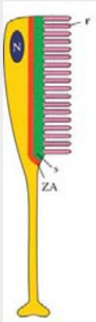
Sensory Systems – Basics and Principles I
Photoreceptors and Phototransduction
Retinal Pigment Epithelium & Visual Cycle

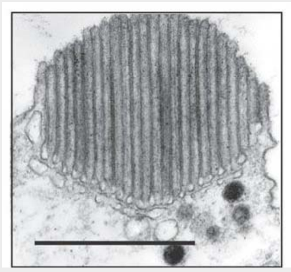
Bernd Wissinger
Molecular Genetics Laboratory
Institute for Ophthalmic Research

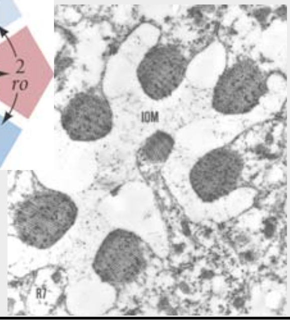
- Evolution of Photoreceptors
- Photopigments
- Rod and Cone Photoreceptors
- Phototransduction in Vertebrates
- Phototransduction in other light-sensitive Cells
    - PTD in Drosophila
    - Rarietal Eye
    - Melanopsin - ipsRGCs
- The Visual Cycle

Evolution of Photoreceptors - Two principle Types:

- Rhabdomeric PRs: Predominante in Protostomia
  - Apical Microvilli Membrane Folds
  - expresses R-Opsins
  - employ Gq/Phospholipase signalling >> Depolarization







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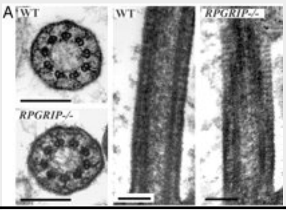
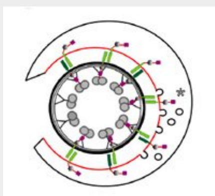
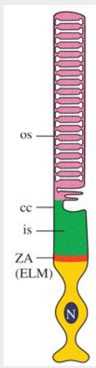
Evolution of Photoreceptors - Two principle Types:

- Rhabdomeric PRs: Predominante in Protostomia
  - Apical Microvilli Membrane Folds
  - expresses R-Opsins
  - employ Gq/Phospholipase signalling >> Depolarization
- Ciliary PRs: Predominate in Chordata
  - Cilium with Evaginations of the ciliary Membrane
  - expresses C-Opsins
  - employ Gt/PDE signalling >> Hyperpolarization







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Evolution of Photoreceptors

- Common origin of Photopigment (Retinal + Opsin)
- Rhabdomeric and Ciliated PRs coexist in some early Bilateria: Platynereis – „Borstenwurm", scallop – „Kammmuschel", Planarians
- Ciliary PRs with C-Opsin in box yellyfish !!

➢ Monophyletic origin of RPs (C-PR first ?)
(Gene Duplications & Cell Type Differenciation)
➢ Long parallel Evolution of C-PRs and R-PRs in Metazoa

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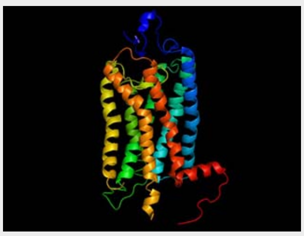
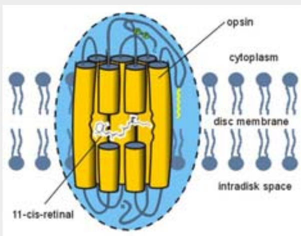
Photopigments

- **Type II Opsins** predominate in Metazoa (monophyletic)
- **G-Protein Coupled Receptor** (GPCR) Family
  Membrane Protein with 7 TM-Helices
- Apoprotein: **Opsin** or **Rhodopsin**
  Chromophore: mostly **Retinal**
- Internal Retinal Binding Pocket with Schiff Base covalent Link
- Range of λ_{max}: 340 – 630 nm





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Opsin Phylogeny

- Deeply rooted, but **monophyletic Tree**
- Dates back to early metozoan species (> 600 Mio years)
- Two main Groups :
  - **C-Opsins** in ciliary PR
  - **R-Opsins** in rhabdomeric PR
  (molecular data on opsins matches cell type differentiation)
- Additional distant related Opsins:
  - Go coupled Opsins
  - Photoisomerase (RGR)

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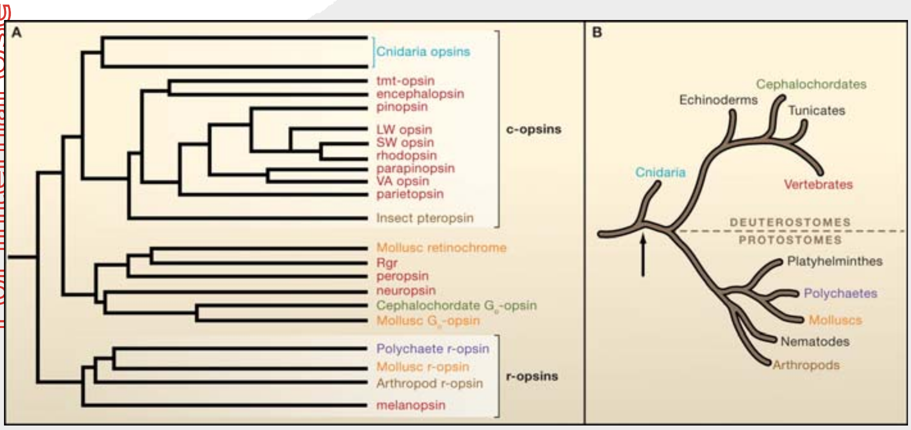
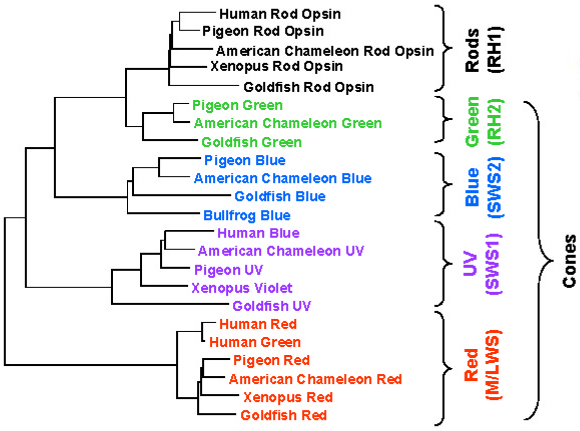
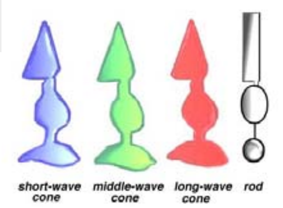
Opsin Phylogeny

Better Figure 1 from Yau and Hardie)



Opsin Phylogeny in Vertebrates





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MPI - Kolloquium
Monday, 30 November 2009, 8.15 pm
Dan-Eric Nilsson, Lund University, Sweden
Theme: "***The origin and evolution of vision***"

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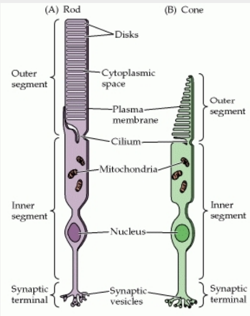
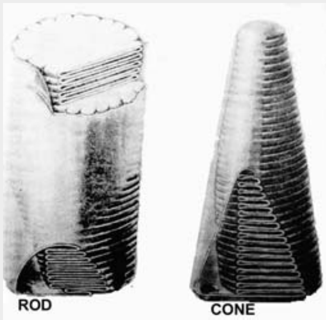
Rod and Cone Photoreceptors

- Rods accomodate Vision under low Light
- Cones for Daylight Vision (adapt over magnitudes of LI)
- Cones with Plasma Membrane Lamellae at OS
- Rods with internal Membrane Discs





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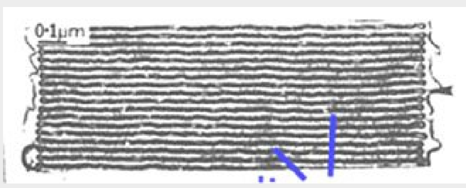
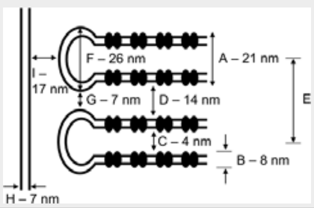
Rod Outer Segment

- Rods with internal Membrane **Discs**
- Rod Outer Segment Dimension:
  Length x Diameter: 20-25 µm x 1-1,5 µm (Mouse)
  40 µm x 1-2 µm (Human)
  50-60 µm x 6-9 µm (Xenopus)
- ROS Discs diameter: 5-20 nm





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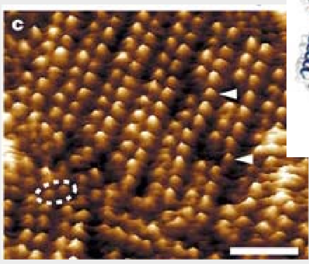
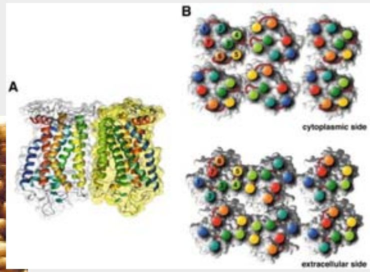
Rhodopsin in the Outer Segment

- High density in the Disc Membrane (~ 25-50.000 / µm²)
- Paracrystalline Structure
- Arrays of Dimers
- 70-90% of ROS protein
- 3 mM in ROS (~ 120 mg/ml)





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Phototransduction in C-PRs

- Basic Principle of Phototransduction
- (Rhod)Opsin Excitation
- G-Protein Coupling
- Phosphodiesterase Activation
- cGMP and CNG channel

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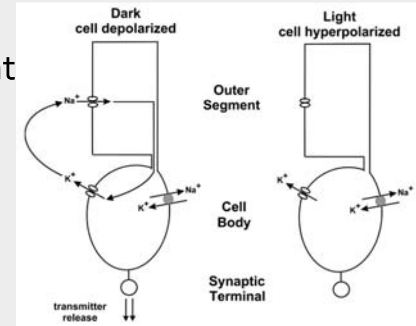
Basic Principle of Phototransduction in C-PRs

- In the Dark:
  - Steady Inward Current in OS (Dark Current)
  - Membrane Potential at ~ -30mV
  - Steady Glutamate Release at the Synapse
- Light Stimulation:
  - Cessation of Inward Current
  - Mem. Potential < -30mV
  - graded Decrease of Glutamate Release



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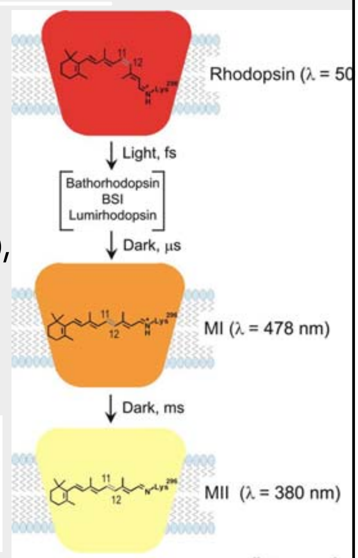
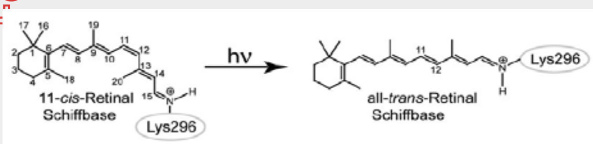
(Rhod)Opsin Excitation

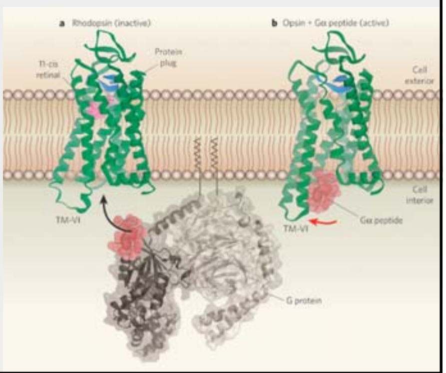
- Photoisomerization of Chromophor: **11-cis-Retinal → all-trans Retinal**
- Induces conformational Changes within the Protein
- Intermediates: Photo~ (s), Batho~ (s), Lumi- (s), Meta I –Rhodopsin (l)
- Stable „active" Form: **Meta-Rhodopsin II**





G-Protein Coupling

- M-II activates a **heterotrimeric G-Protein** through diffusional Encountering
- **Transducin (G_t)** in Rods and Cones
- Induces a **GDP-to-GTP exchange** on α-Subunit ($G_{t\alpha}$) and its **dissociation** from the βγ-Subunits
- A single M-II activates ~20 $G_{t\alpha}$ during a single photon response in rods



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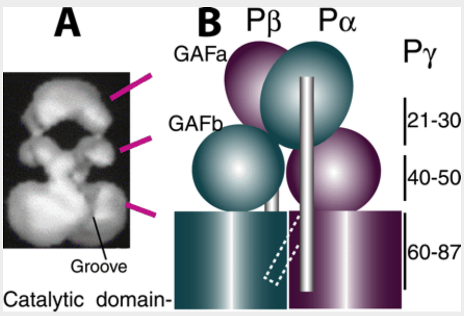
Phosphodiesterase Activation

- $G_{t\alpha}$* stimulate the activity of a retinal cGMP-PDE
- **Typ 6 PDE**: Heterotetramers of αβγγ in Rod, α'α'γγ in Cone
- **$G_{t\alpha}$ bind to inhibitory γ-Subunit** which eventually liberates the activity of the catalytic αβ- or α'α'-Subunits



From: Guo et al. (2006)
J Biol Chem *281, 15412-15422*

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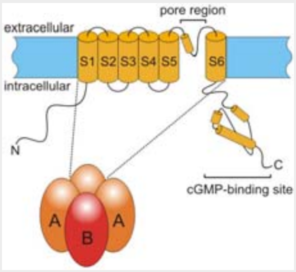
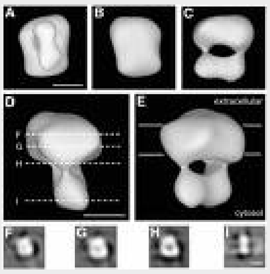
cGMP and CNG Channel

- **PDE hydroloyzes cGMP** to GMP ➢ [cGMP]↓
- cGMP is ligand of a **cGMP-dependent Cation Channel:**
  - in the dark: [cGMP]↑ ➢ Channel open (dark current)
  - light stimulation: [cGMP]↓ ➢ Channel closed
- Sustained net Cation Efflux generates **Hyperpolarization**





From: ISB1-FZ Jülich

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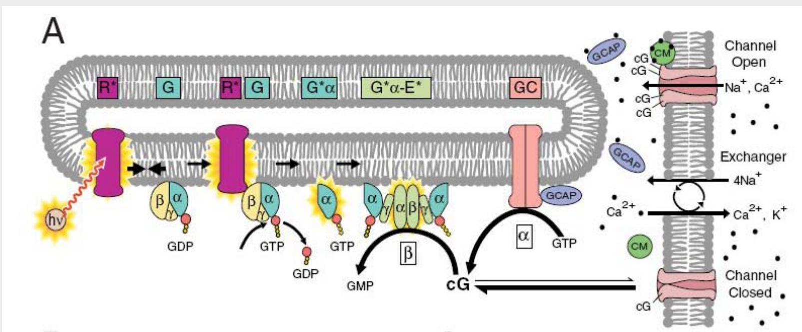
Phototransduction in C-PRs

- Components are integrale Membrane Proteins or membrane-associated (cytoplasmic face of disc membrane), CNG channel in Plasma Membrane
- Encountering through lateral Diffusion



From: Pugh & Lamb (2000)

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Deactivation of Phototransduction in C-PRs

- M-II is phosphorylated by **Rhodopsin-Kinase** which enables Binding of **Arrestin**.
- **Release of all-trans Retinal** from deactivated Photopigment
- $G_{t\alpha}$* inactivates through **intrinsic GTPase** activity and catalyzed by GTPase-activating Complex (**GAP**): RGS9 + R9AP + $G_{\beta 5}$ + PDE_{γ}
  ➢ $G_{t\alpha}$-GDP dissociates from PDE_{γ} and reassemble with $\beta\gamma$-Subunits into the inactive trimeric G_t
  ➢ PDE becomes inactive
- cGMP levels restored by **Guanylate Cyclase** (Ca^{2+}-dep.)
- Reopening of CNG channels

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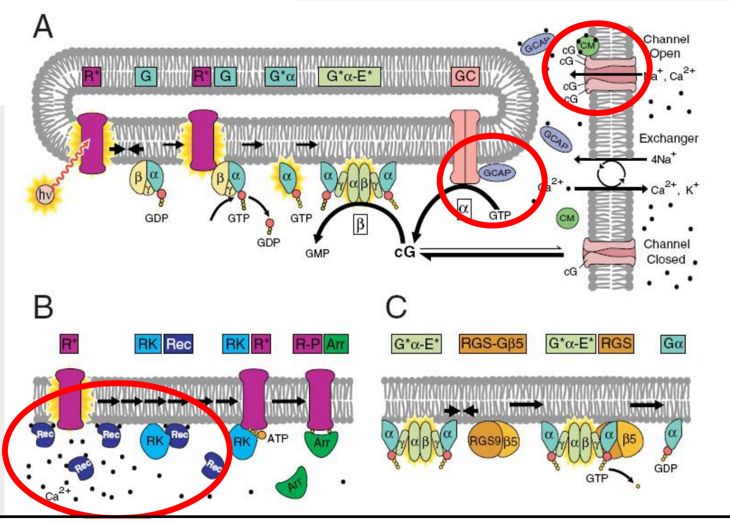
Calcium Feedback and Adaptation Processes

- 10-20% of Dark Current in OS carried by Ca^{2+}
  (600 nM Level stablized by $Na^{+}/Ca^{2+},K^{+}$-Exchanger)
- **Light Stimulation** leads to Closure of CNG channels
  ➢ Cessation of Ca^{2+} Influx, while NCKX is still active
  ➢ **Lowering of [Ca^{2+}] in OS**
- **stimulates Guanylate Cyclase Activity** (through dis-inhibition of GC Activating Proteins (**GCAPs**)
- **stimulates Rhodopsin Kinase Activity** (through Ca-dependent inhibition by **Recoverin**)
- **reduces Ligand Affinity of CNG channel** (through calmodulin)

Calcium Feedback and Adaptation Processes



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Rod vs Cone Phototransduction

- Cones are **30-100 times less sensitive**, but ~ **5 times faster** Response Kinetic
- **- Gt less efficiently activated**
  - Increased RK-Activity > shorted Rh* Lifetime
  - Increased GTPase Activity of $G_{T\alpha\text{-}GTP}$ > Shuts off PDE
  - **faster [Ca^{2+}] fluctuation** due to increased surface/vol. ratio

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Phototransduction Cascade in R-Photoreceptors

- **Depolarizing Light Response !!!**
- Employs **G_q-type G-Protein**
- $G_{q\alpha\text{-}GTP}$ activates **Phospholipase C (PLC)**
- PLC hydrolyzes **PIP_2 to $InsP_3$ and DAG**
- Lipid Conversion activates **TRP and/or TRPL channel(s)**
  ➢ **Cation Influx**
- Ca^{2+} feedback on various targets (PLC, ARR⇔Rh*, TRP,..)

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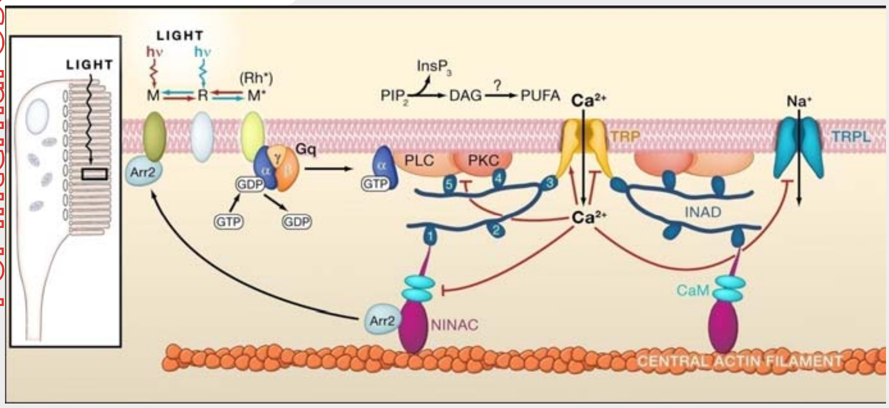
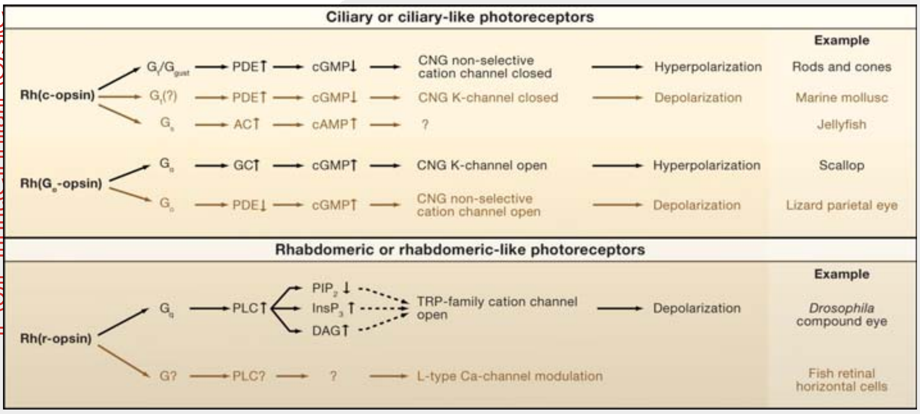
Phototransduction Cascade in R-Photoreceptors
➢ **Drosophila**



Varieties of Photoresponse Signalling



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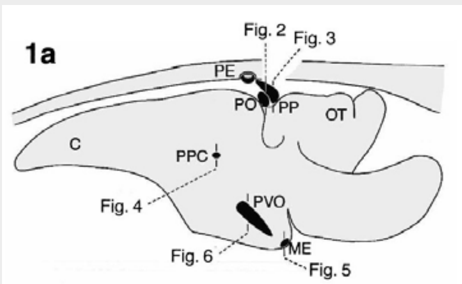
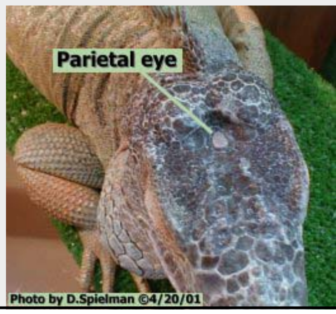
Parietal Eye PR – a Unique Antatonistic Pathway

- Present on Forehead of some Lizards and Amphibians
- Outgrowth of the Diencephalon
- Neuronal Contact to Pineal Organ and other Parts of the Brain
- Function ?, may mediate global detection of dusk and dawn





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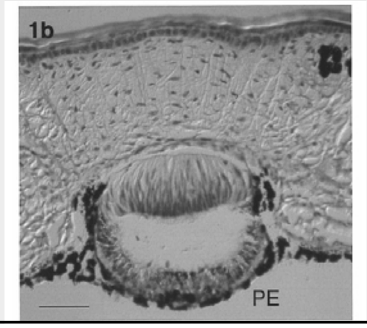
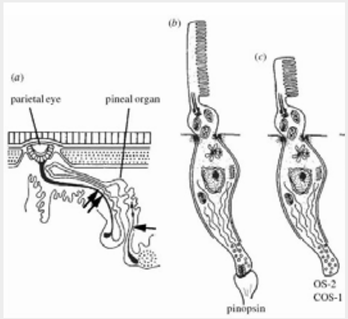
- Primitive Cup-shaped Eye with a simple Lens
- Ciliary-type Photoreceptors similar to Rods Cones with well-developed Outer Segment
- Outer Segments face forward to Light
- No RPE and Interneurons; PR >> GC





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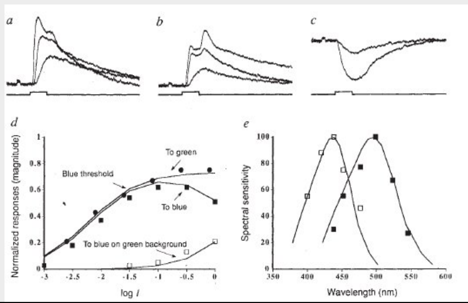
- Capability to discriminate „Colours"
- Unique Mechanisms of **chromatic Antagonism within a single Photoreceptor**
- PR depolarizes upon Stimulation with Green Light and hyperpolarizes upon Blue Light (green adapted)
- Involves a PDE and CNG channel

Depolari-
zation



a - 524 nm flash
b - 452 nm flash
c - 452 nm flash (green adapt.)

Solessio & Engbretson (1993)
Nature 364: 442-445

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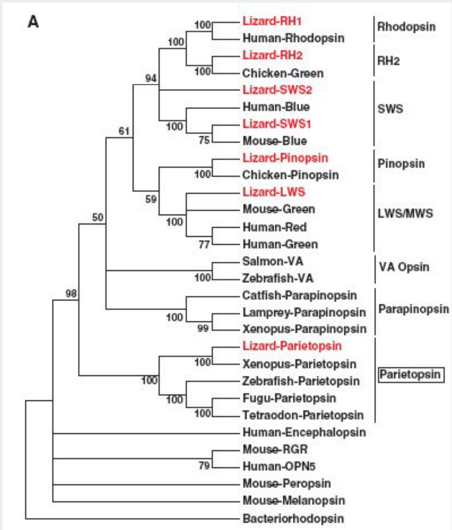
- Two Opsin Genes in PE:
  - Pinopsin
  - Parietopsin
- Parietopsin constitutes new, distant class of opsins
- λ_{max}= 522 nm



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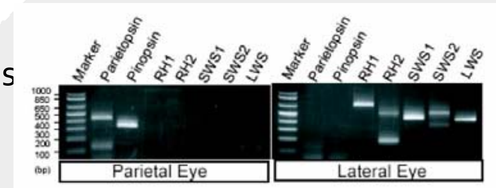
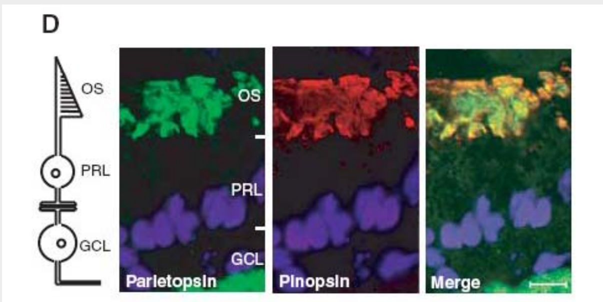
- Lateral Eyes and PE express different Opsins



- PE Photoreceptors express both Pinopsin & Parietopsin



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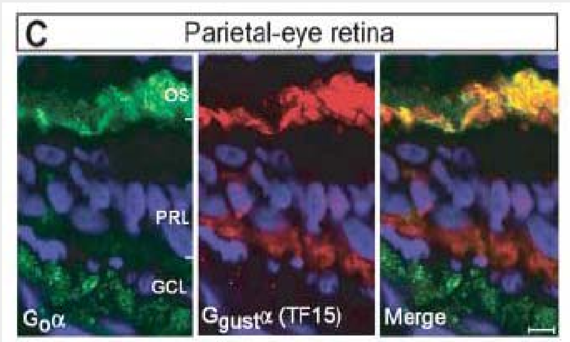
- Rod and Cone α-Transducin absent in PE
- Presence of α-Gustducin and $G_{\alpha o}$
- α-Gustducin and $G_{\alpha o}$ expressed in same PE- Photoreceptor



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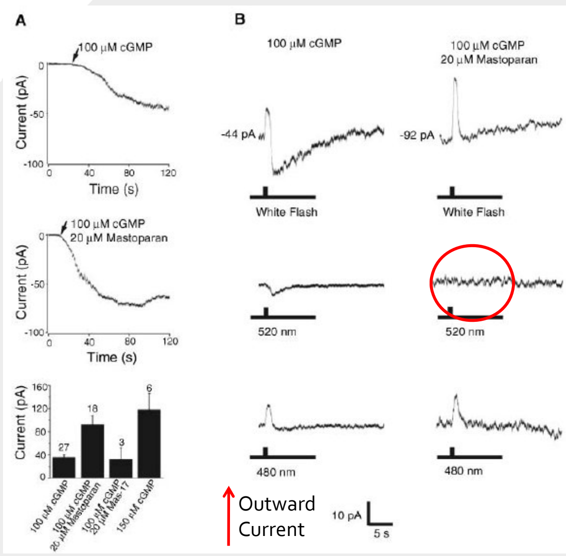
- Which PE Opsin couples to which G-Protein ?
- Whole Cell Recordings with cGMP and $G_{\alpha o}$ Activator Mastoparan
- Absence of Inward Current elicited by Green Light



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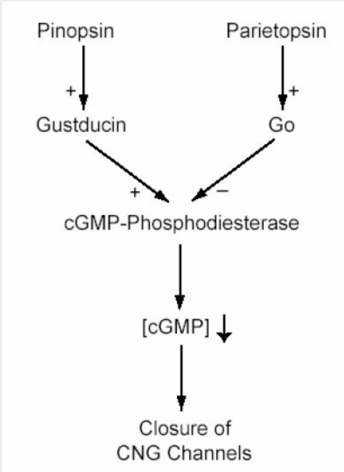
- Hyperpolarizing Light Response is mediated by the blue-sensitive **Pinopsin** which couples to **Gustducin** that **activates the PDE** and induces **Closure of CNG Channels**
- Depolarizing Light Response is mediated by the green-sensitive **Parietopsin** which couples to **G_o** that **inhibit PDE activity** and induces **Opening of CNG Channels**



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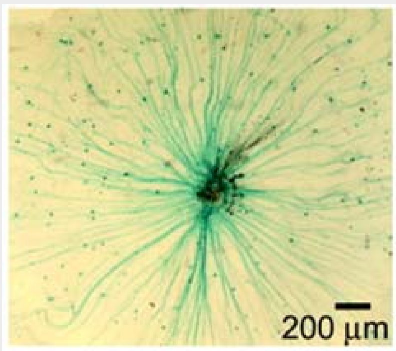
Melanopsin - intrisically photosensitive RGCs

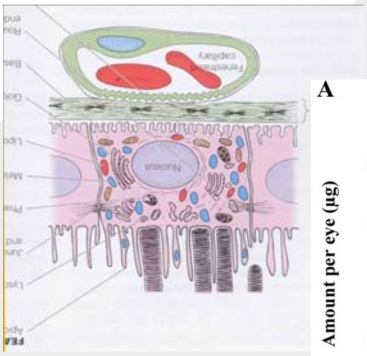
- Third Type of photosensitive Cells in the Mammalian Retina
- Non-Image Vision but participate in circadian photoentrainment (day/night rythm) and pupillary Response
- Much less Light sensitive, slow Response Kinetics
- About 1% of RGCs express Melanopsin (R-type Opsin !!!)
- May use a G_q / PLC Transduction Pathway

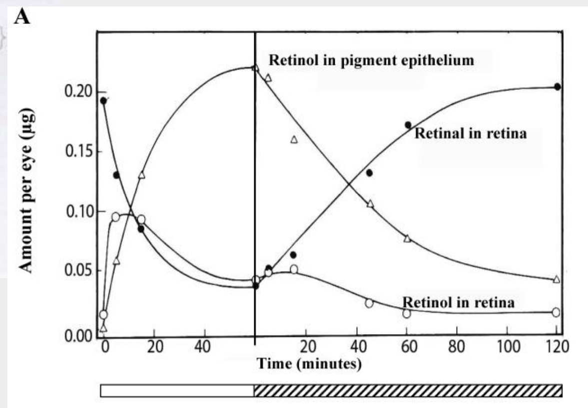


The Problem of Photopigment Recycling

- **Reconversion: All-trans-Retinal ➢ 11-cis-Retinal**
- Different Strategies:
  (1) Photo-Isomerization (Retinal bound to Opsin)
  (2) Photo-Isomerase (RGR), Light-driven
  (3) Elaborated multistep, multisite enzymatic Isomerization
    ➢ Visual Cycle in Vertebrates

Photopigment Recycling utilizes the RPE





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Visual Cycle in the Vertebrate Retina

- M-II decays into M-III ➢ Hydrolysis of SB and Dissociation of Opsin and all-trans Retinal
- Extrusion from intradiscal space (ABCA4) in Cytoplasma and **Export from the Photoreceptor to RPE**
- In RPE: **Isomerization into 11-cis Retinol (RPE65)**
- **Re-Transport from RPE to Photoreceptor**

- Multiple enzymatic converson: R'al ⇔ R'ol ⇔ R-Esters
- Requires Carrier Proteins: CRBP, CRALBP, IRBP

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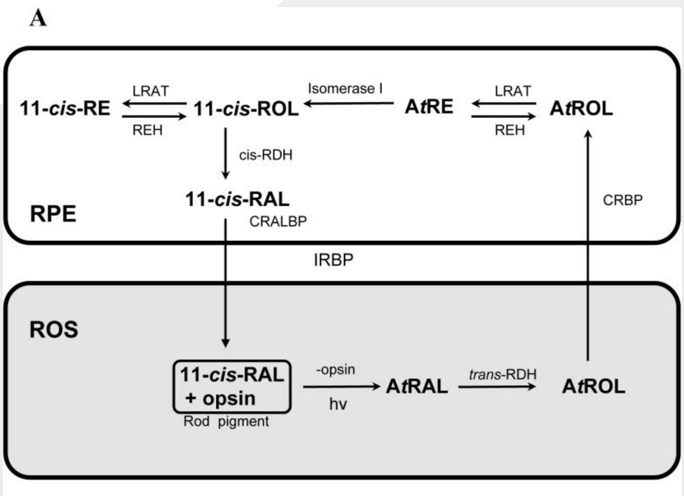
For Internal Use Only

Visual Cycle in the Vertebrate Retina



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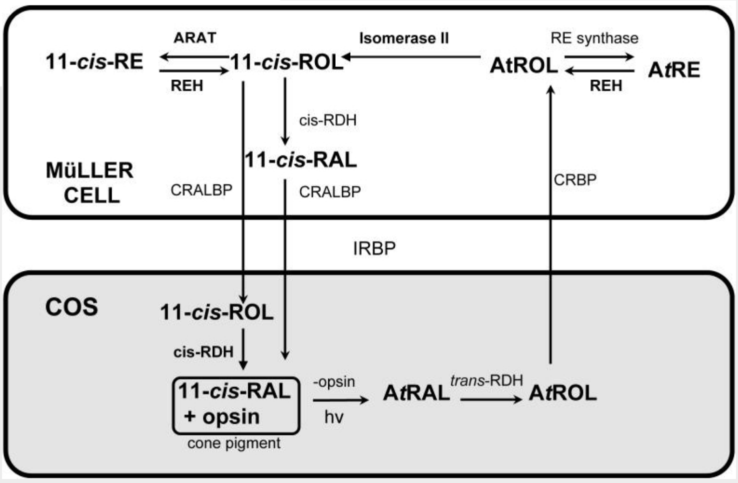
Alternative 2nd Visual Cycle for Cones



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Retinoid Supply

- No *de novo* Retinoid Synthesis in Animals
- Dietary Uptake of Carotinoids (vegetables & fruits) or Retinoids (R‘-Esters from Meat)
- Main Storage in Liver
- Plasma Transport of Retinol: bound to RBP/TTR complex
- Uptake into RPE through a Retinol-Receptor: STRA6
- RPE serves as an intermediate Store for Retinoids


THE END
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