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Review

Molecular Aspects of Eye Evolution and Development: From the Origin of Retinal Cells to the Future of Regenerative Medicine

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A central issue of evolutionary developmental biology is how the eye is diverged morphologically and functionally. However, the unifying mechanisms or schemes that govern eye diversification remain unsolved. In this review, I first introduce the concept of evolutionary developmental biology of the eye with a focus on photoreception, the fundamental property of retinal cells. Second, I summarize the early development of vertebrate eyes and the role of a homeobox gene, *Lhx1*, in subdivision of the retina into 2 domains, the neural retina and retinal pigmented epithelium of the optic primordium. The 2 retinal domains are essential components of the eye as they are found in such prototypic eyes as the extant planarian eye. Finally, I propose the presence of novel retinal cell subtypes with photosensory functions based on our recent work on atypical photopigments (opsins) in vertebrates. Since human diseases are attributable to the aberration of various types of cells due to alterations in gene expression, understanding the precise mechanisms of cellular diversification and unraveling the molecular profiles of cellular subtypes are essential to future regenerative medicine.

Key words: eye, development, evolution, opsin, photoreceptor

Eyes are detectors and convert light waves travelling through the atmosphere into visual images [1, 2]. Vision—the formation of an image or picture from light waves—is the most sophisticated form of light detection. In contrast, the elementary form of light detection might be called simply "light perception." Although elementary light detectors are sometimes differentiated from "authentic" eyes because they do not form images, here I will touch on the idea of light detectors as "ancient eyes" in my brief review of the evolutionary developmental biology of the eye. For the present discussion, I primarily adopt Darwin's definition of an eye as an organ consisting of at least 2 different cell types, photoreceptor cells and pigment cells [3], and introduce our studies regarding how the two retinal components are formed in the vertebrate eye. Finally, I describe the expression of atypical photopigments in the retina, proposing a hypothesis regarding retinal cell diversity and its novel photosensory functions. These studies on the eye and novel photoreceptors will help to elucidate the conserved molecular mechanisms of cellular diversification in the eye, and could pave the way to regenerative ocular medicine and gene therapy for deceased ocular cells.

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Thoughts on the Origin of the Eye

There are various types of eyes in the animal kingdom, such as the 2 compound eyes in honeybees, four pairs of ocellar-type eyes in jumping spiders, and vesicular eyes, which include the distinct camera-type eyes of cephalopod molluscs and the highly evolved camera-type eyes of humans <http://www.brh.co.jp/seimeishi/journal/012/ss_1.html (accessed May 31, 2013.)>. It has been estimated that more than 95% of modern animal species have eyes or some other form of specialized light-sensitive sensory structures. Andrew Parker says in his book, "In the Blink of an Eye," that the birth of so many different types of eyes may have been attributable to a great increase in the amount of light that reached the earth in the late stages of the Precambrian era [2]. It is widely known that the so-called "big bang of evolution" occurred in the Cambrian era, when bizarre animals such as *Opabinia*, the now extinct animal known to us only from its fossils in Burgess shale, lived on the seafloor with 5 eyes at the front of its head [4]. Parker calls this idea the "light switch" theory in evolution of the eye, since it posits the central role played by light in the development of the myriad types of eyes revealed by fossil records. Furthermore, it is intriguing to note that *Trilobites*, which in the past has been considered an ancestor of insects, although this theory is now controversial, already had 2 distinct compound eyes and its size was increased according to the increment of light from the Precambrian era to the early Cambrian era, which occurred, so to speak, just in the blink of an eye [2].

It may have been Charles Darwin who first referred to the diversity in structures of the eye from an evolutional viewpoint. In his *Origin of the Species* (1859), he says that the eye may be exceptional and monophyletic origin of the eye would not be applicable as the structure and morphology of the eye are so diverged in the animal kingdom [5]. There are many types of eyes such as the eyespot in *Euglena*, which senses the direction and intensity of light, and disseminated photoreceptors in the body surface of earthworm, which are reminiscent of light-avoidance-mediating photoreceptors tiling the *Drosophila* larval body wall [6]. In the flat eyes of marine annelid worms, the cupulate eyes of limpets, and the pinhole eyes of *Nautilus*, an image is formed on the retina either by a refractile lens, by refraction at the cornea, or by reflection [7]. The compound eye is found throughout crustaceans and insects. Both compound and vesicular eyes are image-forming devices.

So, the fundamental question arises; did this great variety of eyes arise independently or did they have a common ancestry? As mentioned, looking at the variety of structures and designs, it is conceivable to imagine a separate polyphyletic origin. However, the evidence of common ancestry comes from the discovery of a homeobox gene, *Pax6* [8], which was found to be a causal gene of rodent *small eye* phenotypes [9, 10], aniridia of humans [11], and a fly *eyeless* mutant [12]. In *Drosophila*, the mouse or fly *Pax6* gene can induce ectopic eye formation in imaginal discs of the antenna when overexpressed [13]. The *Pax6/eyeless* gene encodes a transcription factor that binds to DNA with a homeodomain or a paired domain and regulates the transcription of target genes. It has been determined that *Pax6* controls the development of various eyes ranging from planarian to human eyes [3]. In the box jellyfish *Tripedalia*, a *PaxB* gene, which may be a precursor of *Pax6*, was found to be expressed in the eyes [14]. Although the nematode *Caenorhabditis elegans* does not have eyes, Pax6 is involved in the head formation and peripheral sense organs in the tail region of *C. elegans*, implying that photoreception arose from other types of sensing [15, 16]. Since nematodes are one of the most diverse of all animal phyla, eye-bearing nematodes are also known. Their structural and molecular analysis suggests that photoreceptors and eyes may be evolved from ancestral chemoreceptors or thermoreceptors [17]. In this regard, it is interesting that *C. elegans* with a negative phototaxis has ciliary sensory neurons, and that its phototransduction requires a G protein-dependent cAMP pathway through a taste receptor homolog [18].

Pax6 is also involved in congenital eye diseases other than aniridia, such as Peter's anomaly, congenital cataract, late-onset corneal dystrophy, autosomal-dominant keratitis, macular hypoplasia, and optic nerve dysplasia, all of which depend on types of mutations <http://omim.org/entry/607108 (accessed May 31, 2013)>. To date, it has been found that numbers of genes in addition to *Pax6* also contribute to eye development. For example, *Rx/Rax* is required for retinal formation and therefore mutations in *Rx/Rax* result in anophthalmia or microphthalmia [19].

Other examples are *Sox2, Pitx2, Pitx3, Chx10 (Vsx2), Vsx1, Crx*, and *FoxL2*, whose mutations also lead to a variety of congenital eye diseases [20, 21]. Thus, it is now self-evident that genes regulating the developmental processes would be responsible for various congenital anomalies when they are mutated or their expressions are altered.

Again, let's go back to the origin of diversified eyes in the animal kingdom. Since the *Pax6* homologous genes are expressed in the course of eye development in most species, a prototypic eye as found in planaria consisting of photoreceptor and pigment cells [1, 3, 22] would be formed through the action of Pax6. Interestingly, in the aforementioned jellyfish, unicellular photoreceptors contain both the putative photosensory microvilli and the shielding pigment granules within the same cell, which also carries a motor cilium that enables the larva to show phototactic behavior [3, 14]. In view of the evolution and presence of various types of eyes, the most fundamental property of the initial eye is photoreception, which is accomplished by photopigments, including opsins. The opsin is a seven-transmembrane or G-protein-coupled receptor (GPCR) that binds to the retinal chromophore and absorbs photons at a distinct wavelength [23–25]. Thus, it has been proposed that the origin of the eye goes back to the opsin-expressing cells, and that Pax6 would organize the principal components of the photoreceptor and pigment cells (primarily for shielding from light scattering), thereby producing a prototypic eye [26]. In this sense, the opsin protein is key to the origin of the eye at the single cell level, and light is crucial to the evolution and development of the eye. Actually, a recent work has shown that photoreception by melanopsin is required for the development of retinal neurons and elimination of hyaloid vessels [27].

Early Eye Development and the Unexpected Role of Lhx1 in the Optic Primordium

Since the structure of the eye is diversified, we can easily imagine that its different structures developed in distinct ways. Therefore, I will here focus on the developmental processes of vertebrate eyes, particularly at the molecular level. The retinal primordium emerges from the eye pit in the forebrain well before the neural fold closes at around 8 days post-coitum in mice and the third gestational week in humans [28, 29]. As the neural folds meet at the midline, the forebrain evaginates to form the optic vesicle, the retinal anlage. Then the optic vesicle invaginates to form the optic cup and concomitantly induces the lens vesicle from the overlying surface ectoderm in a process known as secondary induction. The induced lens further induces the corneal epithelium from the surface ectoderm. Thus, the retinal component of the eye derives from the neural ectoderm, and the lens and corneal epithelia from the surface ectoderm in the case of vertebrate eyes. The surrounding mesenchyme of the neural crest origin also participates in eye formation and forms the corneal endothelium and stratum, for example.

Recent molecular studies of developmental biology in embryogenesis and organogenesis have led to success in making various organs and tissues from pluripotent cells such as embryonic stem (ES) cells [30–33] as well as induced pluripotent stem (iPS) cells [34–36], and even from differentiated cells, via a process known as direct reprogramming [37]. In one of the more spectacular studies, an optic vesicle was generated from a single layer of neuroepithelium derived from ES cells in culture [31, 38]. Although a self-organizing system of tissue architecture may be useful to produce a whole organ en bloc in culture, it is nevertheless important to obtain single types of cells or tissues very efficiently for cell/tissue implantation therapy, and gene expression profiling of individual cells/tissues is the first step in this process. In this context, the adult retinal stem cells, which resides in the ciliary marginal zone (CMZ) abutting the ciliary epithelium [39], is similar to the embryonic neural retina with regard to the expressions of *Pax6, Chx10 (Vsx2), Rx, Six3, Six6* and other related genes [40]. Therefore, the combinatorial expression of these genes is thought to constitute a molecular fingerprint of retinal stem cells [41], and thus it is crucial to identify a factor(s) or regulatory gene network that controls the transcription of these retinal stem cell marker genes. Intriguingly, all these genes are involved in eye development at very early stages, when a single eye field is present at the most rostral midline, and/or at later stages when various types of retinal neurons differentiate, indicating the reiteration of developmental toolkit genes during development [42].

To elucidate gene expression profiling during retinal development, we performed sequencing analysis of Expressed Sequence Tags (EST) using cDNA from the chick embryonic neural retina [43 and unpublished data]. We first selected 200 known genes and examined their expression patterns on retinal sections from 4 developmental stages, embryonic day 6 (E6), E9, E12, E14 and post-hatching day 1 using a semi-automatic machine for *in situ* hybridization. Through this study, we found that a member of the fibroblast growth factor (FGF) family, *Fgf19*, is expressed by retinal horizontal cells from their early migrating stage to later more mature stages [43]. FGF19 is known as an endocrine hormone-like FGF regulating bile acid metabolism in the liver [44], and it would be intriguing to determine whether FGF19 also exhibits hormone-like activity during early retinal development. We further found that *Fgf19* is expressed by one subset of horizontal cells [45]. It is known that retinal horizontal cells have 3 subtypes: one is axon-bearing, brush-shaped cells, which express a LIM-type homeobox gene, *Lhx1*, and the other 2 are axon-less, *Lhx1*-negative, *Islet1*-positive cells, which are further divided into 2 subtypes by the expression of GABA (gamma-aminobutyric acid) or TrkA (a receptor for nerve growth factor) [46]. *Fgf19* is expressed by *Lhx1*-expressing retinal horizontal cells.

The Lhx family, LIM-type homeobox-containing genes are transcription factors which contain a protein-binding domain of LIM and a DNA-binding homeodomain. The Lhx family consists of 6 groups of 12 members in vertebrates [47]. Since the *Lhx1*-null mice exhibit a headless phenotype, Lhx1 is required for head formation [48]. In the mouse retina, *Lhx1* is expressed in the horizontal cells and the knockout of this gene in retinal progenitor cells leads to defects in horizontal cell migration, showing that Lhx1 is required for correct positioning of retinal horizontal cells [49]. To explore the relationship between *Lhx1* and *Fgf19* in the formation of retinal horizontal cells, we overexpressed *Lhx1* in the emerging optic vesicles of chick embryos (Hamburger-Hamilton's stage 9–10, after one and a half days of incubation). Incidentally, the chick embryo is a classical model animal in developmental biology, as it develops in the egg independently from hens, its embryonic tissues can be easily manipulated by cutting and grafting of other tissues/cells, it can be manipulated by the placement of protein-containing beads, and it can now be subjected to ectopic gene expression using simple electroporation methods. Although we were expecting the production of an additional population of horizontal cells after *Lhx1* overexpression, we found a morphologically distinct thickening of the outer layer of the optic cup at 24 hours post-electroporation. Histological analysis showed that portions of the outer layer, *i.e.*, the prospective pigmented epithelium (RPE), began to resemble a neural retina (NR) (Fig. 1A–C).

This histological change is reminiscent of the second NR formation from the outer layer of the optic cup after ectopic FGF application [50]. It has been postulated that FGF secreted from the overlying surface ectoderm acts as a positive regulator for induction of the NR in the distal portion of the optic vesicle and for its separation of the NR and RPE domains [50]. Other studies, on the other hand, support the idea that the TGF beta-like factors from the extraocular mesenchyme surrounding the optic vesicle have a positive effect on RPE induction in the dorsal portion of the optic vesicle [51]. These ideas seem to be incompatible with recent studies of ES cell-derived optic vesicle formation in culture, as the optic primordium self-differentiates NR and RPE without the overlying ectoderm or surrounding mesenchyme [31]. Our results concerning Lhx1 also support a different mechanism of NR/RPE differentiation, since *Lhx1* is expressed in the proximal region of the emerging optic vesicle (Fig. 1D–F) and overexpression of *Lhx1* is not mediated by the early induction of *Fgf* expression [52]. Since the second NR induced by *Lhx1* overexpression expresses *Pax6, Chx10 (Vsx2), Rx, Six3*, and *Six6*, and NR differentiation markers in later stages, *Lhx1* is a candidate gene capable of regulating the aforementioned retinal stem cell marker genes. We also performed *in ovo* RNA interference (RNAi) to reduce the expression of *Lhx1* in the proximal region of the optic vesicle, resulting in formation of a pigmented vesicle with up-regulation of RPE maker genes such as *Otx2* and *Mitf*. In a severe case, there was no lens formation due to the absence of neural retina formation (unpublished data). From these data, we suggest that the proximal region of the emerging optic vesicle is essential for NR formation, possibly via diffusible factors regulated by the transcription factor, Lhx1.

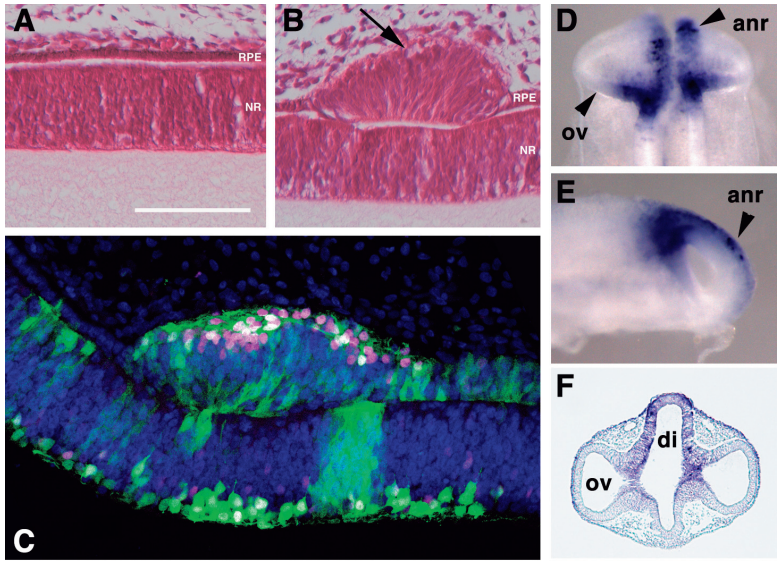

Fig. 1 *Lhx1*-overexpressed optic cup of the chick via electroporation [52]. **A-C**, *Lhx1* overexpression induced a second NR formation from the presumptive RPE. The histologies of the control (**A**) and *Lhx1*-overexpressing (**B**) embryonic retina, where the outer layer of the optic cup is partly thickened (arrow) are shown. (**C**) Overexpression of *Lhx1* (magenta) in the outer layer of the optic vesicle. An EGFP expression plasmid (green) was co-electroporated with an *Lhx1* expression vector. Nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI); **D-F**, *Lhx1* was expressed in the proximal region of the optic vesicle (dark blue). Whole mount *in situ* hybridization (WISH) of embryos around Hamburger and Hamilton's stages 10. (**D**) Dorsal view. (**E**) Lateral view. (**F**) A transverse section cut after WISH is shown at the middle of the optic vesicle (ov). *Lhx1* is distinctly expressed in the proximal region of the ov and dorsal diencephalon (di). Outside these regions, *Lhx1* is expressed in the anterior neural ridge (anr). Bar, 100 μm.

Diversified Retinal Neurons and Assumed Roles of Atypical Opsins

Photosensory cells develop from the NR, and NR-derived retinal cells were well observed in a series of studies by Santiago Ramón y Cajal. He depicted the structures of the mammalian retina, and his sketch distinctly showed 8 types of retinal cells: retinal ganglion cells, amacrine cells, bipolar cells, a horizontal cell, rod cells, cone cells, a Müller glial cell, and an RPE cell <http://en.wikipedia.org/wiki/Santiago_Ram%C3%B3n_y_Cajal (accessed May 31, 2013)>. All these retinal cells except for the RPE cell showed orderly formation in the NR [53]. How these morphologically and functionally diverse retinal cell types are determined is a central issue of developmental biology. In the ongoing effort to address this question, findings on transcription factors and growth factors elaborating the cellular specification and differentiation of individual retinal cells continue to accumulate. However, there continues to be a dearth of ideas regarding the unifying developmental mechanisms or strategies that govern the retinal cell diversification. Arendt (2003) postulated an attractive hypothesis that various retinal cell types in vertebrates have been diversified from 2 prototypic photoreceptors (PRs): rhabdomeric and ciliary PRs [54]. The ciliary (c-) PR corresponds to the rod and cone PRs of vertebrate retina or photosensitive cells in the invertebrate brain, while the rhabdomeric (r-) PR is typical in the invertebrate retina of compound eyes, or intrinsically photosensitive ganglion cells of the vertebrate retina, which express melanopsin. R-PRs are derived from microvilli cells, while c-PRs are derived from ciliated cells. Interestingly, r-PRs and c-PRs have distinct GPCR signaling cassettes [54–56]: r-PRs have the Gq-type alpha-subunit of trimetric G-proteins that has been linked with phospholipase c activity, resulting in depolarization, while c-PRs have the Gt-type alpha-subunit with phosphodiesterase activity, resulting in hyperpolarization. Arendt (2003) summarized the expression patterns of various transcription factors, neurotransmitter-related molecules, and opsins known to be expressed in retinal cells, find-

ing that they are differentially expressed and never overlapped [54]. For example, *Crx, Otx2, Chx10, Mash1*, and rod/cone opsins are expressed by rod/cone photoreceptors and/or bipolar cells, but never expressed by horizontal, amacrine or ganglion cells. Conversely, *Pax6, Brn3, Mash5, BarH, Prox1*, dopamine, acetylcholine, melanopsin, and VAL opsin are expressed by horizontal, amacrine, and retinal ganglion cells, but not expressed by rod/cone PRs or bipolar cells. This expression profile implies that 2 distinct retinal cell lineages may exist in the vertebrate retina, leading to the rhabdomeric and ciliary PRs with distinct molecular fingerprints, respectively.

This hypothesis is associated with the discovery that melanopsin is expressed in the vertebrate retina by a small subset (1–2%) of retinal ganglion cells, *i.e.*, intrinsically photosensitive retinal ganglion cells (ipRGCs) [57, 58]. Melanopsin is phylogenetically grouped into the so-called rhabdomeric opsins (r-opsins), which also include *Drosophila* Rh1 being expressed by invertebrate retinal cells. On the other hand, Arendt *et al.* (2004) found an opsin that is phylogenetically related to ciliary opsins (c-opsins) such as rod/cone opsin, and that is expressed in the brain of a marine worm, *Platynereis dumerilii* [55]. These findings have now led to the idea that there are 2 ancestral photoreceptors in the animal kingdom, rhabdomeric and ciliary, at least in the retina and brain. Other studies have also suggested that photosensitive cells are present in the vertebrate brain as well, and that actually melanopsin, VAL opsin, opsin 5, and encephalopsin* (*whose photosensitivity has not been characterized), are expressed in small subsets of cells of the vertebrate brain [59–64].

I have become interested in Arendt's working model and first anticipated that melanopsin, a rhabdomeric opsin, might be expressed by all the cells other than future ciliary PRs, rods and cones during development, and that melanopsin-expressing immature retinal cells may be rhabdomeric in origin. However, the expression patterns of melanopsin in the differentiating retina did not agree with this hypothesis, as a melanopsin, *cOpn4x* [65], is expressed by small subsets of cells in the ganglion cell layer and the inner nuclear layer [66], while a second melanopsin in chicks, *cOpn4m*, is expressed by a subset of cells in the inner nuclear layer [67]. These data prompted us to explore other atypical opsins that have been identified in the genome but whose photosensitivity or functions have not been determined. We therefore focused on the expression of *opsin 5* in the developing retina.

In the human genome, there are 9 opsin genes, as shown in Table 1. It is known that color blindness and retinal degeneration diseases are caused by mutations/variations of classic visual opsin genes, while direct evidence showing the involvement of atypical opsin genes in retinal diseases has not been uncovered. It is noteworthy that a missense variant of the melanopsin gene is found in seasonal affective disorder [68]. Based on the phylogenetic analysis of the opsin family, Rrh (retinal pigment epithelium derived rhodopsin; peropsin) and Rgr (retinal G-protein-coupled receptor), are likely photoisomerases that convert a chromophore, all-trans-retinal, to 11-*cis* retinal without activation of the G-protein after photo-absorption [23]. On the other hand, it was not known whether or not opsin 5 was a photoisomerase or a GPCR, the latter of which would have the ability to activate the G-protein after photoabsorption, or there was any information about the maximum wavelength of photoabsorption by opsin 5. Opsin 5 was originally identified by genome mining as a novel GPCR-like molecule that belongs to the alpha group of the rhodopsin family [69–71]. We found that there are at least three *opsin 5* genes in the chick genome and obtained the cDNAs with full coding sequences. The three *opsin 5* genes are designated as *Opn5m* (a mammalian-type *opsin 5*), *Opn5L1* (*opsin 5* like 1), and *Opn5L2* (*opsin 5* like 2) [72]. We found that mammalian species have only one *opsin 5* gene, while nonmammalian vertebrates such as birds, frogs, fish, and reptiles have more than three *opsin 5* genes. This tendency is also seen for other *opsin* genes—that is, there are gene losses in the lineage leading to eutherian mammals and nonmammalian genes such as those of the chicken without eutherian orthologs [73].

Furthermore, the platypus, one of the few living species of monotremes, the only mammals that lay eggs, has two *opsin 5* genes, one of which corresponds to the fourth *opsin 5* clade in nonmammalian vertebrates [74]. After laborious experiments, Yamashita and colleagues determined that Opn5m is an ultraviolet (UV) sensor coupled with Gi-type G proteins [62]. Interestingly, another opsin 5, Opn5L2, has the ability to bind exogenous all-trans retinal with a similar photo-spectrometric property to Opn5m [75].

Table 1 The human Opsin family <http://www.ncbi.nlm.nih.gov/omim/>

Opsin Names	Gene Symbols	Gene Loci	Human Diseases	Absorption Maximum (nm)
Red Cone Opsin	***Opn1lw***	Xq28	Red Colorblindness, Blue Cone Monochromatism	**563**
Green Cone Opsin	***Opn1mw***	Xq28	Green Colorblindness, Blue Cone Monochromatism	**534**
Blue Cone Opsin	***Opn1sw***	7q32.1	Blue Colorblindness	**420**
Rhodopsin (Rod Opsin)	***Rho, Opn2***	3q22.1	Retinitis Pigmentosa 4 (Autosomal Dominant/ Recessive), Night Blindness (Congenital Stationary, Autosomal Dominant 1), Retinitis Punctata Albescens	**498**
Opsin 3 (Encephalopsin)	***Opn3***	1q43		**(~460nm)***
Opsin 4 (Melanopsin)	***Opn4***	10q22		**484**
Opsin 5	***Opn5***	6p21-p12		**360**
Retinal Pigment Epithelium Derived Rhodopsin (Peropsin)	***Rrh***	4q25		
Retinal G Protein Coupled Receptor	***Rgr***	10q23.1	Retinitis Pigmentosa 44	

*Data from pufferfish (*Takifugu rubripes*) Opn3 homolog TMT (PufTMT) and the mosquito (*Anopheles stephensi*) Opn3 homolog [82].

Thus, it seems that the opsin 5 family may occupy a pivotal place in the evolution of GPCRs, although this awaits further elucidation. The physiological functions of the *opsin 5* genes have not been fully illuminated, but it was shown that Opn5m is involved in seasonal growth of the testes in quails [61]. Notably, the Opn5m mRNA and protein are distinctly present in bipolar neurons of the paraventricular organ (PVO) of the hypothalamus as well as in the retina in birds (Fig. 2) [61, 62]. The PVO of the hypothalamus is known to be a photoreceptive organ and is only observed in nonmammalian species [76, 77]. There are morphologically distinct neurons in the PVO, having immunoreactivity against the light signal-transducing G-protein (transducin, Gt), and characterized as bipolar, cerebro-spinal fluid (CSF)-contacting neurons. Since whether or not a short UV wavelength can penetrate the skull and reach the deep brain is still controversial, the *opsin 5*-expressing CSF-contacting neurons might detect flow, ions or chemicals of the CSF, other than photons. Although the mammalian brain has no PVO in the hypothalamus except during development [78], our preliminary data show that *opsin 5* is expressed in the hypothalamus of mammals, suggesting its relationship to the neuroendocrine system.

Opn5m and Opn5L2 proteins are localized in different subsets of retinal ganglion cells and inner nuclear layer cells of the chick (Fig. 2A, B) [62, 75]. Another family of non-canonical opsins, the opsin 3-related proteins, are also present in subsets of retinal neurons (our unpublished data). A recent paper [79] showed that a chick melanopsin, Opn4x protein, is localized in the axon-less candelabrum retinal horizontal cells (HCs), which are known to be an Lhx1-negative, Islet1-positive, TrkA-positive HC subset. Importantly, the appearance of atypical opsin expression in subsets of retinal cells suggests the presence of novel types of photosensitive cells, at least in birds, and clearly shows further subtypes within the classical retinal cell types. Considering all these results together, there are clearly more than 2 types of photoreceptors (rhabdomeric and ciliary) in the vertebrate retina with respect to opsin expression.

Perspectives

Here I briefly reviewed evolutional and developmental aspects of the eye, a visual organ, mainly focusing on vertebrate retina and emphasizing retinal cell diversity labeled by expression of non-canonical photopigments, opsins, which also suggests novel functions of opsin-expressing cells in the retina, brain and other organs. From another point of view, the retinal cells can be thought of as a model for studying the mechanisms underlying cellular differentiation and diversification. The number of cell types in the human body is said to be 256 or more [80]. Of course, this number would be much higher if all the cell subtypes were included. Human diseases are thought to be

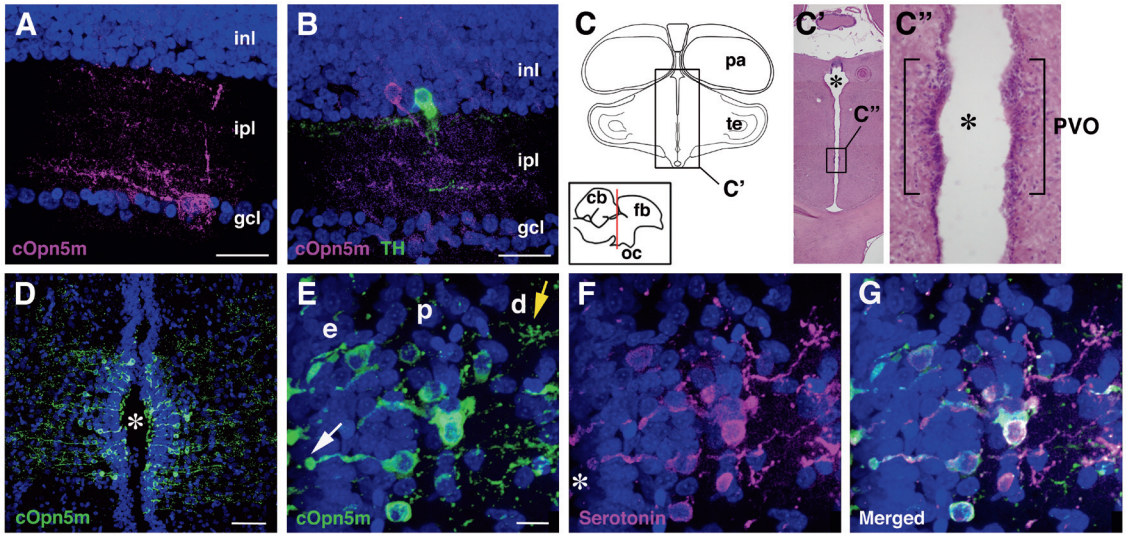

Fig. 2 A mammalian type opsin 5, Opn5m, is localized in the retina and hypothalamus [62]. **A**, A cOpn5m-immunoreactive (IR) cell (magenta) in the ganglion cell layer of the retina at posthatching day 14 (P14); **B**, A cOpn5m-IR cell (magenta) in the vicinity of tyrosine hydroxylase (TH)-IR cells (green); **C**, Schematic diagram of the chick brain showing a coronal plane of the posterior hypothalamus; (Inset) sagittal plane; **C'**, Histology of the chicken (P11) hypothalamus shown in (**C**); **C"**, High magnification of the boxed area in (**C'**), showing the paraventricular organ (PVO) (brackets). The PVO is composed of a columnar ependyma and of CSF-contacting neurons [76]; **D**, cOpn5m-IR cells (green) were predominantly present in the chicken PVO. The nuclei of the cells were stained with DAPI (blue); **E**, High magnification of cOpn5m-IR cells showing their bipolarity, with their club-like projections (white arrow) lining the third ventricle. These cells were present in the ependymal layer (e) and proximal part (p) of the PVO. In the distal (d) part of the PVO, a dendrite of the cOpn5m/serotonin double-positive cell was observed (yellow arrow); **F**, Serotonin-IR cells; **G**, Merged view of (**E**) and (**F**). Most of the Opn5m-IR cells in the PVO overlap serotonin-IR cells. The asterisks in **C'**, **C"**, and **D**, **F** represent the third ventricle. Abbreviations: cb, cerebellum; fb, forebrain; gcl, ganglion cell layer; ipl, inner plexiform layer; inl, inner nuclear layer; oc, optic chiasm; pa, pallium; te, tectum. Scale bars: 25μm (**A**, **B**); 50μm (**D**); 10μm (**E-G**).

attributable to aberrations of various types of cells due to gene mutations and alterations in gene expression. Understanding the precise mechanisms of cellular diversification and unraveling the molecular profiling of cellular subtypes will pave the way to regenerative medicine and gene therapy for deceased cells, including therapies using cancer stem cells, via the recently developed targeted genome editing technique [81].

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