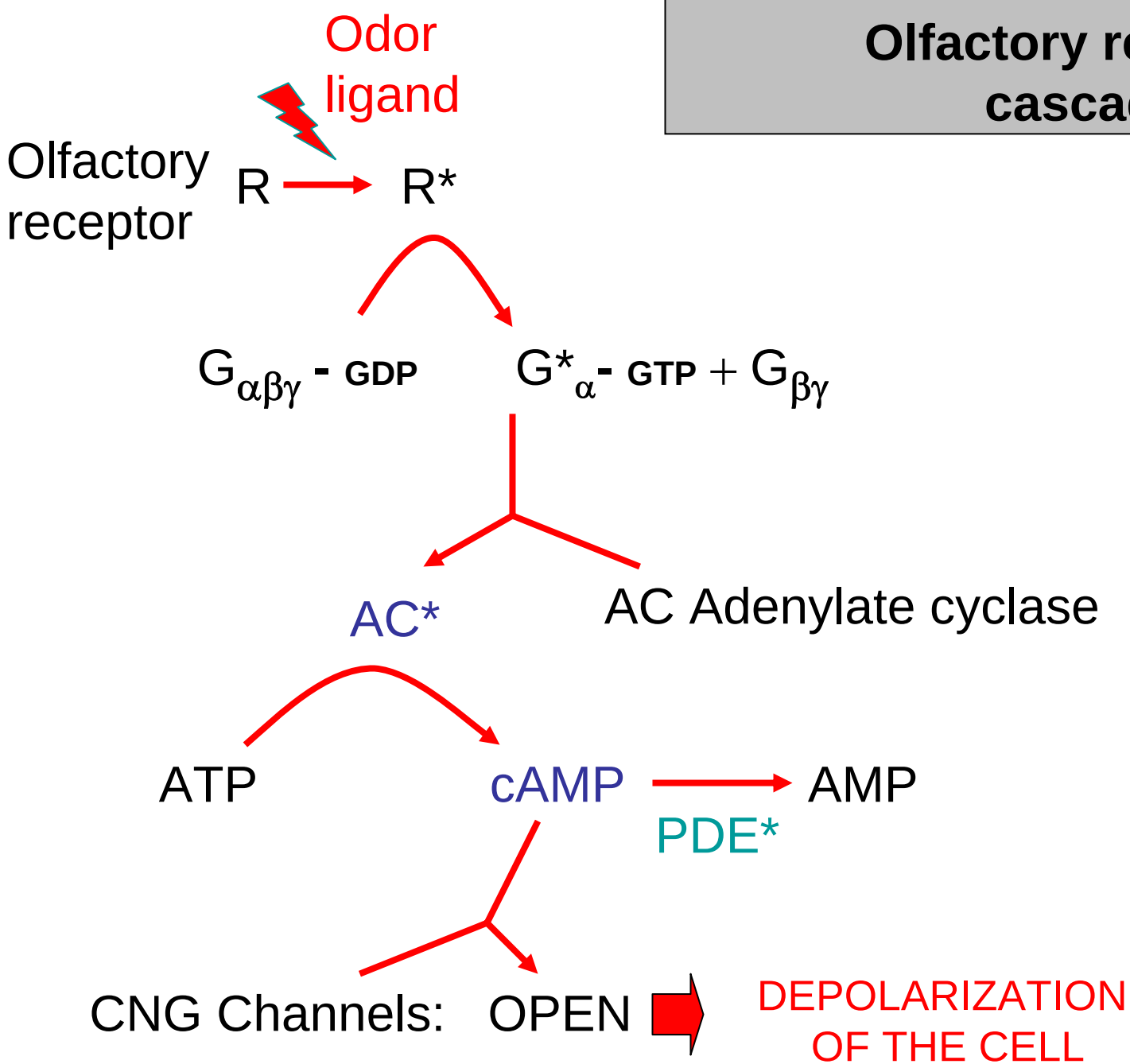
Phototransduction Cascade as an enzymatic amplifier



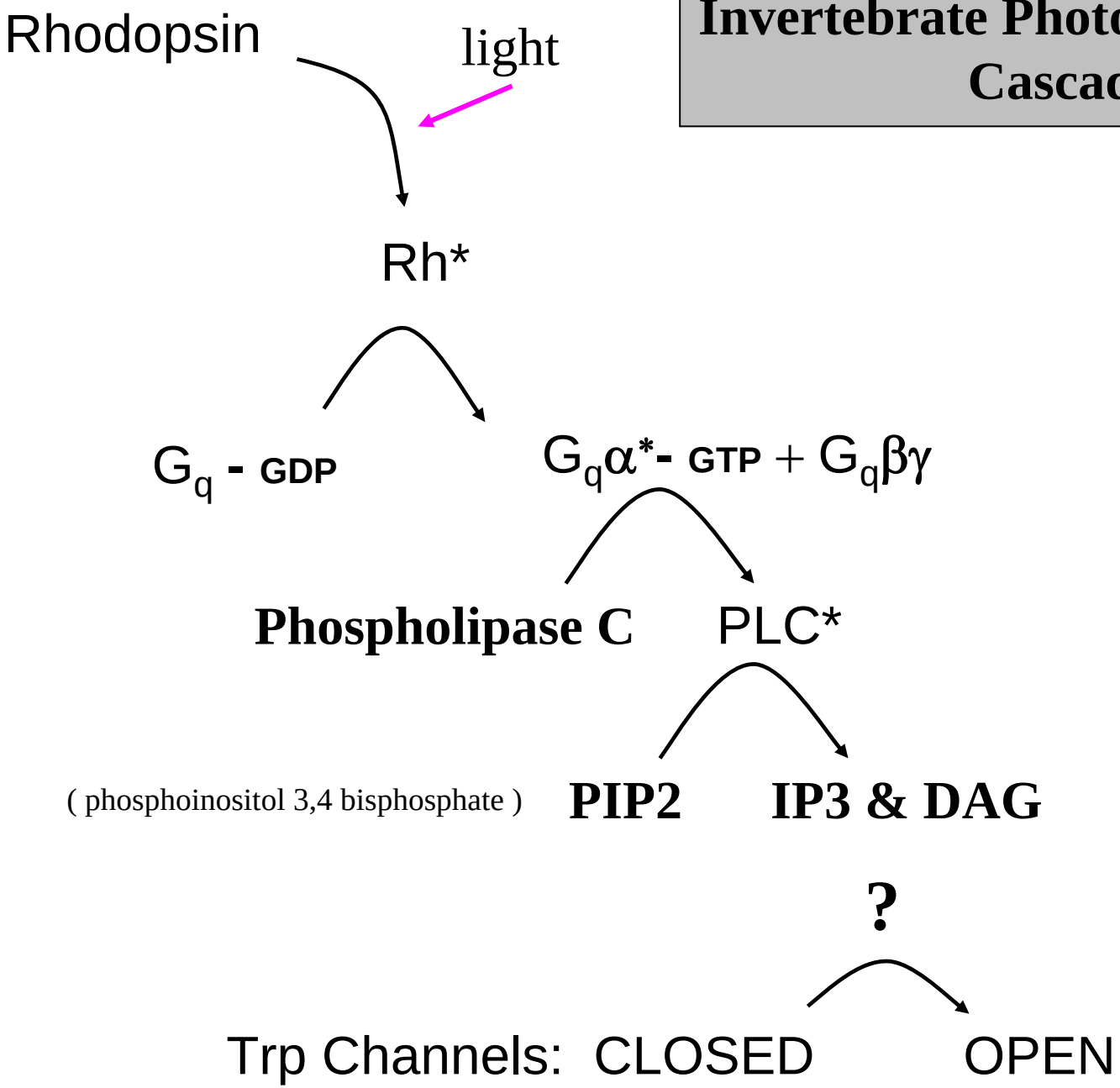
Olfaction:


mucus film
olfactory cilium
tight junction
olfactory knob
supporting cell
receptor dendrite
cell body
basement membrane
axon

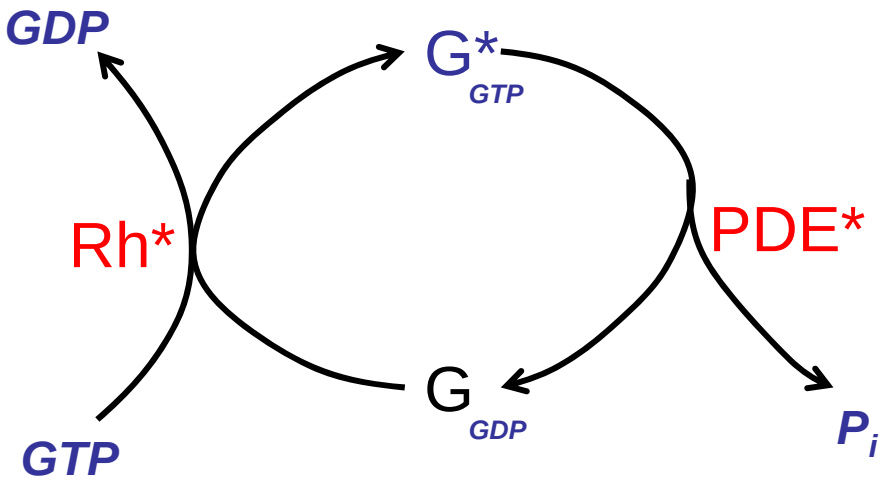
Olfactory receptor cascade



Invertebrate Phototransduction Cascade

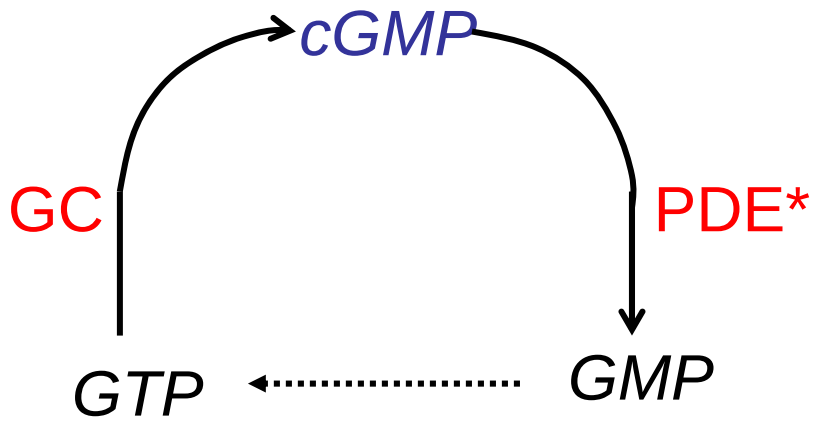


Enzymatic amplifier modules



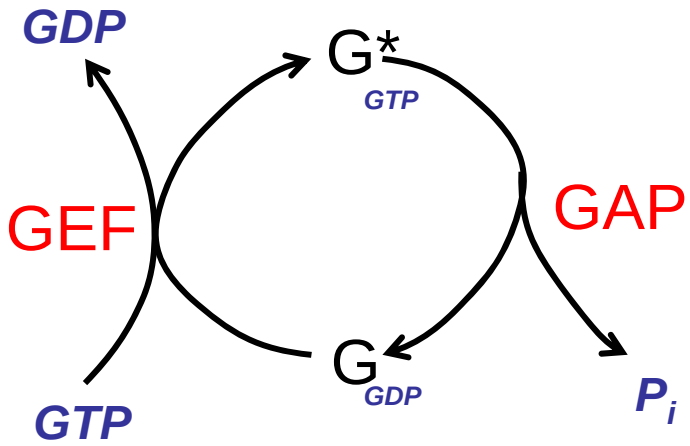
1st stage:
G-protein (Transducin)
activation and deactivation

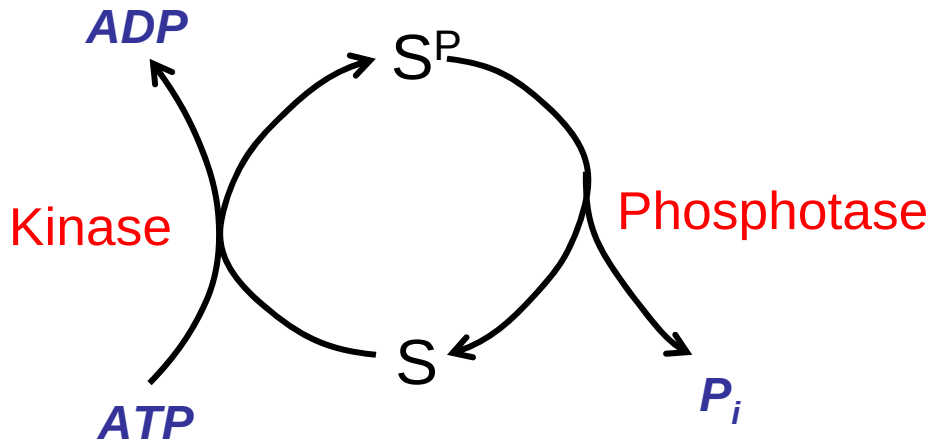
2nd stage:
cGMP hydrolysis
and synthesis



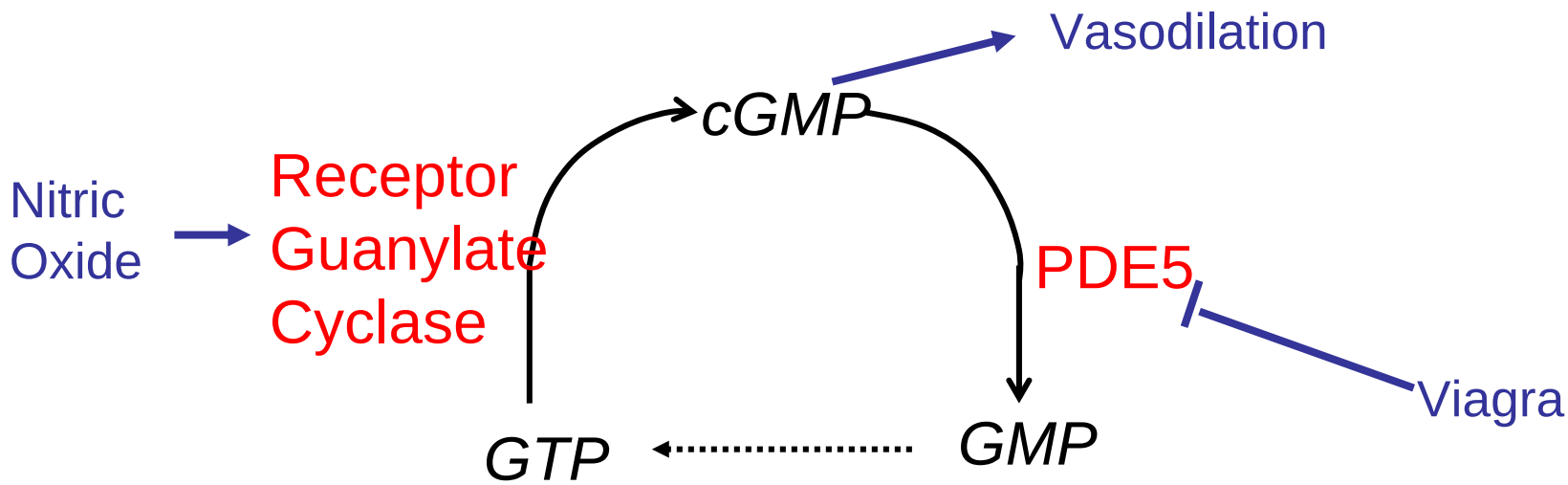
Note: [GTP] / [GDP] & [GMP] acts a metabolic "power supply"

More examples of amp modules

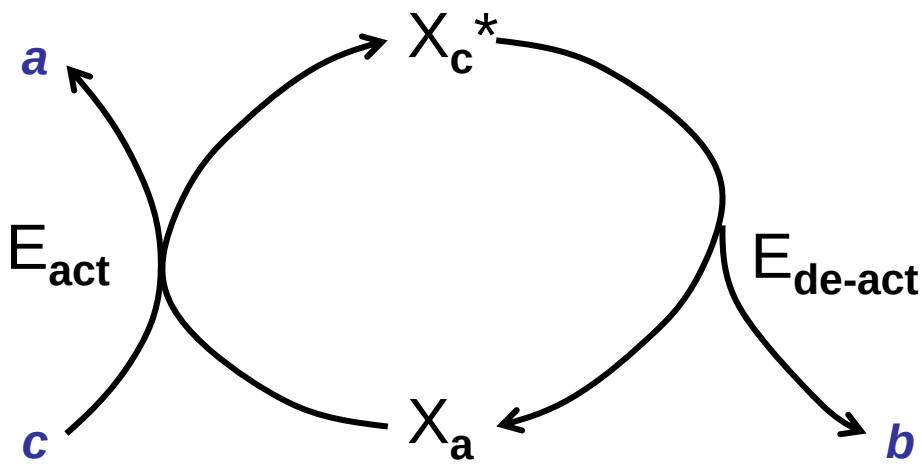




Small GTPases:
Ras, Rho, Rab, Rap, Reb,…



General "push-pull" amplifier circuit



"Power supply":

a+b ⟶ ***c***

maintains high [c]/[a]

$$\frac{d}{dt}[X^*] = k[X][E_{act}] - k'[X^*][E_{de-act}]$$

Steady state:

$$[\overline{X}^*] = \frac{k[E_{act}]}{k[E_{act}] + k'[E_{de-act}]}[X_{tot}]$$

Note: $[X^*]/[X] \neq e^{-\beta\Delta E}$ because the system is held out of equilibrium by fixed [c]/[a] maintained by metabolism

Amplifier gain and time constant

Small change in $[X^*]$ induced by a small change in $[E_{act}]$:

$$\frac{d}{dt}\delta[X^*] + \tau^{-1}\delta[X^*] = k[X_{tot}]\delta[E_{act}]$$

Linear response (in the Fourier domain):

$$\frac{\delta[X^*](\omega)}{\delta[E_{act}](\omega)} = \frac{\tau k[\overline{X}]}{1 + i\omega\tau}$$

Static gain $g = \tau k[\overline{X}]$

Time constant

$$\tau = k[E_{act}] + k'[E_{de-act}]$$

Note: g ~ τ the gain-bandwidth "theorem" !!

Linear analysis of vertebrate phototransduction

Rh* deactivation + 2 amplifier “modules”
with negative feedback via [Ca^{++}]

Good approximation to weak flash (single photon) response

Linear analysis of the cascade

$$\frac{d}{dt}\delta G^* = -\tau_G^{-1}\delta G^* + \tau_G^{-1} g_G \delta Rh^*$$

$$\frac{d}{dt}\delta[cGMP] = -\tau_{cGMP}^{-1}\delta[cGMP] - \tau_{cGMP}^{-1} g_{cGMP}\delta G^*$$

Assuming stoichiometric processes of PDE and Ch* activation are "fast" :*

$$\delta PDE^* \sim \delta G^* \qquad \delta Ch^* \sim \delta cGMP^*$$

$$\delta Ch^* \sim \delta[cGMP](\omega) = \frac{g_G g_{cGMP}\delta Rh^*(\omega)}{(1+i\omega\tau_G)(1+i\omega\tau_{cGMP})}$$

"Hard" and "Soft" parameters

Kinetic constants/affinities -- "hard" parameters change on evolutionary time scale

Enzyme concentrations -- "soft" parameters that can be regulated by the cell.

e.g. $g = \tau k[\overline{X}]$ $\tau = k[E_{act}] + k'[E_{de-act}]$

Golden rule of biological networks:

"anything worth regulating is regulated"

General behavior

$$\frac{\delta X^*(\omega)}{\delta Y^*(\omega)} = \frac{g_1 \ldots g_n}{(1+i\omega\tau_1)\ldots(1+i\omega\tau_n)}$$

Impulse response in the time domain:

$$\frac{\delta X^*(t)}{\delta Y^*(0)} = \begin{cases} g_1 \ldots g_n \dfrac{t^n}{\tau_1 .. \tau_n} & \text{at short times} \\ g_1 \ldots g_n e^{-t/\tau_{Max}} & \text{at times times} \end{cases}$$

For:

$$\tau_1 = \ldots = \tau_n = \tau \qquad \frac{\delta X^*(t)}{\delta Y^*(0)} \sim g \frac{t^n}{\tau^n} e^{-t/\tau}$$

With n=3
- good fit to single-photon response

Case of feedback

$$\tau_G \frac{d}{dt}\delta G^* = -\delta G^* + g_G \delta Rh^*$$

$$\tau_{cGMP} \frac{d}{dt}\delta[cGMP] = -\delta[cGMP] - g_{cGMP}\delta G^* + g_F \delta[Ca]$$

feedback

$$\tau_{Ca} \frac{d}{dt}\delta[Ca] = -\delta[Ca] + g_{Ca}\delta[cGMP]$$

via δCh*

$$\frac{\delta cGMP(\omega)}{\delta Rh^*(\omega)} = -\frac{g_G g_{cGMP}(1+i\omega\tau_{Ca})}{(1+i\omega\tau_G)[(1+i\omega\tau_{cGMP})(1+i\omega\tau_{Ca}) - g_{Ca}g_F]}$$

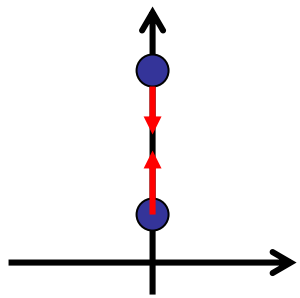
Consequences of negative feedback

$$\frac{\delta cGMP(\omega)}{\delta Rh^*(\omega)} = -\frac{g_G g_{cGMP}(1+i\omega\tau_{Ca})}{(1+i\omega\tau_G)[(1+i\omega\tau_{cGMP})(1+i\omega\tau_{Ca}) - g_{Ca}g_F]}$$

Reduction of static gain:

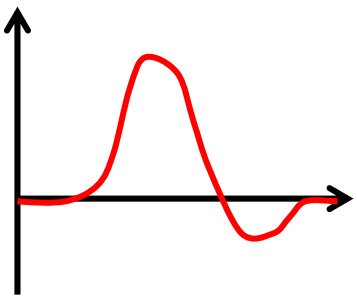
$$g_G g_{cGMP} \rightarrow \frac{g_G g_{cGMP}}{(1-g_{Ca}g_F)}$$

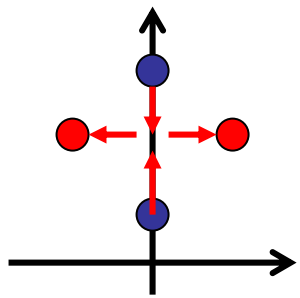
Accelerated recovery:



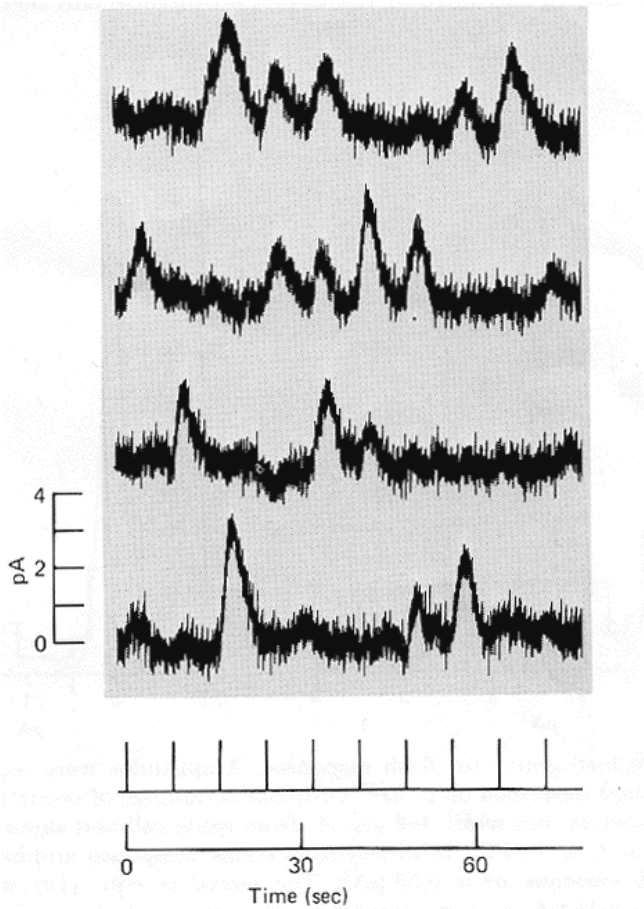
"Ringing":

$$g_{Ca}g_F > \frac{(\tau_{Ca}-\tau_{cGMP})^2}{4\tau_{Ca}\tau_{cGMP}}$$





Signal and Noise: How much gain is enough?



Single photon "signal":

$$\left.\begin{array}{l} \delta I \sim 2 \text{ pA} \\ I_{Dark} \sim 60 \text{ pA} \end{array}\right\} \quad \delta I / I_{Dark} \sim 3\%$$

What's the dominant source of background noise?

Thermal fluctuations?

$$\frac{CV^2}{2} \rightarrow CV_{Dark}\delta V = k_B T \rightarrow \frac{\delta V}{V_{Dark}} = \frac{k_B T}{CV_{Dark}^2} \sim 10^{-6}$$

ROS capacitance C ~ 20pF

Negligible !

Reaction shot noise

$$\frac{d}{dt}X^* = \Gamma_+(X,..) - \Gamma_-(X^*,..) + \eta(t)$$

# of molecules

Langevin noise
(i.e. Gaussian, White)

$$< \eta(t)\eta(0) >= (\Gamma_+ + \Gamma_-)\delta(t)$$

E.g. if $\Gamma_- = 0$ consider ΔX^* produced over time Δt

$$< \Delta X^* >= \int dt \Gamma_+$$

$$< (\Delta X^*)^2 > - < \Delta X^* >^2 = < [\int dt \eta(t)]^2 >= < \Delta X^* >$$

Poisson process

Channel opening noise

$$\frac{d}{dt}Ch^* = \gamma([cGMP])Ch - \tau_{Ch}^{-1}Ch^* + \eta(t)$$

$$Ch_D^* = < Ch^* > = \tau_{Ch}\gamma([cGMP]_D)Ch_{Tot}$$

Channel flicker noise:

$$< (\delta Ch^*)^2 > = < Ch^* > = Ch_{Dark}^*$$

Note: this Poisson law holds generally for mass action

$$\frac{< (\delta Ch^*)^2 >^{1/2}}{< Ch^* >} = \frac{1}{\sqrt{Ch_D^*}} \sim \frac{1}{\sqrt{10^4}} = 1\%$$

< 3% signal

Plus fluctuations of $\delta[cGMP]$:

$$< (\delta Ch^*)^2 > = Ch_D^* + f(\tau_{cGMP} / \tau_{Ch}) < (\delta[cGMP])^2 >$$

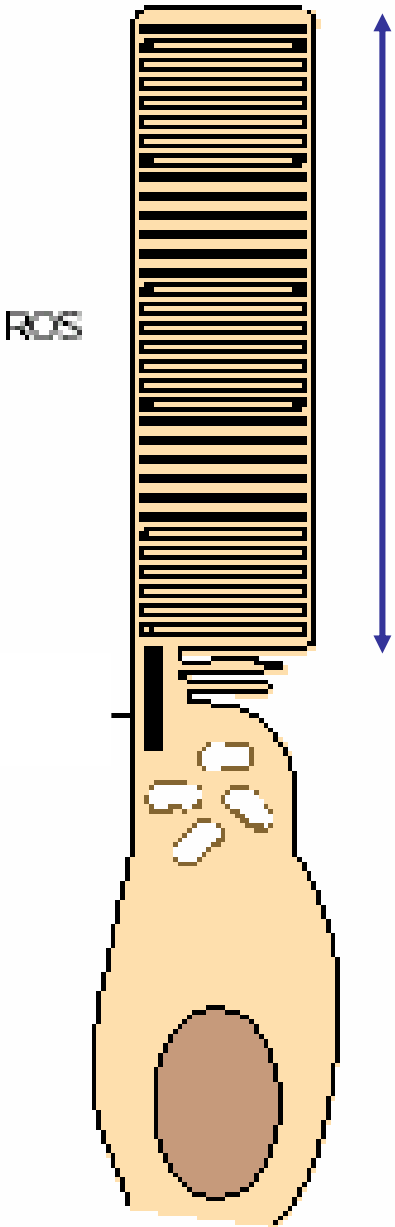
cGMP fluctuations

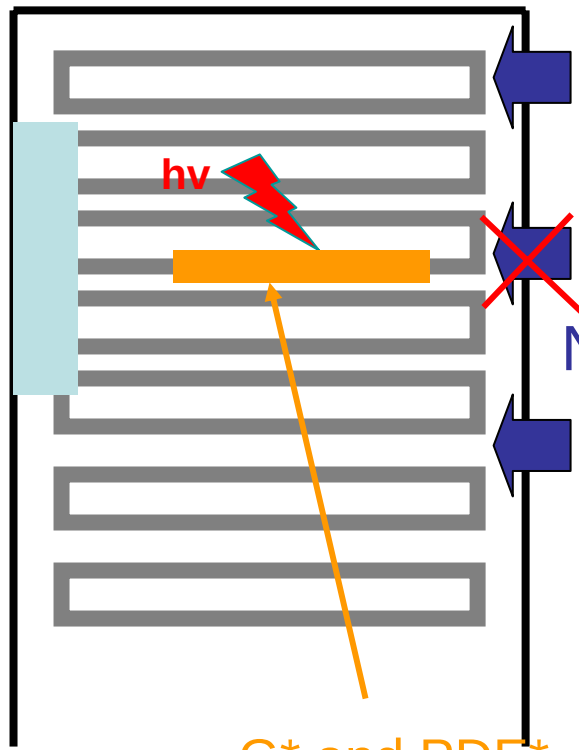
Note: voltage response is coherent over the whole rod outer segment, hence must consider the whole volume $V_{ros} \sim 10^4 \mu m^3$

$\#cGMP = [cGMP]\, V_{ros} \sim 10 \mu M\ 10^4 \mu m^3 \sim 10^7$

Negligible fluctuations …

Locality of single photon response





cGMP depletion

Na^+, Ca^{2+}

G* and PDE* activity

$$l_{cGMP} \sim \sqrt{D_{cGMP}\tau_{cGMP}} \sim \sqrt{10^3 \mu m^2 s^{-1} \times .5s} \sim 20\mu m$$

$$l_{G^*} \sim \sqrt{10\mu m^2 s^{-1} \times .5s} \sim 2\mu m$$

Single photon response variability

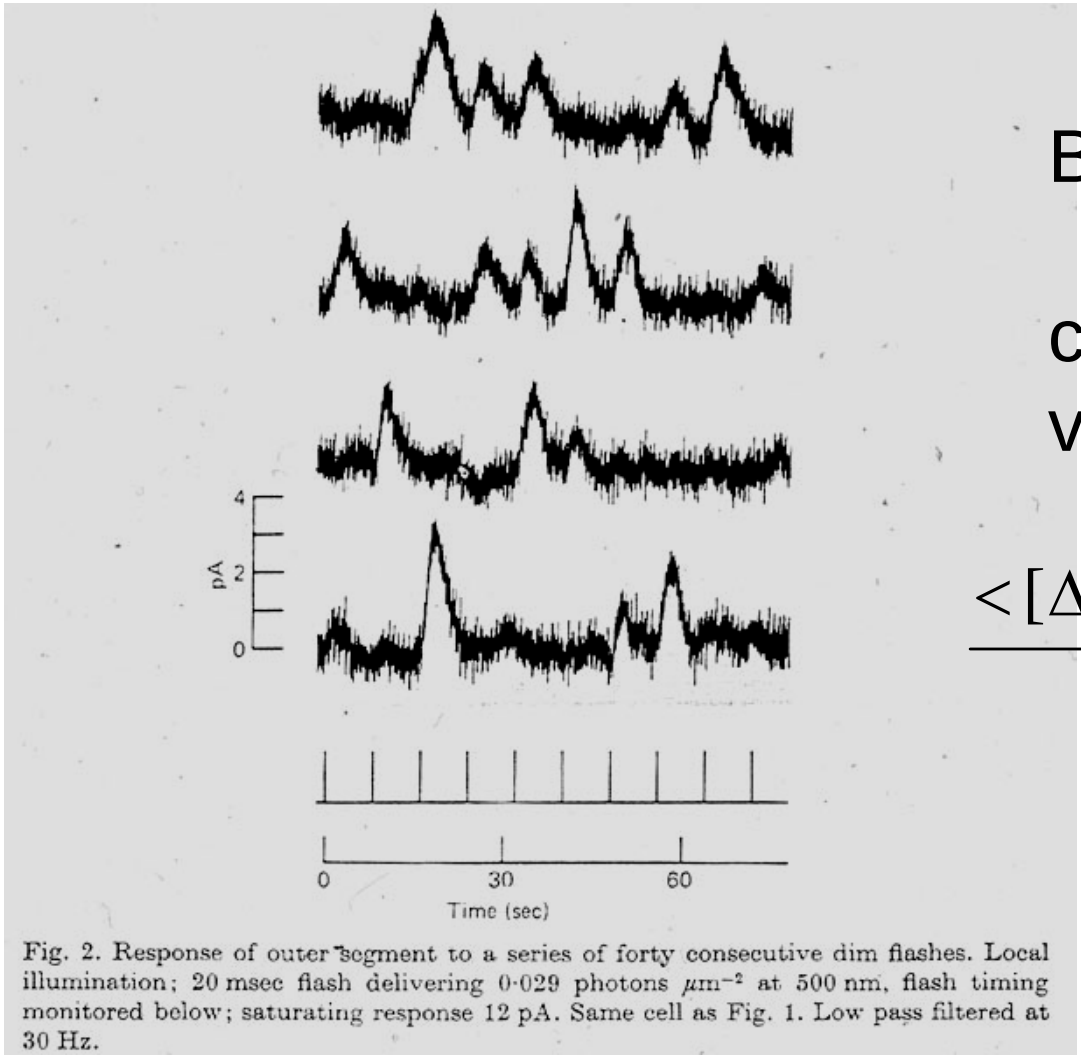


Fig. 2. Response of outer segment to a series of forty consecutive dim flashes. Local illumination; 20 msec flash delivering 0·029 photons μm^{-2} at 500 nm, flash timing monitored below; saturating response 12 pA. Same cell as Fig. 1. Low pass filtered at 30 Hz.

Baylor-Riecke:

coefficient of variation

$$\frac{< [\Delta I_p - < \Delta I_p >]^2 >^{1/2}}{< \Delta I_p >} \sim 0.25$$

What is the largest source of response variability?

Spontaneous activation G -> G* ??

$< 10^{-7}\ s^{-1}$ Negligible even with 10^8 G molecules !

Variability of Rh* "on"- time Δt ??

$$\Delta G^* \sim k\Delta t \qquad <\Delta t> = \tau_{Rh^*}$$

1st order Poisson process:

$$\frac{<(\Delta t)^2> - <\Delta t>^2}{<\Delta t>^2} = 1$$

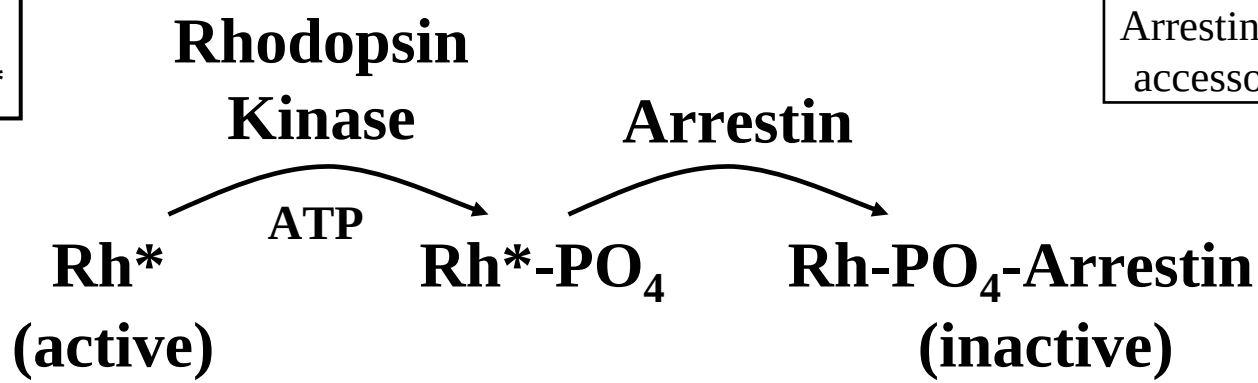
Baylor& Reicke measured: $\frac{<(\Delta I_p)^2> - <\Delta I_p>^2}{<\Delta I_p>^2} \sim .05$

Poisson process of order 20 !!!

Multistep deactivation of Rh*

Meta-Rhodopsin shut-off:

Rh Kinase is turned on by binding to Rh*



Arrestin is a 48kD accessory protein

Summary:

What we have learned:

- GPCR / cyclic nucleotide cascade
- Enzymatic amplifier module
  and linear response
- Noise analysis

Tomorrow…

from frog to fly and from linear to non-linear

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