Visual pigments

NS, Biochemistry
Dr. Mamoun Ahram
Third year, 2014
References

• Photoreceptors and visual pigments
  – Webvision: The Organization of the Retina and Visual System
    (http://www.ncbi.nlm.nih.gov/books/NBK11522/#A127)
  – Molecular Biology of the Cell.
    (http://www.ncbi.nlm.nih.gov/books/NBK26912/#A2826)
  – Biochemistry
    (http://www.ncbi.nlm.nih.gov/books/NBK22541/#A4618)

• Vitamin A and Carotenoids
  – Lippincott Williams & Wilkins, p.381-383
Lecture outline

• Visual transduction (dim vs. bright light)
  – Components (cells and molecules)
  – Mechanisms of activation, amplification, and termination

• Color blindness

• Metabolism of vitamin A
Basics of human vision

- X-rays
- Visible light
- Radio waves

Wavelength (m)
Rods and cones

Dim Light (1 photon)

Bright Light

Rods: 120 million
Cones: 7 million
How they really look like…
More on rod cells
The dark current

1. Na+ and a lesser amount of Ca2+ enter through cyclic nucleotide-gated channels in the outer segment membrane.
2. K+ is released through voltage-gated channels in the inner segment.
3. Rod cells depolarize.
4. The neurotransmitter glutamate is released continuously.

1. Channels in the outer segment membrane close, the rod hyperpolarizes.
2. Glutamate release decreases.
GENERATION OF VISION SIGNALS
The players

- Rhodopsin
- Transducin
- Phosphodiesterase
- $\text{Na}^+$-gated channels
- Regulatory proteins
Rhodopsin

Phosphorylation sites
Sites of interaction with cytoplasmic proteins

Retinal attachment site
chromophore

Region containing oligosaccharides

adapted from Hargrave et al. 1984
Piantanida, 1991
Light absorption by rhodopsin
11-cis-retinal
Rhodopsin intermediates

- Rearrangements in the surrounding opsin convert it into the active $R^*$ state.
- The chromophore converts the energy of a photon into a conformational change in protein structure.
G proteins are heterotrimeric, consisting of $\alpha$, $\beta$, and $\gamma$ subunits. In its inactive state, transducin’s $\alpha$ subunit has a GDP bound to it.
Transducin

R* binds transducin and allows the dissociation of GDP, association of GTP, and release of the α subunit.
Phosphodiesterase (PDE)
Activation of phosphodiesterase

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

- When activated, PDE hydrolyzes cGMP to 5'-GMP
- The cGMP concentration inside the rod decreases
- Cyclic nucleotide-gated ion channels respond by closing
1. Light stimulation of rhodopsin leads to activation of a G-protein, transducin.


3. PDE hydrolyzes cGMP, reducing its concentration.

4. This leads to closure of Na^+ channels.
Animation movie

SIGNAL AMPLIFICATION
Rhodopsin (1) $\rightarrow$ Transducin (500)
Transducin (1) → PDE (1 x 1 catalytic subunits)
PDE (1) $\rightarrow$ cGMP ($10^3$)
Facilitation of transduction

1. 2-dimensional surface
2. low in cholesterol and have a high content of unsaturated fatty acids
Cooperativity of binding

The binding of one cGMP enhances additional binding and channel opening ($n = \text{about 3}$)

- Overall, a single photon closes about 200 channels and thereby prevents the entry of about a million Na+ ions into the rod.
Signal termination
Mechanism I

Arrestin binding

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

- In dark, the outer segment contains high levels of transducin and low levels of arrestin.
- In light, it is the opposite.
Mechanism III
GTPase activity of G protein

- Transducin has an intrinsic GTPase activity that hydrolyzes GTP to GDP.
- Upon hydrolysis of GTP to GDP, transducin $\alpha$ subunit releases the PDE $\gamma$ subunit that re-inhibits the catalytic subunit.
- Transducin $\alpha$-GDP eventually combines with transducin $\beta\gamma$
Mechanism IV

Unstable all-trans rhodopsin complex

11-cis-Retinal + Lysine side chain $\rightarrow$ Opsin

Rhodopsin $\rightarrow$ Light-induced isomerization ($<10^{-2}$s)

All-trans-retinal $\rightarrow$ Spontaneous dissociation (100s)

Meta-rhodopsin II (activated opsins)
A role for calcium ions

When the channels close, $\text{Ca}^{2+}$ ceases to enter, but extrusion through the exchanger continues, so $[\text{Ca}^{2+}]_{\text{int}}$ falls.
Mechanism V

Guanylate cyclase

- In the dark, guanylate cyclase-associated proteins (GCAPs) bind Ca\(^{2+}\) and inhibit cyclase activity.
- A decrease in [Ca\(^{2+}\)]\(_{\text{int}}\) causes Ca\(^{2+}\) to dissociate from GCAPs, allowing them to dimerize.
- Dimerization of GCAPs leads to full activation of guanylate cyclase subunits, and an increase in the rate of cGMP synthesis.
Mechanism VI

Ca-calmodulin

- In the dark, Ca$^{2+}$-Calmodulin (CaM) binds the channel and reduces its affinity for cGMP.
- During visual transduction, the decrease in [Ca$^{2+}$]$_{int}$ causes CaM to be released, increasing the channel’s affinity for cGMP so that during recovery, the channel reopens at lower levels of cGMP.
COLOR VISION
Cone photoreceptor proteins
How different are they?

- Cone opsins have similar structures as rhodopsin, but with different amino acid residues surrounding the bound 11-cis retinal; thus they cause the chromophore’s absorption to different wavelengths.
- Each of the cone photoreceptors vs rhodopsin $\approx 40\%$ identical.
- The blue photoreceptor vs green and red photoreceptors $= \approx 40\%$ identical.
- The green vs. red photoreceptors $> 95\%$ identical.
A hydroxyl group has been added to each amino acid in the red pigment causing a $\lambda_{\text{max}}$ shift of about 10 nm to longer wavelengths (lower energy).
Rods vs. cones

- Light absorption, number, structure, photoreceptors, chromophores, image sharpness, sensitivity
COLOR BLINDNESS
Chromosomal locations

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

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

(A) Recombination between genes

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

(B) Recombination within genes
Genetic probabilities

Representative X chromosomes
(each male has only one)

1. Normal vision
2. Normal vision
3. Normal vision
4. Severe red-green color blindness
5. Moderate severe
6. Mild

- green-pigment gene
- red-pigment gene
* = mutation in red-pigment gene
Pedigree

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

= Carrier of Trait
Examples

Red blindness

Green blindness
Single nucleotide polymorphism

<table>
<thead>
<tr>
<th>Location</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA change</td>
<td>Serine → Alanine</td>
</tr>
<tr>
<td>Wavelength</td>
<td>560 nm → 530 nm</td>
</tr>
</tbody>
</table>

![Graph showing absorption spectra with wavelengths 560 nm and 530 nm]
METABOLISM OF VITAMIN A
Forms of vitamin A

11-cis-retinal

Retinol

Retinoic Acid
Source of vitamin A

- All derived from the β-carotene
- Beta-carotene (two molecules of retinal)
Absorption, metabolism, storage, action of vitamin A
Deficiency of vitamin A

- Night blindness, follicular hyperkeratinosis, increased susceptibility to infection and cancer and anemia equivalent to iron deficient anemia

- Prolonged deficiency: deterioration of the eye tissue through progressive keratinization of the cornea (xerophthalmia)