

The opsins

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Summary

The photosensitive molecule rhodopsin and its relatives consist of a protein moiety - an opsin - and a non-protein moiety - the chromophore retinal. Opsins, which are G-protein-coupled receptors (GPCRs), are found in animals, and more than a thousand have been identified so far. Detailed molecular phylogenetic analyses show that the opsin family is divided into seven subfamilies, which correspond well to functional classifications within the family: the vertebrate visual (transducin-coupled) and non-visual opsin subfamily, the encephalopsin/tmt-opsin subfamily, the G_q-coupled opsin/melanopsin subfamily, the G_o-coupled opsin subfamily, the neuropsin subfamily, the peropsin subfamily and the retinal photoisomerase subfamily. The subfamilies diversified before the deuterostomes (including vertebrates) split from the protostomes (most invertebrates), suggesting that a common animal ancestor had multiple opsin genes. Opsins have a seven-transmembrane structure similar to that of other GPCRs, but are distinguished by a lysine residue that is a retinal-binding site in the seventh helix. Accumulated evidence suggests that most opsins act as pigments that activate G proteins in a light-dependent manner in both visual and non-visual systems, whereas a few serve as retinal photoisomerases, generating the chromophore used by other opsins, and some opsins have unknown functions.

Opsins are membrane proteins with molecular masses of 30-50 kDa that are related to the protein moiety of the photoreceptive molecule rhodopsin; they typically act as light sensors in animals [1-4]. Photoreceptive proteins similar to the animal opsins in three-dimensional structure but not in amino-acid sequence have been found in archaea, bacteria, fungi, and a green alga, *Chlamydomonas reinhardtii* [5,6]. These non-animal opsins function as light-driven ion pumps or light sensors but there is no evidence that they are structurally related to animal opsins, so they are not considered further here.

Gene organization and evolutionary history

Since the first sequence of an opsin, bovine rhodopsin, was determined by conventional protein sequencing in 1982 [7,8] and cDNA sequencing in 1983 [9], more than 1,000 opsins have been identified. The molecular phylogenetic tree shows three large clusters, and detailed analyses have revealed that the opsin family is divided into seven

subfamilies; there is less than about 25% amino-acid similarity between subfamilies but more than about 40% among members of a single family (Figure 1). The division into subfamilies corresponds well to functional classification of opsins, which is based partly on the type of G protein coupled to each of these G-protein-coupled receptors (GPCRs). The seven subfamilies are as follows: the vertebrate visual (transducin-coupled) and non-visual opsin subfamily; the encephalopsin/tmt-opsin subfamily; the G_q-coupled opsin/melanopsin subfamily; the G_o-coupled opsin subfamily; the peropsin subfamily; the retinal photoisomerase subfamily; and the neuropsin subfamily. Members of the G_q-coupled opsin/melanopsin, G_o-coupled opsin, encephalopsin/tmt-opsin and retinal photoisomerase subfamilies are found in both deuterostomes (such as cephalochordates and vertebrates) and protostomes (such as molluscs and insects; Figure 1), suggesting that diversification of the subfamilies occurred much earlier in animal evolution than the deuterostome-protostome split [10].

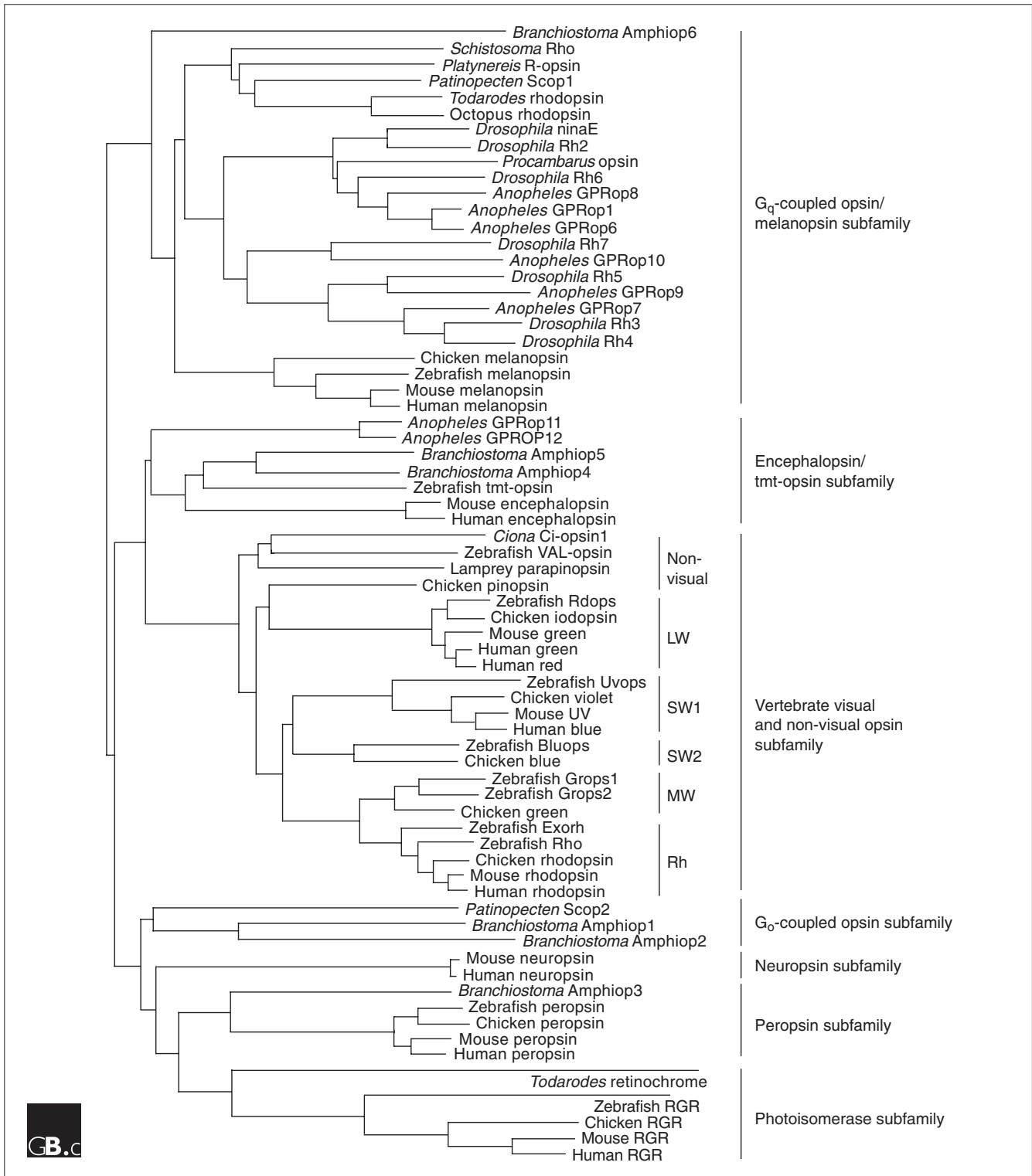


Figure 1

A molecular phylogenetic tree of the opsin family. The tree was inferred by the neighbor-joining method [81]. It shows that members of opsin family are divided into seven subfamilies, whose names are given on the right of the tree. Common names of species shown: *Anopheles*, mosquito; *Branchiostoma*, amphioxus; *Ciona*, ascidian; *Drosophila*, fruit fly; *Patinopecten*, scallop; *Platynereis*, polychaete annelid worm; *Procambarus*, crayfish; *Schistosoma*, blood fluke; *Todarodes*, squid. Abbreviations: LW, long-wavelength-sensitive opsin; SW, short-wavelength-sensitive opsin; MW, middle-wavelength-sensitive opsin; Rh, rhodopsin; RGR, retinal G-protein-coupled receptor. Other abbreviations are protein names; where only a color is given for a protein name, it refers to a cone opsin that detects that color.

The visual and non-visual opsin subfamily contains vertebrate visual and non-visual opsins. The visual opsins can be further subdivided into cone opsins and rhodopsin, which have distinct molecular properties arising from differences in the residues at positions 122 and 189 of the amino-acid sequence [11,12]. The cone opsins can be further divided into four subgroups, which correspond well with their absorption spectra: long-wavelength opsins (LW or red), short-wavelength opsins (SW1 or UV/violet and SW2 or blue), and middle-wavelength opsins (MW or green; see Figure 1) [1,3,13]. Note that other nomenclatures are also used to specify these four groups. Most vertebrates, including the lamprey [14], have four kinds of cone-opsin genes, whereas mammals lack the SW2 and MW genes. Interestingly, humans have regained the green-sensitive opsin by duplication of the LW gene, so the green cone opsins of humans and lower vertebrates belong to different opsin subgroups (LW and MW) [15,16]. In the human genome, the red and green opsin genes are localized in tandem.

Lower vertebrates, including lampreys, have several non-visual opsin genes that are members of the same subfamily as the vertebrate visual opsins. The first non-visual opsin to be discovered was pinopsin [17], which is involved in photoreception in the pineal organs of birds [17,18] and lizards [19]. Parapinopsin was first found in the pineal complex of the catfish [20], and it has also been found in zebrafish and *Xenopus* and more recently in the lamprey pineal [21]. 'Vertebrate ancient' opsin (VA-opsin) was first found in the salmon retina [22]; the lamprey also has an ortholog of VA-opsin, called P-opsin [23]. The ascidian chordate *Ciona* has an opsin (Ci-opsin1) that is closely related to the vertebrate non-visual opsins [24].

Within the other six subfamilies of opsins, members of the encephalopsin/tmt-opsin subfamily were first found in mouse and human [25], and homologs were recently identified in the teleosts [26] and interestingly in invertebrates, the mosquito *Anopheles* [27] and the marine ragworm *Platynereis* [28]. Phylogenetic analysis shows that encephalopsin/tmt-opsin subfamily probably clusters most closely with the vertebrate visual and non-visual opsin subfamily (see Figure 1). Melanopsin is an important vertebrate non-visual opsin, but because it is more similar in amino-acid sequence to invertebrate G_q-coupled visual opsins, it is not classified as a member of the vertebrate visual and non-visual opsin subfamily (see Figure 1); melanopsins have been found in many vertebrates, from fish to humans [29,30]. Members of the G_o-coupled opsin subfamily have been found in molluscs and in the chordate amphioxus [10,31] but not in human, mouse, zebrafish or *Drosophila*. Neuropsins, recently identified in mouse and human [32], are phylogenetically distinguishable as a subfamily but little is known about them. Peropsins are known from a range of vertebrates, from fish to human [33], and an ortholog was recently found in amphioxus [31]. Finally, members of the

Table 1**Chromosomal locations and numbers of introns of the nine human opsin genes**

Opsin	Chromosomal location	Number of introns
Rhodopsin	3q22.1	4
Blue opsin	7q32.1	4
Red opsin	Xq28	5
Green opsin	Xq28	5
Encephalopsin	1q43	3
Melanopsin	10q23.2	9
Peropsin	4q25	6
RGR	10q23.1	6
Neuropsin	6p12.3	6

retinal-photoisomerase subfamily, which includes retinal G-protein-coupled receptor (RGR) and retinochrome, are found in vertebrates and molluscs [34,35]; an RGR homolog has also been found in an ascidian [36].

The gene organization of different vertebrate opsins provides further information about relationships among the subfamilies [32,37,38]. The numbers of introns in the human opsin genes are shown in Table 1 as an example. Three of the four or five introns in the vertebrate visual and non-visual opsin genes are shared at conserved positions with encephalopsin/tmt-opsin genes, consistent with the close relationship between these subfamilies found by phylogenetic analysis. The peropsin, retinal photoisomerase (RGR) and neuropsin subfamily genes have six introns, which are at positions different from those of vertebrate visual and non-visual opsin genes. Two and three of the peropsin introns are conserved in the RGR of the retinal photoisomerase subfamily and the neuropsin gene, respectively, again confirming a close evolutionary relationship between these subfamilies. The melanopsin gene has nine introns at positions different from those of other opsin genes.

Recent genome studies have also provided us with information on the loss of opsin genes during animal evolution. No opsin gene has been found in *Caenorhabditis elegans* [39,40]. *Drosophila* has seven opsin genes, all of which belong to the G_q-coupled opsin/melanopsin subfamily [41]. In comparison, humans have nine opsin genes (Table 1), which are spread over six of the seven subfamilies (Figure 1). A PCR study [31] revealed that amphioxus has at least six opsin genes from four subfamilies (Figure 1); deuterostomes therefore appear to have opsins from more subfamilies than do protostomes.

Characteristic structural features

Opsins share several amino-acid motifs, including seven transmembrane helices, with other G-protein-coupled

receptors (GPCRs) of the rhodopsin superfamily. The first primary sequence of a member of the rhodopsin superfamily, the β -adrenergic receptor, was determined in 1986 [42], and since then, the opsin family has been considered one of the typical members of the superfamily. As shown in Figure 2a, several amino-acid residues are highly conserved among the opsin family members; about half of these are conserved in all GPCRs of the rhodopsin superfamily [43]. All opsins bind a chromophore: the vertebrate visual and non-visual opsins, the invertebrate G_q -coupled opsins, and the G_o -coupled opsins all bind 11-*cis*-retinal, whereas the photoisomerases and the peropsins bind all-*trans*-retinal (Figure 2b). The chromophores of the other opsins are uncertain.

The crystal structure of bovine rhodopsin has been solved [44-46] (Figure 2c). K296 (in the single-letter amino-acid code) in helix VII binds retinal via a Schiff-base linkage, in which the nitrogen atom of the K296 amino group forms a double bond with the carbon atom at one end of the retinal (Figure 2d). The key residue K296 is important for light absorption and its presence or absence can be used to judge whether or not a newly found rhodopsin-type GPCR is really an opsin. The counterion is another important residue: it is a negatively charged amino acid that helps to stabilize the protonated Schiff base (see below). In the vertebrate visual and non-visual opsin subfamily, the highly conserved residue E113 serves as the counterion [47-49], whereas in other opsins position 113 is occupied by other amino acids (tyrosine, phenylalanine, methionine, or histidine) and the highly conserved E181 serves as the counterion. This difference suggests that counterion replacement has occurred during the molecular evolution of vertebrate visual and non-visual opsins [50,51].

Localization and function

Functions of the vertebrate visual and non-visual opsins

Two photoreceptor cells are involved in vision in most vertebrates - rod and cone cells - and they are distinguishable by their shapes. The rod and cone cells contain different opsins: rods have rhodopsin, which underlies twilight vision, and cones have cone opsins, which underlie daylight (color) vision [1]. When excited by light in rod and cone cells, rhodopsin and cone pigments drive an enzyme cascade involving G proteins and their effectors: the excited pigments activate the G-protein transducin, which stimulates cGMP phosphodiesterase, resulting in a decrease in intracellular cGMP concentration. This decrease leads to closure of a cGMP-gated cation channel, leading to the hyperpolarization of the visual photoreceptor cell. In general, rods and cones contain distinct sets of phototransduction molecules (transducin, phosphodiesterases and channels) [52]. It should be noted that the visual opsins are also expressed in non-visual photoreceptor cells, including the pineal photoreceptor cells that are found in most non-mammalian vertebrates.

The lower-vertebrate non-visual opsin genes are expressed in photoreceptor cells other than rods and cones. For example, pinopsin is involved in photoreception in the pineal organs of birds [17,18] and lizards [19]. It is suggested to activate both transducin and the G-protein G_{11} and therefore to drive two different phototransduction cascades [53,54]. The parapinopsin recently found in the pineal organ of the lamprey [21] is a UV-sensitive and bistable opsin with stable dark and light-activated states. VA-opsin is found in the salmon retina [22] but in amacrine and horizontal cells (two kinds of neural cell in the retina), not in rod and cone visual cells [55]. A splice variant of VA-opsin called VAL-opsin is localized to deep parts of the brain and the horizontal cells of the zebrafish [56].

Functions of other subfamilies

The visual opsins of arthropods and molluscs belong to the G_q -coupled opsin group, which is different from the vertebrate visual opsin group. They are localized to the microvilli of the rhabdomeric photoreceptor cells, which are typical visual cells of arthropods and molluscs and are morphologically different from vertebrate rods and cones. These opsins are coupled to the signal-transduction cascades involving the G protein G_q and phospholipase C [2,57-60] and leading to depolarization of the cells in response to light. The different subgroups of insect opsins have distinct absorption spectra; this underlies insect color vision. Vertebrate melanopsins are very similar to the G_q -coupled invertebrate opsins [29,30]; mouse melanopsin has been reported from knockout studies to be involved in the response of the pupil to light [61] and in the entrainment of circadian rhythm by light [62]. As suggested by their close relationship to the G_q -coupled opsins, melanopsin can be coupled to a G_q /phospholipase-C cascade, similar to that used by the invertebrate opsins [63-65].

Mouse encephalopsin (also called panopsin) is strongly expressed in the brain and testes and weakly in other tissues [25], and the teleost homologs are localized to multiple tissues (they are therefore named teleost multiple tissue (tmt) opsins) [26]. The functions of the encephalopsins and tmt-opsins are unknown, but their close but distinct position in the phylogenetic tree relative to the vertebrate visual and non-visual opsins may mean that they are more likely to have distinct functions.

Some invertebrates have photoreceptor cells - distinct from the rhabdomeric photoreceptors - that are called ciliary photoreceptors because their photoreceptive portions originate from cilia. Interestingly, the scallop (a bivalve mollusc) has both kinds, and in the ciliary photoreceptor cells a novel opsin has been found that is different from the G_q -coupled one [10]. It colocalizes with a large amount of G_o -type G protein and is thought to activate G_o *in vivo*; it is therefore named G_o -coupled rhodopsin (or G_o -coupled opsin). Electrophysiological evidence suggests that scallop G_o -coupled

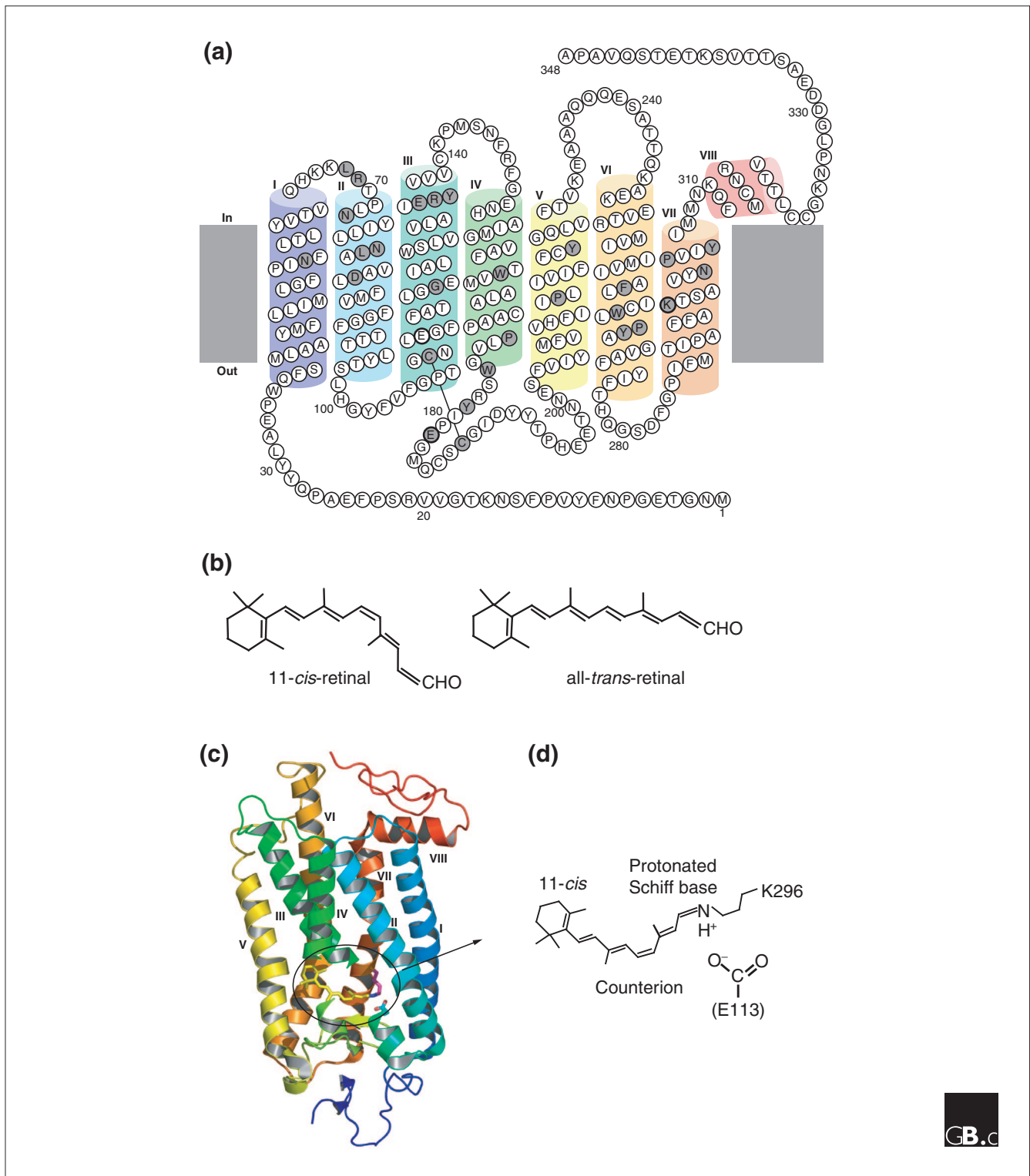


Figure 2

Structures of opsins and of the chromophore retinal. **(a)** A model of the secondary structure of bovine rhodopsin. Amino-acid residues that are highly conserved in the whole opsin family are shown with a gray background. The retinal-binding site (K296) and the counterion position (E113) are marked with bold circles, as is E181, the counterion in opsins other than the vertebrate visual and non-visual ones. C110 and C187 form a disulfide bond.

(b) The chemical structures of the 11-cis and all-trans forms of retinal. **(c)** The crystal structure of bovine rhodopsin (Protein DataBank ID: 1U19 [PDB: 1U19]). The chromophore 11-cis-retinal, K296 and E113 are shown in stick representation in the ringed area. **(d)** The structure of the Schiff base linkage formed by retinal within the bovine opsin, together with the counterion that stabilizes it.

rhodopsin elevates the intracellular cGMP concentration through light-dependent activation of G_o, which leads to hyperpolarization of the cell [66].

Neurospins are localized to the eye, brain, testes and spinal cord, but their functions are unknown. Peropsin was first found in the RPE of the mammalian eye [33]. It binds all-*trans*-retinal as a chromophore, and light isomerizes it to the 11-*cis* form [31] (Figure 2b). This photochemical property indicates that peropsin may serve as a retinal photoisomerase, like retinochromes and RGRs [34,35]. Retinochrome and RGR, the members of the retinal-photoisomerase subfamily, bind all-*trans* retinal (Figure 2b) as a chromophore [67,68] and are not coupled to G proteins, unlike the visual opsins, which bind the 11-*cis* form of retinal. Retinochrome and RGR have been identified in the mollusc and vertebrate retinas, specifically in the inner segments of the visual cells [69,70] and in the retinal pigment epithelium (RPE) [34], respectively. Irradiation of these two pigments causes the isomerization of all-*trans* retinal to the 11-*cis* form [67,68], suggesting that these opsins enzymatically generate the chromophore and supply it to the visual opsins [70,71].

Mechanism

The function of most opsins except for the photoisomerases can be divided into two parts: light absorption and G-protein activation. Most opsins function through absorption of visible light, but the chromophore retinal itself has an absorption maximum in the UV region, not in the visible region. This potential problem is solved by the opsins as follows. As previously described, retinal binds to K296 in helix VII through the protonated Schiff base (Figure 2d); the protonation, which results in the delocalization of π electrons within the retinal molecule, shifts its absorption spectrum towards visible light. In the protein, the proton on the Schiff base is unstable and a counterion, a negatively charged amino-acid residue, therefore needs to be present in order to stabilize it.

Absorption of light (a photon) by retinal results in its photoisomerization from the 11-*cis* to the all-*trans* form (Figure 2b). This is followed by a conformational change of the protein moiety, eventually resulting in activation of the G protein. Photochemical studies have identified some spectroscopically distinguished intermediates that form during bleaching of the vertebrate rhodopsin - 'batho', 'lumi', 'meta I', and 'meta II' - which appear on the picosecond, nanosecond, microsecond and millisecond timescales after light absorption, respectively [1]. Many biochemical and biophysical studies have focused on the question of what conformational changes take place in the protein moiety during the formation of the active state of opsins, especially the meta II intermediate of bovine rhodopsin. The most notable hypothesis is that light triggers the relative outward movement of helices III and VI [72,73] to form meta II, most likely following flipping-over of the retinal

ring [74]. This movement of the helices could expose G-protein-binding sites, such as the cytoplasmic loop between helices V and VI [75,76]. This loop varies in sequence among the different subfamilies and underlies their selective coupling to different subtypes of G protein [77,78]. It is believed that a similar helix motion occurs in most members of the rhodopsin superfamily.

Frontiers

There are many unanswered questions concerning the functions of the different opsins. Recently, genetic approaches using knockout and/or transgenic animals have been used to understand the function and/or the expression mechanism of some opsins. Recent progress with RGR knockout mice concluded that RGR serves as the photoisomerase of retinal *in vivo* [71], and the function of melanopsin in the circadian clock has been studied with mutant mice lacking photoreception through rods and cones [62]. One interesting approach for investigating why there are so many kinds of opsins is functional replacement of one opsin with another and observing the altered phenotype. The first example of this approach was the experimental replacement of rhodopsin with cone opsin in transgenic *Xenopus*, by selective stimulation of the cone opsin in a single rod cell that contained both cone opsins and rhodopsins [79]. The 'knock-in' technique could be most useful for this kind of opsin-replacement experiment (H. Imai and Y. Shichida, unpublished observations).

GPCRs are important targets for drug discovery, and the opsin family is currently a good subject for such studies because it is the only family for which the structure of a member has been solved at high resolution. Structural studies of opsins could provide valuable information for understanding how GPCRs in general activate G proteins. Okada *et al.* [45] have investigated the photochemistry of the rhodopsin crystal, which raises the possibility of solving the structure of an active form of rhodopsin (meta II) at high resolution (2.5 Å). The crystal structure of the meta I photointermediate of rhodopsin has recently been solved to around 5.5 Å resolution [80]. The crystallization of a complex of active rhodopsin (meta II) with a G protein could be one of the breakthroughs that help to elucidate the G-protein-activating mechanism.

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