UNIVERSITY OF PAPUA NEW GUINEA
SCHOOL OF MEDICINE AND HEALTH SCIENCES
Division of Basic Medical Sciences
Discipline of Biochemistry and Molecular Biology
PBL SEMINAR MBBS III
BIOCHEMISTRY OF VISION: An Overview

What is the general concept of vision?

- Eye focuses on an Image by projection on Retina
- Series of events begins:
  - Photochemical events: Transformed Physical event to Chemical reactions via series of Biochemical reactions
  - Biochemical events amplifies the signals, and converts to Electrical events
  - Electrical impulses sent to Brain
  - Image reconstructed in "Mind's eye"
  - Conscious acknowledgement of the presence of an object: Thus, we see the object

To see an object:

- Light enters the eye passes through:
  - Cornea, Aqueous humor in anterior chamber, Lens, Vitreous humor (vitreous body), Focused on **Retina** (contains visual sensing apparatus)

Other basic components of the eye:

- Conjunctiva, Iris, Cillary body, Sclera, Choroid, Fovea Centralis, etc.

Does metabolism occurs in the basic components of the eye?

- Basic components in eye are living tissues thus require regular supply of nutrients for:
  - Energy metabolism, Growth, Maintenance of structural integrity
- Biochemical process occurs in various components without interference with visual process

What metabolic processes occur in some basic structures in eye?

- **Aqueous humor**:
  - Iso-osmotic fluid that contains:
    - Salts, Albumin, Globulin, Glucose, and other constituents
  - Supplies nutrients to Cornea and Lens
  - Removes end products of metabolism from Cornea and Lens

- **Vitreous humor:**
  - Collagenous or Gelatinous mass that helps to maintain shape of the eye while allowing it to remain flexible

- **Cornea**:
  - Requires constant supply of energy mainly via **Aerobic** metabolism
  - Glucose and O_2 are major substrates for energy production in Cornea
  - Rate of Hexose Mono-Phosphate Pathway (HMP Pathway) is very high
    - About 65% of Glucose used by Cornea is metabolised via HMP pathway, **Why?**
- Cornea contains high activity of **Glutathione Reductase (GSH Reductase**) that utilizes **NADPH** produced by the **HMP** pathway
- Epithelium of Cornea is permeable to Atmospheric Oxygen, used for Oxidative processes occurring in Cornea

- Absorption of O_2 in Cornea can results in formation of Reactive Oxygen Species (ROS) and other Free Radicals that can damage the Cornea
  - Thus, Cornea needs protection (**How?)**
- Cornea is protected by active GSH Reductase system
- GSH Reductase system neutralizes negative effects caused by ROS and Free Radicals

Reaction sequence of GSH Reductase system:

- Reaction catalysed by **Glutathione Peroxidase** (Requires trace element called **Selenium**):

RS-SR + 2GSH ====➔ 2R-SH + GSSG

- Reaction catalysed by Glutathione Reductase (**GSH Reductase**):

GSSG + NADPH + H^+ ====➔ 2GSH + NADP

(Where RS-SR = oxidized membrane structure; GSH = reduced Gluatathione; GSSG = oxidized Gluatathione)

What is the major role of HMP pathway and GSH Reductase in Cornea?

- Protect Cornea from damage by effectively neutralizing negative actions of ROS and Free Radicals formed by Corneal absorption of molecular Oxygen

- **Lens**:
  - Lens has no blood supply but it is metabolically active
    - Energy is obtained via **Anaerobic** metabolism
    - Nutrients obtained from Aqueous humor
  - Structurally Lens is composed of:
    - Water, Specific proteins (Alpha, Beta, and Gamma – Crystallins), Albuminoids, Enzymes and membrane proteins
  - Proteins are synthesized in Epithelial layer around edge of the lens

What are some of the specific properties of the Lens?

- Proteins in lens must maintain Clear Crystalline State and also must be in Native un-aggregated state {**Why?}**
- Proteins in lens are sensitive to:
  - Changes in Redox state,
  - Osmolarity,
  - Excessively increased concentrations of metabolites,
  - Physical insults such as UV irradiation
- Some processes that help to maintain structural integrity of lens:
  - Na-K – ATPase reaction for osmotic balance,
  - GSH reductase reaction for redox state balance,
  - Protein synthesis for growth and maintenance

- Human lens increases in Weight and Thickness with age and become less Elastic
  - This Characteristic of human lens is accompanied by loss of near vision – condition known as **Presbyopia**

What is the major function of the Lens?

- To focus light onto the Retina
- Image of object focused on Retina is Inverted and Reversed

What do you understand by the following; **Emmetropia, Myopia, Hyperopia, Presbyopia, Astigmatism?**

- **Emmetropia (Normal sight)**: Lens properly focused light onto Retina
- **Myopia (Nearsighted)**: Lens focuses light in Front of Retina
- **Hyperopia (Farsighted)**: Lens focuses light behind Retina
- **Presbyopia**: Loss of ability of Lens to accommodate with age, leads to loss of near vision
- **Astigmatism**: Curvature of the Lens is not uniform (oblong shape of lens), leads to refractive error of the lens

What is the general characteristic of the Retina:

- Highly vascular tissue that contains Visual Sensing Apparatus in Eye
  - Essentially no blood vessels in area on Retina (**Fovea Centralis**) where visual acuity is highest
  - Rods are not found in the Fovea
- Energy production in Retina mainly via **Anaerobic** Glycolysis, although cells in Retina including visual cells (Rods and Cones) contain mitochondria
- Mitochondria are absent in the Outer Segments of Rods and Cones where the Visual Pigments are located
- Rods and Cones form synapses with Bipolar Cells, which in turn interact with other nerve cells (Horizontal cells, Amacrine cells, Ganglion cells) in Retina
- Many Rods synapse on a single bipolar cell, but a few Cones synapse on a single bipolar cell (this may account for the high acuity of Cones compared to Rods)
- Nerve cells transmit electrical signals generated by Photoreceptors (Rods and Cones) to Brain via Optical nerve
- Pigment epithelial cells have two major functions:
  - Prevent scatter of light by absorbing stray light
  - Convert 11-cis Retinal to all-trans Retinal (Photo-isomerization)

Retina has dual functions:

- To transform light into nerve impulse and
- To integrate visual formation

What are some characteristics of Photoreceptor cells (Rods and Cones)?

- **Rods** and **Cones** are Photoreceptor (visual pigment) cells in Retina
- **Rod Cells**:
  - Provide Black and White Vision
  - Respond to Dim Light
  - Do not perceive colour
- Rhodopsin is Photoreceptor Pigment in Rod cells
- Rhodopsin consists of Two component parts:
  - **Protein: Opsin and Cofactor: 11-cis-retinal**

Opsin + 11-cis-Retinal ===➔ Rhodopsin

Structurally Rod cell can be separated into two segments (**Fig 1**):

- **Outer Segment:**
  - Specialized for Photoreception and contains Photoreceptor proteins (Rhodopsin)

- **Inner Segment:**
  - Contains Mitochondria, Nucleus, Ribosomes and other Organelles, for production of Energy, Synthesis of Proteins, and other Metabolic processes

- **Cone cells**:
  - Function in Bright Light
  - Responsible for Colour Vision
- Photoreceptors in Cone cells are **Red**, **Green** or **Blue pigments**
- Protein molecules that form these pigments are different from each other and from Opsin

{Note: **Fovea Centralis** is the centre of the **Macula**, which is a circular area of the Retina that contains the greatest concentration of **Cones** and has the greatest visual acuity}

Compare the photoreceptor characteristics of Rods and Cones?

- Rods have Low Acuity
- Cones have High Acuity

- Rods are sensitive to Low-intensity light
- Cones are sensitive to High-intensity light

- Rods detect Black and White but not Colour Vision
- Cones detect Colour Vision

- Rods have Slow Dark Adaptation
- Cones have Fast Dark Adaptation

How are the Carotenoid related to Vitamin A (Retinol)?

- Carotenoids (eg. Beta-carotene) are provitamins
- Retinol and Retinal are forms of Vitamin A used by the body
  - {NB: **1 Retinol equivalent = 1 ug Retinol = 6 ug beta carotene**)
- Cleavage of beta-carotene yields 2 molecules of All-trans-retinol

What are some major sources of Carotenoids and Vitamin A?

- Carotenoids are found in Green Leafy and Yellow Vegetables
- All-trans-retinyl-esters occur in:
  - Fish Liver Oils, Liver, Kidney, Butter Fat

How are Carotenoids and Vitamin A absorbed and metabolized?

- In GIT Carotenoids are absorbed directly in the presence of Bile
- Most Carotenoids are cleaved to Retinal and then converted to Retinol, which is absorbed via GIT
- Retinyl-esters are hydrolysed to give Retinol, which is then absorbed via GIT
- Retinol is re-esterified with Palmitate in Intestinal mucosal cells to form Retinyl-esters and transported to Liver, the main storage site for vitamin A
- In Pigmented epithelial cell layer of Retina, All-trans-Retinol is converted to 11-cis-Retinol

- 11-cis-Retinol is oxidized to 11-cis-Retinal, which then forms a complex with Opsin to form Rhodopsin (Visual pigment in Rod Cells)

What is the direction of movement of light rays entering the eyes?

- Photons of light enters eye via Lens
- Photons passes via
  - Optic Nerve Fibres, Ganglion Neurons, Bipolar Neurons, Nuclei of Rods and Cones
- Reaching the Outer Segment of Rods and Cones where Signal Transduction process begins

THE VISUAL CYCLE (Fig 2)

Give a brief explanation of the Visual Cycle

- Retinol is transported to Retina via blood Circulation,
- Retinol moves into Pigment epithelial cells and esterified to Retinyl-ester for storage
- When needed, **Retinyl esters** are hydrolysed and Isomerized to form **11-*cis* Retinol**, then Oxidized to **11-*cis* Retinal**
- **11-*cis* Retinal** is then shuttled across Inter-photoreceptor matrix to the Rod cell, where it binds to Opsin to form visual pigment, **Rhodopsin** (Visual purple)
- Absorption of Photon of light causes Conversion of **11-*cis* Retinal** to **All-trans Retinal** resulting in break up of **Rhodopsin** to **All-trans Retinal** and **Opsin**
- Process triggers a cascade of events, leading to generation of Electrical signals that are sent to Optic Nerve
- Nerve impulse generated by Optic nerve is conveyed to Brain for Integration and ultimately Interpreted as Vision
- Once released **All-*trans* Retinal** is converted to **All-*trans* Retinol**, which can be transported across Inter-photoreceptor matrix to Retinal epithelial cell to complete the visual cycle
- Inadequate amount of Retinol in the Retina results in Impaired Dark Adaptation, known as "Night Blindness."

How does Transduction of light signal leads to generation of impulse?

- Conversion of light energy to Nerve Impulses (Action Potential) involves Three Interconnecting Cycles:
  - Cycles involve reactions of: **Rhodopsin**, **Transducin** and **Phosphodiesterase**

- Net result of these reactions is to cause Hyperpolarization of Plasma membrane of Rod (or Cone) cells

  - From Resting Potential of – 30 mV to Hyperpolarized Potential of approximately - 35 mV

R + Light ===➔ R* ===➔ T-GTP ==➔ PDE* → **Cyclic-GMP =➔ open channel**; **5'-GMP =➔ close channel**

{Diagram is simplified schematic representation of how Sodium channels in Rods are regulated:
R = Rhodopsin; R* = Activated Rhodopsin; T = Transducin;
PDE* = Activated Phosphodiesterase}

What sequence of events occurs in the diagram above?

- **Plasma membrane of Rod cell contains Cation-specific channels that are kept Open in the Dark by Cyclic-GMP**
  - **Thus Na^+ ions rapidly flow into Outer segments of Rods in the Dark, because the channels are highly permeable to Na^+ ions and the electrical gradient is large.**
  - **Na^+ - K^+ - ATPase Pumps located in Inner Segments are responsible for maintaining Electrical gradient in Rod cells**
- **Absorption of light by Rhodopsin blocks these Cation-specific channels in the Outer Segment by reducing the concentration of Cyclic-GMP**
- **This ultimately reduces Influx of Na^+ ions, causing Plasma membrane to become Hyperpolarized (i.e. more negative on the inside compared to the outside)**
- **Light-induced Hyperpolarization is Transmitted by Plasma membrane from the Outer Segment to the Synaptic body, and then to other Neurones in the Retina and via the Optic nerve fibres to the Brain**

What are some of the characteristics of Cone cells?

- Cone cells are used to distinguish Colours
- Number of cone cells is less than Rod cells
- Mechanism of stimulation of Cone cells (Red, Blue or Green) by Photons is similar to that for Rod cells
- Normally only one type of Visual pigment occurs in a single Cone cell type
- Optimum absorbance:
  - **Blue pigment = 420 nm, Green pigment = 535 nm, Red pigment = 565 nm**

- Each protein pigment forms complex with 11-cis-Retinal
- Colours other than those of the Visual pigments are distinguished by graded stimulation of different Cone Cells and comparative analysis by the Brain
- Colour vision is **Trichromatic**
- Loss of one Colour pigments results in **Dichromatic** vision
- Genes encoding Visual pigments are located in specific Chromosomes
  - **Rhodopsin** gene is located on **3rd Chromosome**
  - Gene encoding **Blue pigment** is located on **7th Chromosome**
  - Genes encoding **Red pigment** is located on **X-chromosome**
  - Genes encoding **Green pigment** also located on **X-chromosome**

What is the Biochemical basis of Cataract?

- Cataract is a disease that affects the Lens
- Cataract is **Opacity** of Lens caused by loss of Osmolarity and change in solubility and aggregation state of Lens Crystallins (proteins in lens)
  - Changes result in Light Scatter in affected regions of Lens

Basically there are two types of cataracts:

- **Senile Cataracts**:
  - Age-related, usually caused by changes and breakdown of Lens proteins

- **Diabetic Cataracts**:

- Due to loss in Osmolarity of Lens cause by Activity of enzymes in Polyol (Aldose) metabolic pathway
  - **Aldose Reductase,** and
  - **Polyol (Aldose) Dehydrogenase**

- **Diabetic Cataract**:
  - Glucose accumulate in Lens and induces production of Aldose Reductase, which then converts Glucose to Sorbitol
  - Sorbitol is slowly converted to Fructose by Polyol Dehydrogenase
  - Resultant effect is accumulation of Sorbitol in Lens,
    - Osmolarity of Lens is increased,
    - Structural organization of Crystalline Proteins in Lens are altered
    - Rate of protein aggregation and denaturation is enhances causing light scattering
  - Light scattering effect increases in areas of Lens affected

(NB: Cataract: Scattering of Light)

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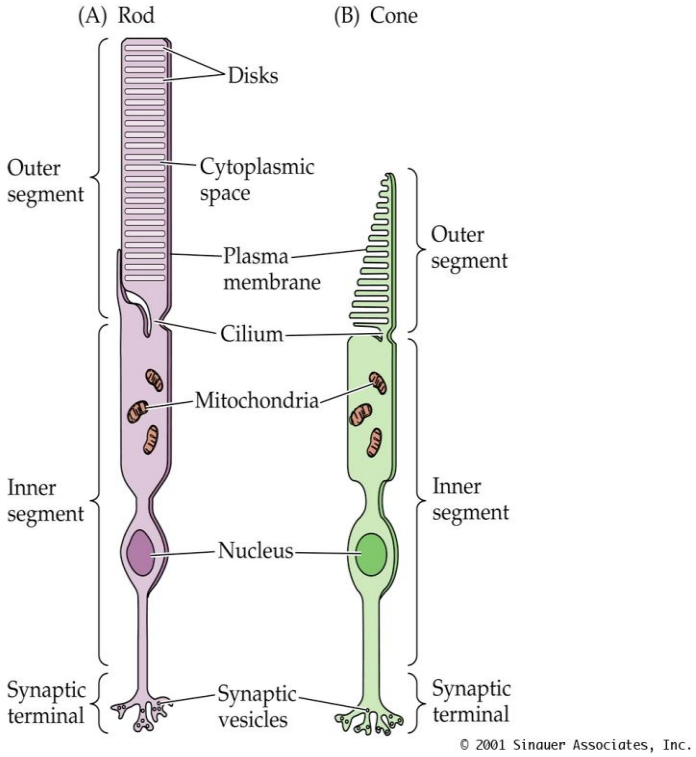

Fig. 1: Schematic diagram of Rod and Cone cells
(http://www.ic.ucsc.edu/~bruceb/psyc123/Vision123.html.pdf)

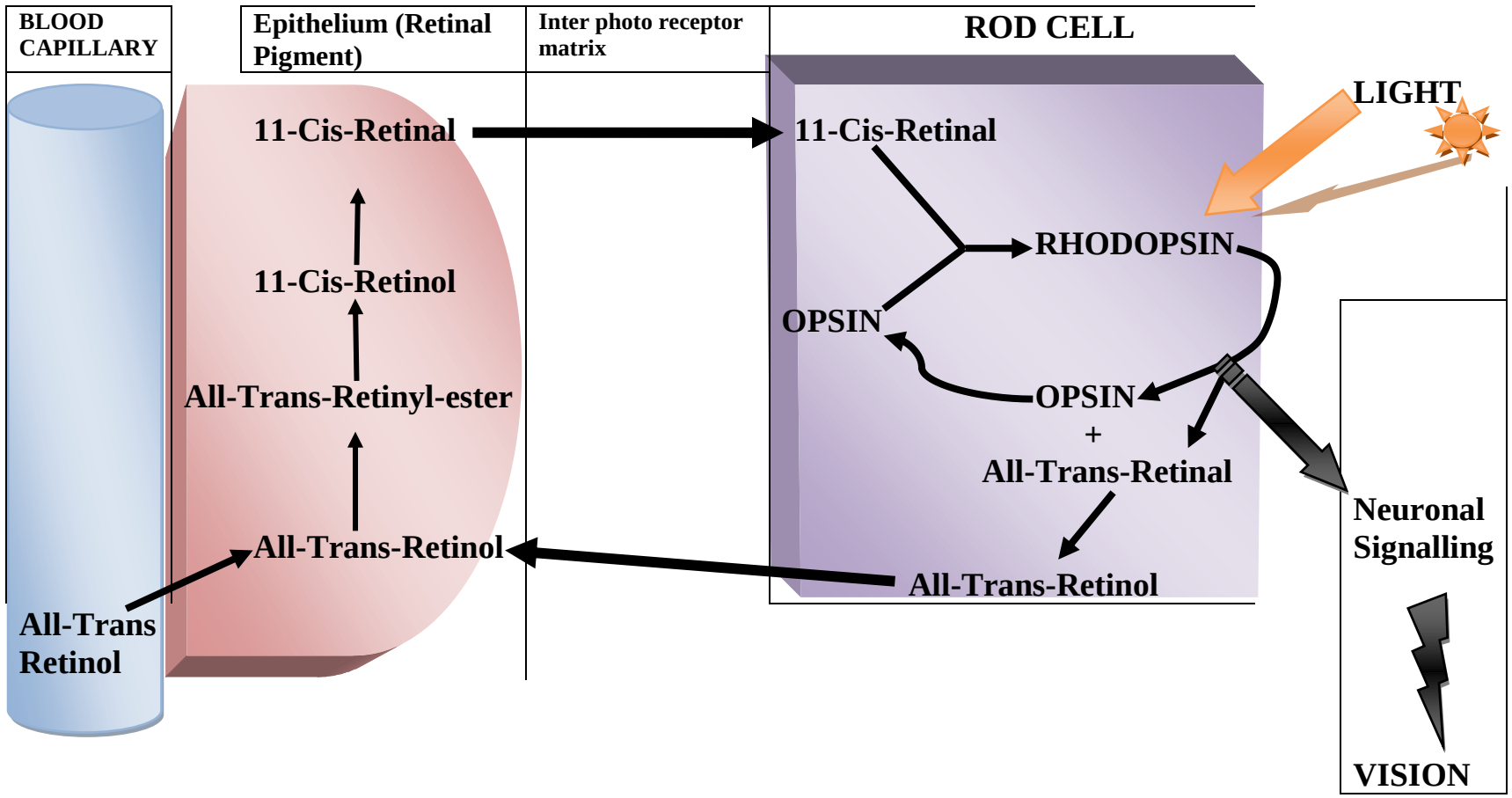

Fig. 2: Schematic diagram of the Visual Cycle