ARTICLE

Present State of Knowledge of Chemistry of Our Vision: Photoreceptor Molecules and Vision Cycle†

Ankita Das[1], Udita Das[2] and Asim K. Das[2,✉]

A B S T R A C T

Vision process involves the participation of two types of retinal photoreceptor cells: rod cells respond to dim light while cone cells respond to bright light and colours. The visual pigments in both types of photoreceptor cells contain the common chromophore, 11-*cis*-retinal, linked through a Schiff base linkage to the opsin protein, a member of G-protein coupled receptor (GPCR) family, composed of 7-transmembrane helices (a 7TM receptor). Rhodopsin is the visual pigment present in the rod cells while three distinct types of visual pigments known as photopsins (red cones, green cones and blue cones absorbing red, blue and green parts of the visible spectrum respectively) are present in the cone cells. Absorption of light by the visual pigment causes the photoexcitation followed by photoisomerization, 11-*cis*-retinal (*Z*) to all-*trans*-retinal (*E*) with a high quantum yield through a number of reactive intermediates characterized by low temperature and picosecond (ps) time resolved spectroscopies coupled with femtosecond spectroscopy. This photoisomerization leads to a change in the conformation of opsin GPCR and a signal transduction cascade by activating transducin, a heterotrimeric G-protein, to breakdown the cGMP to close the cGMP-gated cation channels resulting in hyperpolarization of the photoreceptor cell. This action potential creates a nerve signal that is transmitted to the brain to produce the sense of vision. The photoisomerized pigment undergoes rapidly hydrolysis to produce the opsin protein and all *trans*-retinal, which can be reconverted enzymatically to 11-*cis*-retinal for recharging opsin to generate the active visual pigment to maintain the vision cycle (Wald cycle). This brief review highlights the state of our understanding of the biochemistry behind the art of vision in humans and other organisms.

K E Y W O R D S

Author affiliations:

[1]School of Chemical Sciences, Indian Association of Cultivation for the Science, Kolkata-32, India
[2]Department of Chemistry, Visva Bharati, Santiniketan-731235, India
†This article is dedicated to Prof. Krzysztof (Kris) Palczewski, the world renowned vision scientist, who made a significant contribution in understanding the science behind the vision in the vertebrate eye, a biochemical process.

✉To whom correspondence to be addressed:

E-mail: asimkumar.das@visva-bharati.ac.in

I N T R O D U C T I O N

Vision, the art of seeing by eyes, is one of the most important activities of the human life. Eye, a photochemical organ, acts as a transducer to convert the visible light energy (*ca.* 300–800 nm) to produce an electrical signal as a visual signal that passes through the neurone to brain. Brain translates the signal and we see the object and its colour. Wald established [1] that retinol derivatives constitute the chemical basis of our vision and this contribution was subsequently recognized by a Nobel

Prize award in 1967. To understand the chemistry behind the vision signal generation, both the chemists and biologists have carried out a lot of investigations for a long time and it is still an active area of research to explore the details of visual cycle and to improve our understanding level [1-12]. In fact, application of the knowledge of laser spectroscopy, femtosecond (fs) spectroscopy and picosecond (ps) time resolved spectroscopies at low temperature in the last two decades have characterized the basic photochemical reaction followed by the rapid non-photochemical processes and revealed a great deal of information regarding the structural features of the intermediates involved having lifetime ranging from femtosecod to millisecond in the process [3].

The visual pigments are the Schiff bases produced from the opsin protein, a member of G-protein coupled receptor (GPCR) family and 11-*cis*-retinal acting as the chromophore. The visual pigments in rod and cone photoreceptor cells isomerize 11-*cis*-retinal (Z) to all-*trans*-retinal (E) configuration upon absorption of photons. This photochemical isomerization reaction with a very high quantum yield (*ca.* 0.7) [3,11,12] occurs through different intermediates which are now well characterized by the application of ultrafast spectroscopic techniques. This isomerization process of the chromophore associated with a change in the tertiary structure of the protein of the visual pigment is responsible to activate transducin, a heterotrimeric G-protein, to generate the visual nerve signal transmitted to brain for translation. After photoisomerization responsible for the generation of visual nerve signal, the photo-excited pigment undergoes rapidly hydrolysis to produce the opsin protein and all-*trans*-retinal. For the eyes to restore light sensitivity, opsins require recharging with 11-*cis*-retinal through Schiff base condensation. This *trans-cis* back conversion is attained through a series of enzymatic reactions [13] to constitute the retinoid (visual) cycle [7,12,14,15].

This concise review on the multidisciplinary area reflects and summarizes the present day knowledge of the biochemistry behind the art of vision in humans and all other living beings using the eyes as the seeing organ. This concise but complete tutorial type review on the vision process targets to benefit the readers of different disciplines of different levels. It is also expected to attract the attention of the students at the advanced level.

Visual pigments

In vertebrates, there are two kinds of photoreceptor cells [16-22] called rod cells and cone cells (named so because of their distinctive shapes) in retina. The rod cells function in dim light vision while the cone cells are responsible for bright light and colour vision. In species like owls and cats, the rod cells are mainly responsible for their night vision or twilight vision.

The visual pigments present in both the rod and cone cells contain 11-*cis*-retinal (acting as the chromophore), which is linked to the opsin glycoproteins through the Schiff base imine linkage. In rod cells, the visual pigment rhodopsin is the Schiff base produced from 11-*cis*-retinal and opsin glycoprotein embedded in the rod cell membrane. Rhodopsin acts as the primary photoreceptor in the whole animal kingdom. It suggests

that the vision pigment, rhodopsin, emerged at the very early stage of evolution for the purpose of vision [14,22].

The membrane protein, opsin, a glycoprotein, the protein part of rhodopsin, is a classic example of 7TM receptor characterized by a seven-transmembrane-helix (7TM helix). A 7TM receptor possesses seven helices spanning (*i.e.* single polypeptide chain snaking the membrane seven times) the lipid bilayer membrane [6,7,22]. The 11-*cis* retinal forms the Schiff base linkage with a lysine residue of the 7th helix. Ligand binding to 7TM receptors leads to the activation of membrane bound heterotrimeric G proteins. In fact, opsin, the protein part of rhodopsin, acts as a light sensitive G-protein coupled receptor (GPCR) protein [12,23-26]. It may be observed that rhodopsin (having GPCR structure) is the first member of 7TM receptor family to have the three dimensional structure determined. Its crystal structure was determined by Palczewski *et al*. in 2000 [24]. The rhodopsin crystal structure provides a structural basis for understanding the mechanism and function of this and other G protein-coupled receptors (GPCRs).

11-*cis*-retinal is a form of fat soluble vitamin A (more correctly vitamin A_1). In fact, vitamin A_1 exists in different forms like retinal$_1$ (aldehydic form), retinol$_1$ (alcoholic form) and retinoic acid$_1$ (carboxylic acid) [14] (Fig. 1). The structure of vitamin A_1 consists of a β-ionone ring (derived from cyclohexene ring) with a side chain bearing two isoprenoid units (*i.e.* 4 conjugated double bonds) ending with –CHO (retinal) or –CH$_2$OH group (retinol) or –CO$_2$H (retinoic acid). Vitamin A_2 where the C_3–C_4 bond of β-ionone ring is dehydrogenated can also exist in different forms also like retinol$_2$ and retinal$_2$ (Fig. 1). Thus in contrast to vitamin A_1, in vitamin A_2, the cyclohexane ring contains two conjugated double bonds. Different forms of vitamin A_3 are also known. In vitamin A_3, at the C_3 position of β-ionone cyclohexene ring, there is an OH group.

β-Carotene (provitamin A_1) is the precursor of vitamin A_1. It undergoes the oxidative cleavage in the intestine to produce two moles of retinal$_1$ [14,15]. In fact, the skeleton of vitamin A_1 represents exactly half of the carotene molecule (Fig. 2). For the sake of simplicity, in the next discussion, the subscript 1 is dropped to describe retinal$_1$, *i.e.* retinal represents retinal$_1$.

Retinal is generated from retinol through oxidation catalyzed by the Zn(II) containing metalloenzyme, alcohol dehydrogenase. 11-*cis*-retinal represents the *cis*-configuration with respect to the C_{11}=C_{12} double bond. The terminal aldehyde group (–CHO) undergoes the Schiff base condensation reaction with the ε-NH$_2$ group of the lysine residue 296 located at the centre of seventh transmembrane helix of 7TM opsin glycoproteins embedded into the photoreceptor cell membrane to produce the different visual pigments like rhodopsin and photopsins [7,14] (Fig. 3). The Schiff base rhodopsin pigment present in rod cells is protonated and it is stabilized by a negatively charged carboxylate amino acid residue (glutamate 113 located in the second helix of 7TM opsin protein) through the salt bridge interactions. The β-ionone ring of retinal located in the hydrophobic region of opsin participates in hydrophobic supramolecular interactions. Thus, the retinal chromophore in the visual pigments is stabilized by different types of non-covalent supramolecular interactions [3].
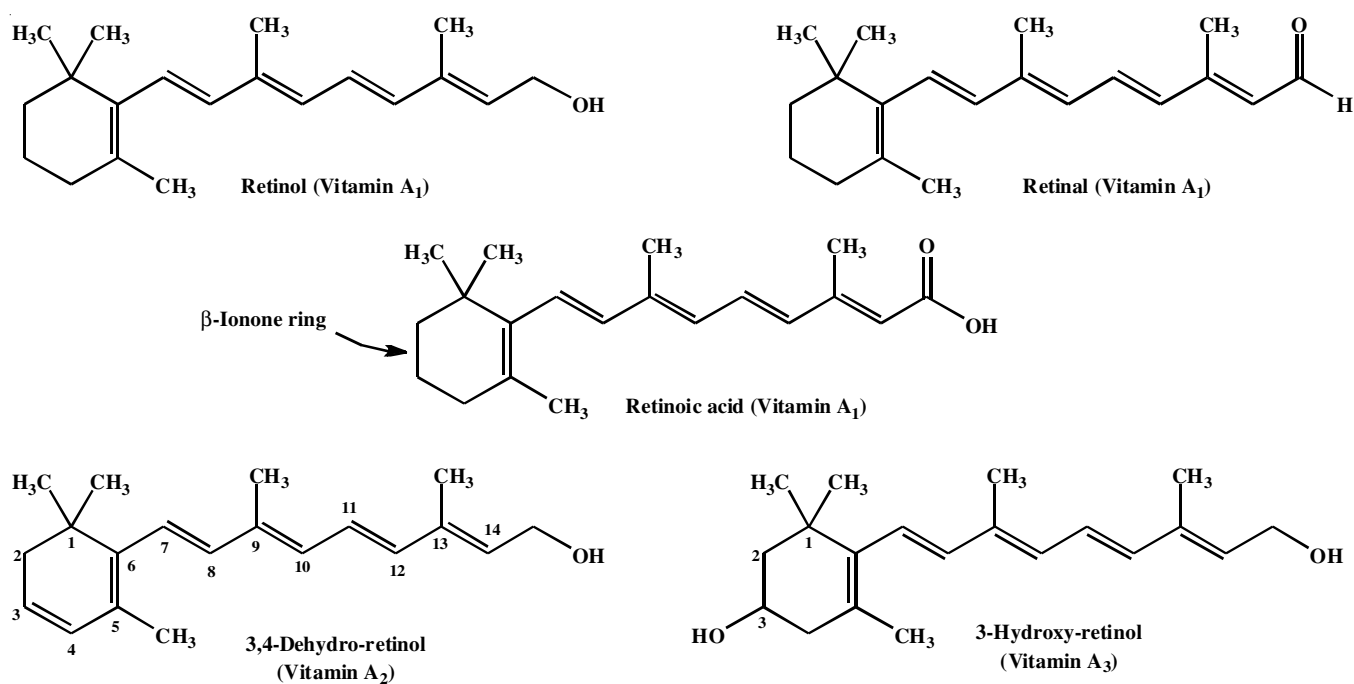
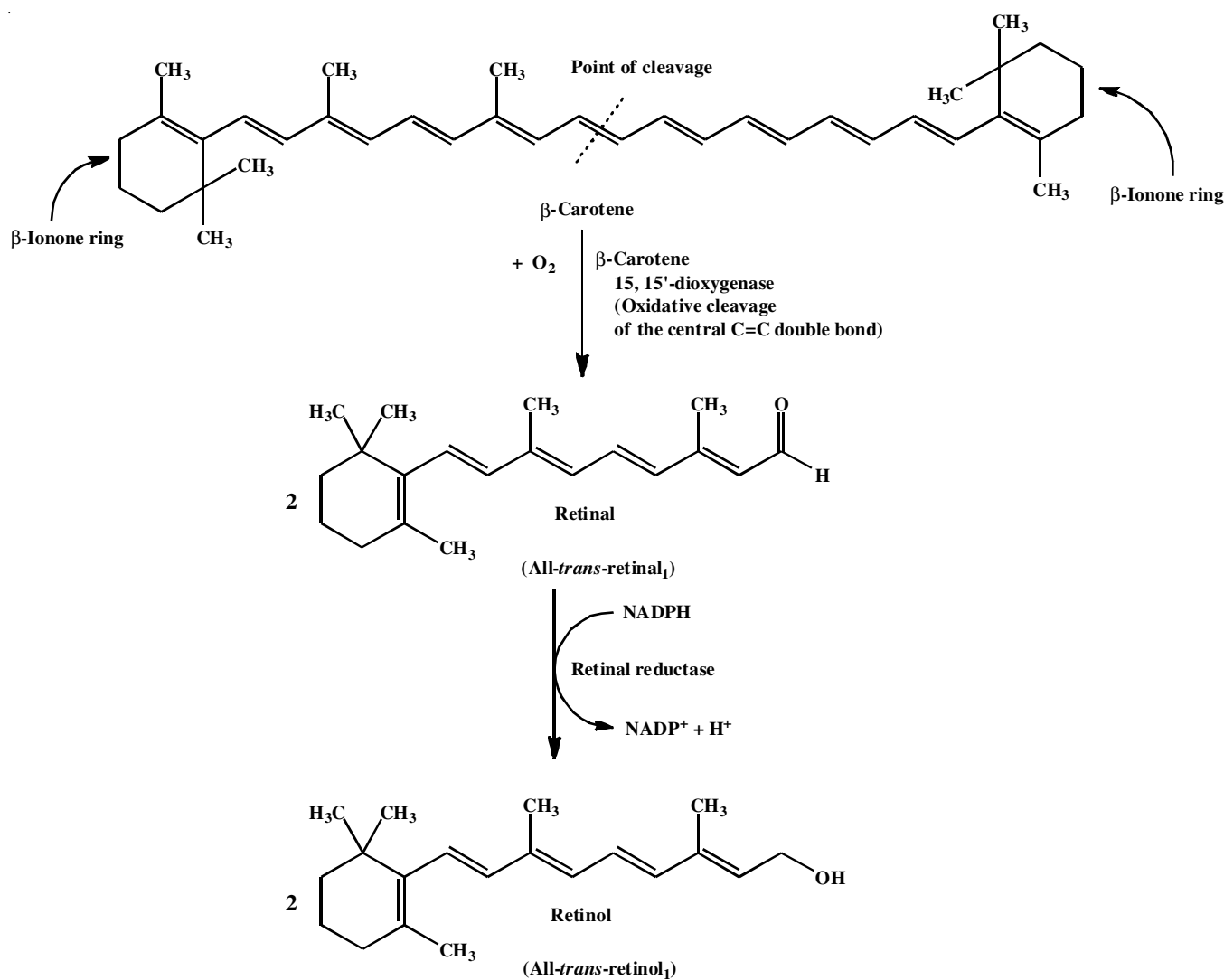Fig. 1. Structures of different types of vitamin A

Fig. 2. Biosynthesis of vitamin A (in the intestinal mucosa) from β-carotene
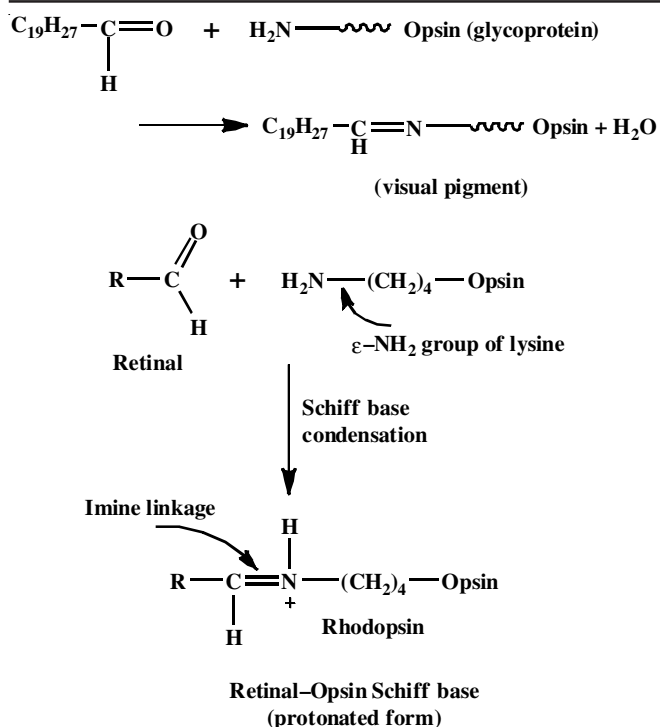
Fig. 3. Illustration of synthesis of the visual pigments (*e.g.* rhodopsin) through the Schiff base condensation between the opsin protein and 11-*cis*-retinal

The visual rhodopsin pigment can be simply described as the retinal-opsin Schiff base where the retinal moiety acts as the chromophore. It indicates the importance of vitamin A, which is required for the biosynthesis of vision pigments [27-30]. In fact, vitamin A deficiency leads to an impaired vision. Rhodopsin undergoes light induced isomerization, 11-*cis*-retinal (Z) moiety → all-*trans*-retinal (E) moiety, through the rotation around the $C_{11}=C_{12}$ double bond leading to the movement of Schiff base N-atom by about 500 pm [3,14] (Fig. 4).

Stereochemistry of visual pigment (rhodopsin)

The retinal-opsin Schiff base visual pigment, rhodopsin produced from retinal can exist as *cis*- and *trans*-isomers with respect to the C_{11}–C_{12} double bond of the retinal moiety, which is the chromophore of the pigment. For free retinal, the *trans*-isomer is more stable than the *cis*-isomer due to the steric hindrance in the *cis*-form (Fig. 5). However, it has been observed that in rhodopsin, in dark, the retinal moiety exists predominantly in the 11-*cis*-configuration [3,14,22].

In the 11-*cis*-configuration of retinal, the CH_3-group of C_{13}-centre and H-atom of C_{10}-centre are placed very close to each other to generate a steric hindrance between them. To minimize this steric destabilization (Fig. 5), the molecule is somewhat twisted and the planarity of the skeleton around the $C_{11}=C_{12}$ double bond is lost to prevent the conjugation (*i.e.* delocalization of the π-electron cloud is prevented to some extent). Thus, the steric relaxation leads to a partial loss of resonance stabilization. However, the loss of this resonance stabilization and the destablilization due to the said steric hindrance in the opsin-retinal Schiff base (*i.e.* rhodopsin) are compensated by a number of noncovalent supramolecualr interactions between the 11-*cis* retinal moiety and opsin protein. It may be noted that in free retinal, no such non-covalent supramolecualr interactions are possible to stabilize the *cis*-configuration and this

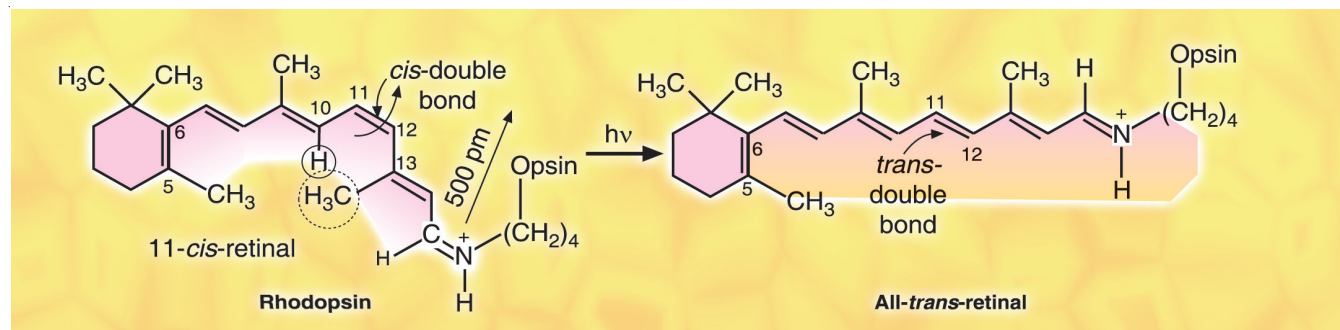
Fig. 4. Structure of the visual pigment, rhodopsin (present in the rod cell membrane) and its light induced isomerisation, 11-*cis*-retinal → all-*trans*-retinal moiety through the rotation around the $C_{11}=C_{12}$ double bond leading to the movement of Schiff base N-atom by about 500 pm
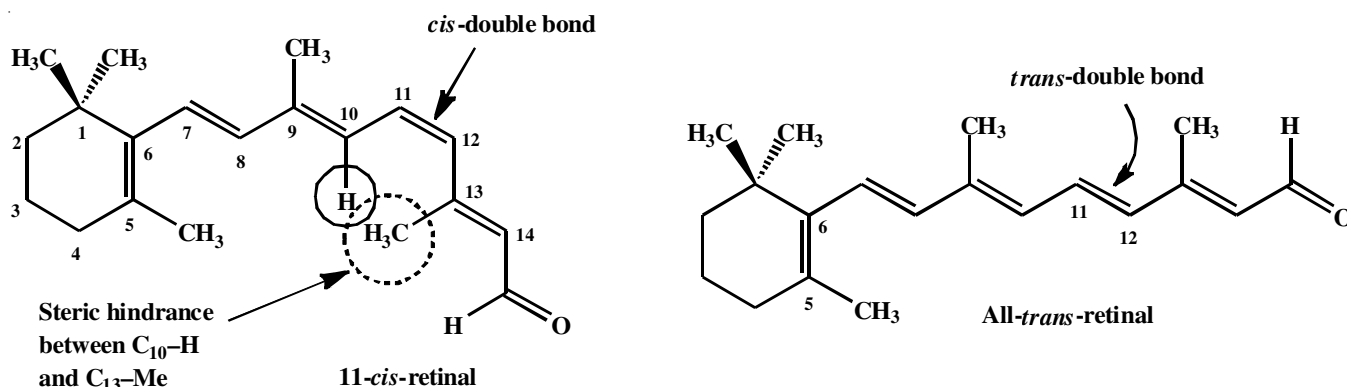

Fig. 5. Two geometric (*cis-trans*) isomers of retinal with respect to the $C_{11}=C_{12}$ double bond

is why, it predominantly exists in the *trans*-form to avoid the steric destabilization and gains resonance stabilization [3].

In fact, these stabilizing non-covalent interactions in rhodopsin are favoured only for 11-*cis*-configuration of retinal but not for all-*trans*-configuration of retinal. This explains why rhodopsin Schiff base complex bears the 11-*cis*-retinal moiety (in dark phase) though all-*trans*-retinal isomer is more stable in free state [3-7,14].

Spectral properties of retinal visual rhodopsin pigment

Rhodopsin absorbs very efficiently in the middle range of visible spectrum having $\lambda_{max} = 500$ nm ($\pi \rightarrow \pi^*$ transition) with a very high molar extinction coefficient ($\varepsilon \approx 10^4 \, M^{-1} \, cm^{-1}$) [14,22,31,32]. This very high molar extinction coefficient value indicates that most of the striking photons of appropriate wavelength are absorbed by rhodopsin [14] (Fig. 6).
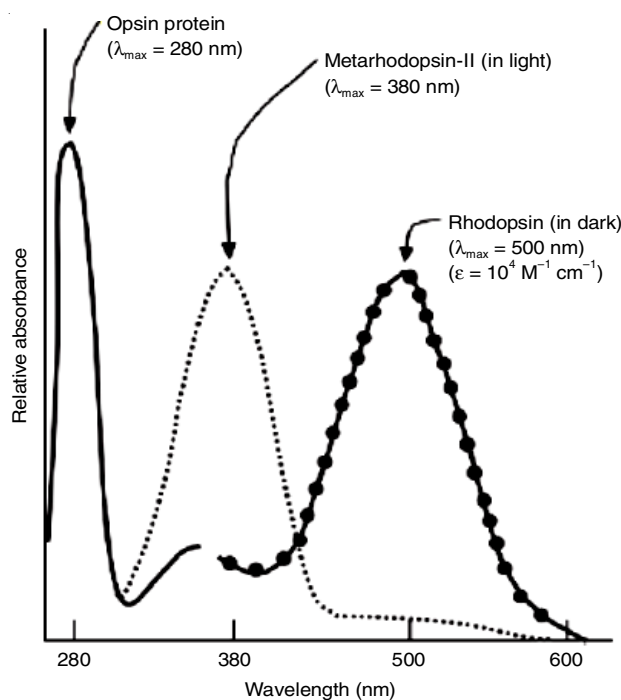

Fig. 6. UV-visible absorption spectra of the visual pigment, rhodopsin and its active from metarhodopsin-II (activating the rod cell G-protein, transducin to generate a nerve impulse) and opsin protein

It has been already stated that 11-*cis*-retinal moiety in rhodopsin is the effective chromophore absorbing the visible light. This chromophore is a polyene consisting of conjugated six double bonds (including Schiff base imine linkage, –CH=N–) and shows the $\pi \rightarrow \pi^*$ transition in the visible range. It may be observed that free retinal possesses $\lambda_{max} = 370$ nm; the unprotonated Schiff base possesses $\lambda_{max} = 380$ nm, while the corresponding protonated Schiff base shows the red shifted $\lambda_{max} = 440$ nm or longer wavelength. Thus, rhodopsin exhibiting $\lambda_{max} = 500$ nm is due to its protonated form of Schiff base of 11-*cis*-retinal and opsin glycoprotein. This red-shift by about 50-60 nm (*i.e.* 440 nm to 500 nm) is probably due to the different noncovalent supramolecular interactions between the 11-*cis*-retinal moiety and opsin protein of rhodopsin to stabilize the photoreceptor pigment. The positive charge of the protonated Schiff base linkage of rhodopsin is neutralized through a salt bridge interaction by glutamate 113 residue of the second helix of 7TM

opsin closely located to the lysine-retinal Schiff base imine linkage of rhodopsin pigment.

Photochemical properties of 11-*cis*-retinal moiety present in the retinal visual rhodopsin pigment

Wald [3,7,14,33] established that light absorption due to the $\pi \rightarrow \pi^*$ transition in the chromophore (*i.e.* 11-*cis*-retinal moiety) of rhodopsin is accompanied with the isomerization of chromo-phore retinal moiety (*i.e.* 11-*cis*-retinal to all-*trans*-retinal). The $\pi \rightarrow \pi^*$ transition increases the electron density in the anti-bonding π-MO (*i.e.* π^*-MO) with the concomitant depletion of electron density in the π-bonding MO. Consequently, the $\pi \rightarrow \pi^*$ transition weakens the C_{11}–C_{12} π-bond and the torsional rigidity of the system is significantly reduced. As a result, the activation energy barrier for *cis (Z) $\rightarrow$ trans (E)* isomerization is significantly reduced [3,14,34]. This is the driving force behind the efficient photochemical *cis*- to *trans*-isomerization (*i.e.* 11-*cis*-retinal $\rightarrow$ all-t*rans*-retinal moiety) in the visual rhodopsin pigment and it makes the quantum yield of the process so high (*ca.* 0.7) [3].

The skeleton of 11-*cis*-retinal moiety is bent (approximately spherical shaped) while the skeleton of all-*trans*-retinal moiety is straight requiring more space. Thus, the photochemical isomerization (*i.e.* 11-*cis*-retinal $\rightarrow$ all-*trans*-retinal) leads to the atomic motion (*i.e.* light energy is converted into mechanical energy causing the atomic motion) to straighten the polyene skeleton and this structural reorganization introduces the steric hindrance between the straight retinal skeleton and opsin protein. The stereochemistry of 11-*cis*-retinal moiety establishes the stabilizing non-covalent supramolecular interactions between the retinal moiety and opsin protein while the streochemistry of all-*trans*-retinal moiety cannot maintain these stabilizing supramolecular interactions but rather establishes a destabilising steric hindrance between the retinal moiety and opsin protein. In fact, to accommodate the said structural change in 11-*cis*-retinal (Z) $\rightarrow$ all-*trans*-retinal (E) isomerization, the tertiary structure of opsin protein changes including the change in the position of ligand binding site of 7TM receptor [12,22,34]. Thus, the photoisomerization leads to a change in the tertiary structure of protein (Fig. 7) and the excited Schiff base (*i.e.* all-*trans*-retinal-opsin pigment, rhodopsin) becomes sterically unstable and eventually, it undergoes the hydrolytic cleavage to give all-*trans*-retinal and opsin protein.

The above mentioned photochemical isomerization of retinal Schiff base occurs within a few picoseconds (1 ps = 10^{-12} s) of photon absorption and produces the unstable strained intermediate, bathrhodopsin bearing the all-*trans*-retinal group, which is subsequently transformed through a number of intermediates into the deprotonated all-*trans*-retinal-opsin Schiff base called metarhodopsin II (deprotonated Schiff base form) possessing the reorganized opsin protein within a few milliseconds (1 ms = 10^{-3} s). The photochemical activation of rhodopsin to metarhodopsin II triggers a number of complex events to produce a nerve signal for visual sensation [3,12,14].

Photochemical activation of rhodopsin to generate a neuronal visual signal

Rhodopsin being exposed to light undergoes to change its colour, within a few milliseconds and this process is called
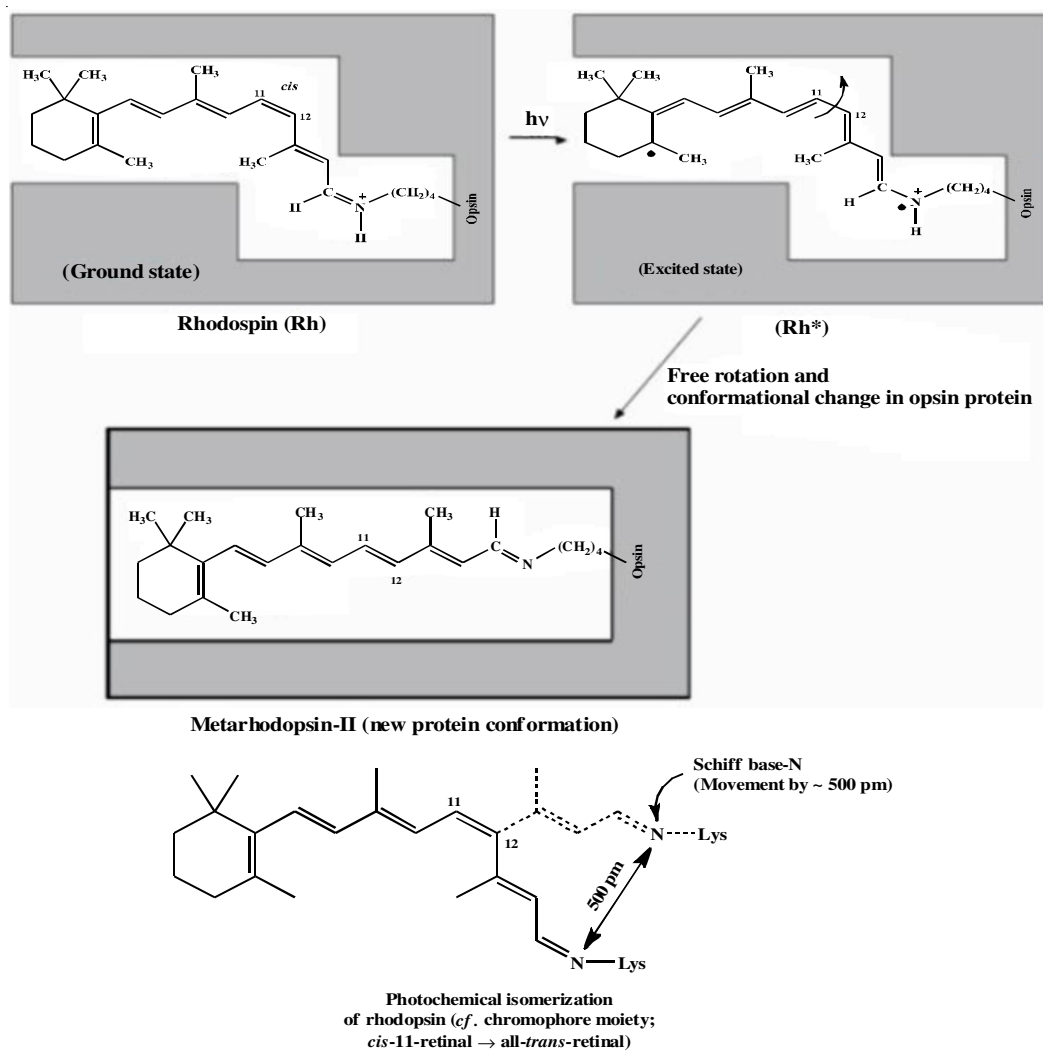
Fig. 7. Schematic illustration of the change in the *tertiary* structure of opsin protein during the photochemical changes in rhodopsin

bleaching of rhodopsin because in this process, rhodopsin loses its colour [3]. It takes place through a number of unstable intermediates. Both low temperature and picosecond (ps) time resolved spectroscopies in the last 20 years have revealed a great deal of information regarding the structural features of the intermediates involved in the *cis-trans* isomerization process of the chromophore as primary reaction in rhodopsin to activate transducin, a heterotrimeric G-protein to generate the visual nerve signal. Femtosecond (fsc) spectroscopy has been applied to rhodopsin to follow the process of isomerization in real time. All the reactive intermediates like photorhodopsin (Photo-Rh), bathorhodopsin (Batho-Rh), lumrirhodopsin (Lumri-Rh), metarhodopsin-I (Meta-Rh-I) and metarhodopsin-II (Meta-Rh-II) have been spectroscopically characterized to understand the process of photoisomerization of rhodopsin (Figs. 8 and 9) [3,12,33]. Formation of photorhodopsin upon photon absorption by rhodopsin occurs within 200 fsec (1 fs = 10^{-15} s) and then bathorhodopsin is formed and stable in the picoseconds (1 ps = 10^{-12} s) domain. Then bathorhodopsin is converted to lumirhodopsin within the time scale of nsec (1 ns = 10^{-9} s) followed by the conversion into the intermediates, metarhodopsin-I (μs time scale, 1 μs = 10^{-6} s) and metarhodopsin-II (ms time scale, 1 ms = 10^{-3} s) successively. Finally, meta-
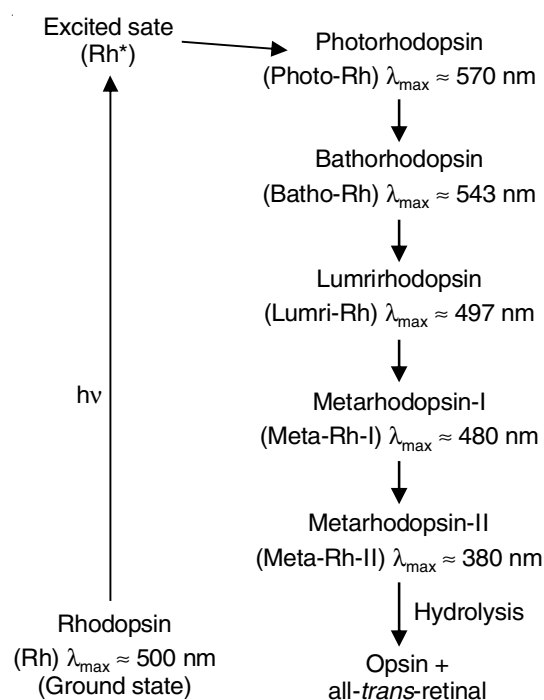

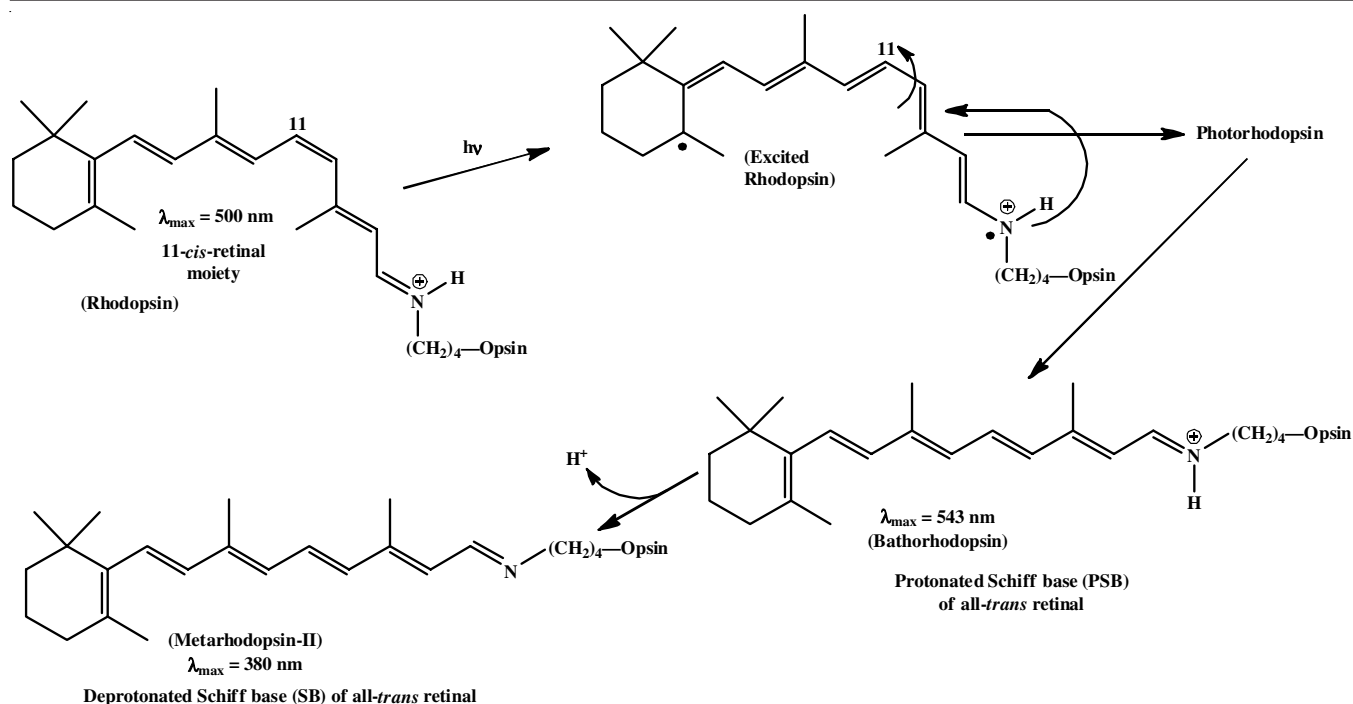Fig. 8. Photobleaching and hydrolysis of rhodopsin after its activation

Fig. 9. Chemical structure of the species involved in the photobleaching process of rhodopsin

rhodopsin-II (all *trans*-form of deprotonated rhodopsin Schiff base with the reorganisd opsin protein) is hydrolyzed to opsin protein and all-*trans*-retinal, which is subsequently enzymaticlly isomerized to 11-*cis*-retinal [13] to regenerate the Schiff base pigment, rhodopsin. The bleaching of rhodopsin and photolysis are schematically illustrated in Fig. 8.

The potential energy diagram of photoexcitation of rhodopsin (primary process *i.e.* Franck-Condon (FC) transition) and subsequent steps [3,33] are shown in Fig. 10. It is important to note that the *cis-trans* isomerization is highly efficient in rhodopsin (quantum yield: 0.67) [3,35]. This highly efficient photoisomerization of the retinal chromophore is attained only in the protein environment of rhodopsin because quantum yield for the said photoisomerization of rhodopsin chromophore in solution is relatively much small. In fact, the protein environment facilitates the isomerization process. Resonance Raman and infrared spectroscopies (vibrational spectroscopies) have revealed that light energy is stored by chromophore distortion and the protein synergistically responds to the chromophore motion (atomic motion) to accommodate the structural change in the chromophore [3]. Actually, tertiary structure of the protein changes accordingly to facilitate the isomerization process.

Bleaching and photolysis of rhodopsin and its resynthesis in retina is schematically shown in Fig. 11. In this process, the heterotrimeric G-protein, transducin, bound to the cell membrane plays a crucial role. The conformational change in the receptor's cytoplasmic domain activates the G-protein [22]. It is called G-protein because it binds guanyl nucleotide like GDP and GTP. The intermediate metarhodopsin-II (deprotonated all-*trans*-retinal-opsin Schiff base bearing the structurally reorganized opsin protein) activates the heterotrimeric G-protein transducin (bearing the α-, β- and γ-subunits; Fig. 12) and it triggers the exchange of GDP (guanosine diphosphate) by GTP (guanosine triphosphate) at the α-subunit of transducin G-
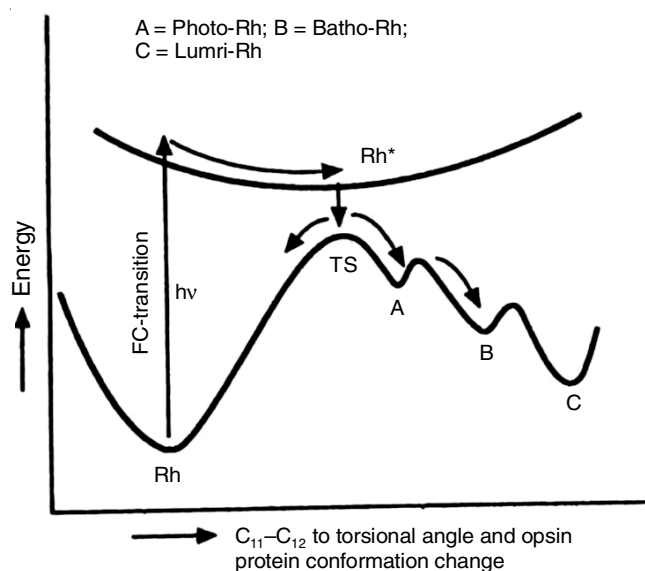

Fig. 10. Schematic representation of potential energy diagram of photo-excitation of rhodopsin (primary process, *i.e.* Franck-Condon (FC) transition) and subsequent steps in the photobleaching process (TS represents the transition state)
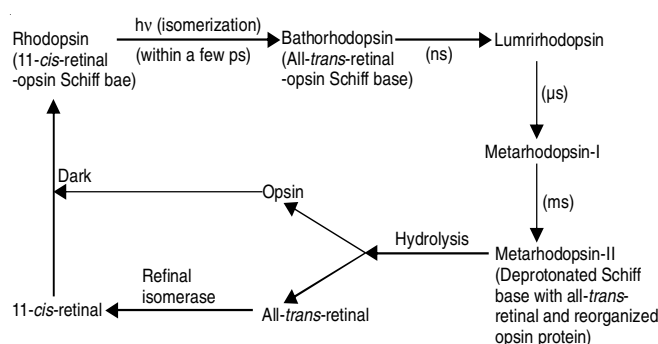

Fig. 11. Bleaching and photolysis of rhodopsin and its resynthesis in retina
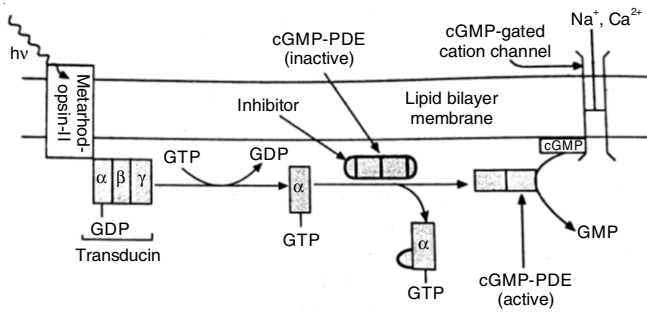
Fig. 12. Activation of the heterotrimeric G-protein transducin (bearing the α-, β- and γ-subunits) through the photoexcitation of rhodopsin to produce a nerve signal by closing the cGMP gated cation channels.

protein. In this event, the β- and γ-subunits of transducin are separated [12,14,22]. The GTP bound α-subunit of transducin removes the inhibitors of cGMP-PDE (cyclic-GMP-phosphodiesterase enzyme) and as a result, cGMP-PDE is activated to hydrolyse the cGMP to GMP *i.e.* guanosine monophosphate. As the level of cGMP (cyclic GMP) is depleted, the cGMP-gated cation channels are closed (Fig. 13) and consequently, the influx of Na^+ and Ca^{2+} ions is prevented. Thus, the reduced cation permeability leads to the condition of an ion imbalance causing the hyperpolarization of the cell membrane to generate an action potential to produce an electrical signal (*i.e.* neuronal signal) as the visual signal transmitted through the neuron network to the visual cortex of brain. After the generation of the nerve impulse, the cGMP-gated cation channels are reopened to restore the initial condition of ion balance rapidly.
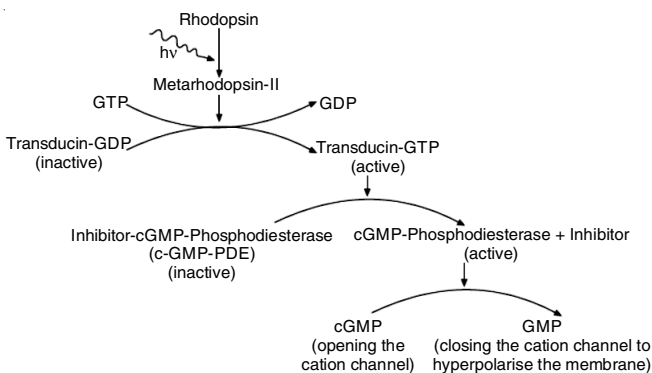

Fig. 13. Visual cascade involving the activation of heterotrimeric G-protein transducin leading to the hydrolysis of cyclic guanosine monophosphate to produce a nerve signal

Restoring of the visual signal transduction

Visual system responds to the environmental changes very quickly. To achieve this, the visual signaling system after excitation must be returned back to its initial state very rapidly. The restoring process starts by preventing the interaction between the activated rhodopsin and transducin G-protein [5,12,14,22]. Rhodopsin kinase catalyses the phosphorylation of the carboxy terminus of the serine and threonine residues of activated rhodopsin. Then the inhibitory protein called arrestin binds the phosphorylated rhodopsin and prevents its further interaction with transducin [36,37]. It is the first step of the restoring process. Secondly, the built-in GTPase in α-subunit of G-protein hydrolyses rapidly GTP to GDP and then the GDP bound α-subunit reassociates with the β- and γ-

subunits of transducin. It brings back the cGMP-PDE in the inactive state. The third step involves the action of guanylate cyclase to increase the level of cGMP by synthesizing cGMP from GTP [14] to reopen the cGMP-gated ion channels. It may be noted that activity of guanylate cyclase depends on the Ca^{2+} ion level because it can inhibit the activity of guanylate cyclase [14,22]. It has been already mentioned that during illumination, the entry of Ca^{2+} ion through the cGMP-gated ion channels stops and thus the reduced cytoplasmic level of Ca^{2+} ion stimulates the catalytic activity of guanylate cyclase to raise the level of cGMP.

Activation: Lowering the cGMP level and closing the ion channel.

Recovery: Guanylate cyclase activity increased at the reduced Ca^{2+} ion level to increase the cGMP level.

Wald's visual cycle

Visual cycle (Fig. 14) is a very efficient process and the activation followed by the restoration of the visual pigment to its initial state is attained very rapidly [1,2,7,14,15,38-43]. The photoisomerization of rhodopsin leads to a conformational change in the opsin membrane protein and this change is responsible for the generation of nerve impulse. Finally, the photoexcited rhodopsin Schiff base pigment having the all-*trans*-retinal moiety responsible for nerve signal generations is hydrolyzed to all-*trans*-retinal and opsin. To regenerate the rhodopsin pigment from the hydrolyzed products, all-*trans*-retinal is immediately but partially isomerized to 11-*cis*-retinal not directly but through different intermediate steps catalyzed by different enzymes (described commonly as retinal isomerase collectively) present in retinal epithelium (RE) to restore the visual cycle called retinoid or visual cycle [15,20]. This multi-step rapid catalytic cycle is primarily responsible to restore the rapid visual activity and for the continuity of vision.
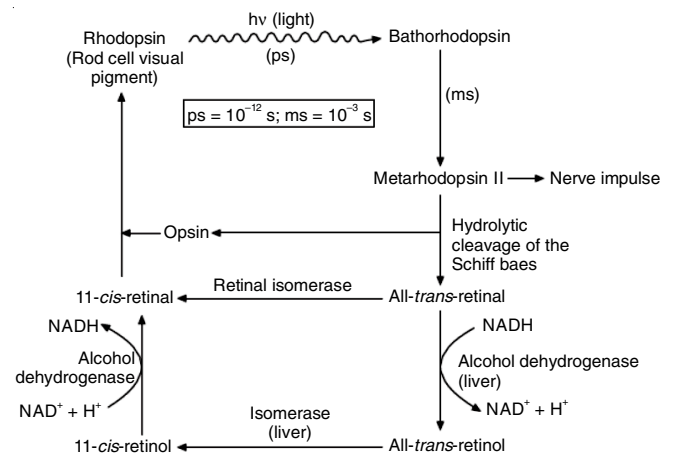
Fig. 14. Schematic representation of Wald's visual cycle

In the multistep retinoid or visual cycle (Fig. 15), the hydrolyzed product, all-*trans*-retinal, is first reduced to all-*trans*-retinol (*i.e.* vitamin A) by NAD(P)H-dependent retinol dehydrogenases (RDHs) enzyme present in the photoreceptor cells and then brought back to the adjacent retinal pigment epithelium (RPE). It is there esterified by lecithin-retinol acyltransferase (LRAT) to all-*trans*-retinyl esters, primarily palmitoyl esters and then isomerized to 11-*cis*-retinol by the retinoid

isomerohydrolase RPE65 (Retinal Pigment Epithelium-specific 65 kDa protein, also known as isomerase I) [20,44-50]. This light-independent retinoid isomerization catalyzed by RPE65 occurs in a complex enzymatic reaction involving simultaneous hydrolysis of the ester moiety (Fig. 15). Thus the isomerized product, 11-*cis*-retinol, is finally oxidized to 11-*cis*-retinal by retinol dehydrogenase. It is then transported back from RPE to the photoreceptors where it undergoes Schiff base condensation with opsin to regenerate the light sensitive visual pigments like rhodopsin. RPE65 is involved for regeneration of both rhodopsin (rod cell) and photopsins (cone cell) visual pigments. Defective generation of RPE65 causes blindness. But, in contrast to the RPE65-dependent chromophore regeneration pathway for rhodopsin, there is an alternative metabolic pathway involving the enzymes located in cone photoreceptors and Müller cells to regenerate the photopsins of cone cells [20].

This conversion of all-*trans* retinal to 11-*cis*-retinal in retina is incomplete and rest of the all-*trans*-retinal is transported to liver (Fig. 14), where it is converted to all-*trans*-retinol through reduction by NADH (reduced form of nicotinamide adenine dinucleotide, NAD^+ *i.e.* $NAD^+ + e + H^+ \rightarrow NADH$) catalyzed by the zinc(II) containing metalloenzyme, liver alcohol dehydrogenase (LADH) [51] and then it is isomerized to 11-*cis*-retinol,

which is oxidized by NAD^+ catalyzed by LADH to 11-*cis*-retinal to participate in the visual cycle to resynthesize rhodopsin, *i.e.* 11-*cis*-retinal-opsin visual Schiff base pigment [14,15].

Dark adaptation time: If a person moves from a bright light zone to a dark zone, the rhodopsin content is almost completely exhausted and the vision is impaired. However, when the person returns to the light zone, within a few minutes, called dark adaption time, rhodopsin is resynthesized and vision is improved [36,37]. In the case of vitamin A (precursor of 11-*cis*-retinal) deficiency, the dark adaptation time is increased.

Colour vision and participation of cone cells

The cone cells are designed by nature for the bright light and colour vision [22,32,38,42,43,52]. Like the rod cell visual pigment, rhodopsin, there are cone cell visual pigments called photopsins which also possess the same 11-*cis*-retinal as the chromophore. Thus, photopsins are the Schiff bases produced from 11-*cis*-retinal and opsin glycoproteins having the different amino acid sequences. In human cone cells, there are three distinct types of visual pigments (Fig. 16): (i) cyanopsin known as blue cones absorbing the blue-violet light ($\lambda_{max} = 425$ nm), (ii) iodopsin known as green cones absorbing the green light ($\lambda_{max} = 540$ nm) and (iii) porphyropsin known as red cones

Fig. 15. Retinoid or visual cycle and the role of RPE65 enzyme
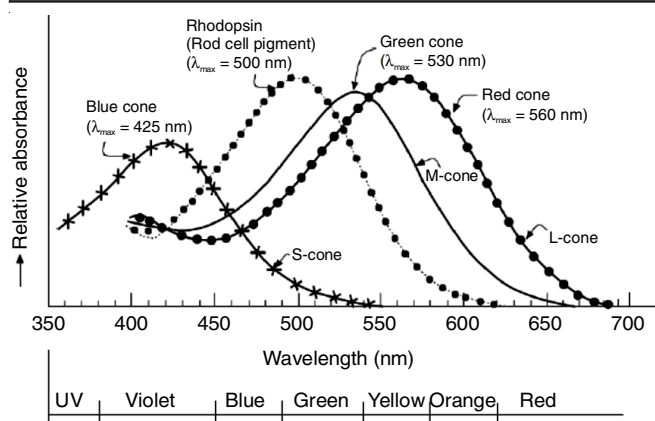
Fig. 16. Absorption spectra of the visual pigments: photopsins (*i.e.* cone cell pigments) and rhodopsin (rod cell pigment)

absorbing the yellow-orange light (λ_{max} = 570 nm) [14,22,31, 32] (Fig. 16). The blue cone (B-cone), green cone (G-cone) and red cone (R-cone) absorbing the short (S), middle (M) and long (L) wavelength portions of the visible spectrum are also described as the S-cone, M-cone and L-cone, respectively. It may be noted that rhodopsin of rod cells shows the λ_{max} = 500 nm. The difference in the photoreceptor properties of these three visual pigments arises from the changes in the sequence of amino acids in the opsin glycoprotein interacting noncovalently with the 11-*cis*-retinal chromophore moiety. Comparison of the amino acid sequence among the rhodopsin and phtopsins is quite striking [22]. The amino acid sequence of the photopsins is almost 40% identical with that of rhodopsin. For photopsins, the G and R photoreceptors are > 95 % identical in terms of amino acid sequence. Defects in the visual cycle appear from the defective pathways of biosynthesis of the visual pigments [53-55]. Colour blindness arises from the impaired genetic synthesis of the visual pigments in the cone cells [22,56-58]. A person lacking in the gene responsible for the synthesis of green cones fails to distinguish between the red and green colour.

Like the rhodopsin pigment of the rod cells, the pigments of the cone cells are also photochemically activated in the same way (through the isomerization in 11-*cis*-retinal chromophore unit) and bleached and eventually hydrolyzed to produce all-*trans*-retinal and opsin. The photoexcitation in the pigments of the cone cells produce a nerve impulse (as in the case of the rhodopsin pigment of rod cells) which is transmitted as a visual signal to brain. Photoexcitation of the pigment, cyanopsin, generates the signal for blue colour sensing. Similarly, the iodopsin and porphyropsin pigments are responsible for sensing the green and red colour, respectively. Simultaneous photoexcitation of these three cone cell pigments in different proportions leads to the perception of different colours by the brain. In fact, according to the RGB colour model, red (R), green (G) and blue (B) are considered as the three primary or basic colours, which can generate all colours of the visible spectrum when mixed together in appropriate proportions [14,22]. On the other hand, mixing of these three primary colours in equal proportions gives the white colour.

Conclusions

The present review on this multidisciplinary area projects in concise, the present status of our knowledge of biochemistry behind our vision, to make it popular to the readers of different levels. It indicates that the present field is still an active area of research to refine our understanding the chemistry and biology involved in the vision process. However, the present day knowledge has attained a position to understand the biochemistry of the vision pigments, involved the reaction intermediates characterized by low temperature and picosecond (ps) time resolved spectroscopies coupled with femtosecond spectroscopy, involved enzymes, the mechanism of nerve signal production and the rapid attainment of the vision cycle (Wald cycle) to maintain the continuity of the vision process, *etc.* This concise review is expected to attract the attention of the students also at the advanced level.

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