

Evolution of Visual Pigments and Related Molecules

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In photoreceptor cells, light activates visual pigments consisting of a chromophore (retinal) and a protein moiety (opsin). Activated visual pigments trigger an enzymatic cascade, called phototransduction cascade, in which more than ten phototransduction proteins are participating. Two types of vertebrate photoreceptor cells, rods and cones, play roles in twilight and daylight vision, respectively. Cones are further classified into several subtypes based on their morphology and spectral sensitivity. Though the diversities of vertebrate photoreceptor cells are crucial for color discrimination and detection of light over a wider range of intensities, the molecular mechanism to characterize the photoreceptor types remains unclear. We investigated the amino acid sequences of about 50 vertebrate opsins, and found that these sequences can be classified into five fundamental subfamilies. Clear relationships were found between these subfamilies and their characteristic spectral sensitivities. In addition to opsins, we studied other phototransduction proteins. The amino acid sequences of phototransduction proteins can be classified into a few subfamilies. Even though their spectral sensitivity is considerably different, cones fundamentally share the phototransduction protein isoforms which are different from those found in rods. It is suggested that the difference in phototransduction proteins between rods and cones is responsible for their sensitivity to light. Isoforms and their selective expression may characterize individual photoreceptor cells, thus providing us with physiological functions such as color vision and daylight/twilight visions.

Key words: Phototransduction; Molecular evolution; Visual system; Opsin; Photoreceptor cells

INTRODUCTION

To receive light under a variety of photic environments, vertebrates have evolved photoreceptors having distinct characters. Two kinds of variability are generally found among vertebrate photoreceptors. One is the wavelength of light sensitivity of each photoreceptor, that is the spectral sensitivity. Many vertebrates have multiple photoreceptors of different spectral sensitivity, and utilize them for the discrimination of colors of incident light.

The spectral sensitivity of a photoreceptor is mainly due to the absorption spectrum of its visual pigment, consisting of the chromophore (11-cis retinal, retinal₁) and protein moiety (opsin). Some vertebrate pigments naturally have 3-dehydro-retinal (retinal₂) instead of retinal₁ as the chromophore. The absorption maximum of retinal₁-based pigment can be estimated from that of retinal₂-based pigment [1]. In this manuscript, we focus on the interaction between opsin and chromophore, and therefore consider only retinal₁-based pigments. Even if only retinal₁-based pigments are being taken into consideration, the absorption

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maxima of vertebrate visual pigments are widely distributed from 350 to 570 nm. This diversity is likely caused by the difference in the chromophore-opsin interaction, and can be understood by a detailed analysis of opsin sequences [2].

The other diversity is the diversity in the range of light intensities detectable by each photoreceptor, that is the sensitivity to light. Many vertebrates have duplicate photoreceptor types (rods and cones) responsible for twilight and daylight vision, respectively. Cones are less sensitive than rods. The light response of cones is faster and is terminated more rapidly than that of rods, and more pronounced adaptation than rods [3].

Photons captured by visual pigments trigger an enzymatic cascade, phototransduction cascade, resulting in closure of a fraction of the cGMP-gated channels in the cell membrane. The phototransduction cascades in cones, are less well understood. However, cones possess similar phototransduction proteins, suggesting that the signal transduction pathway of vertebrate photoreceptors is essentially identical.

To understand the diversity and evolution of vertebrate photoreceptor cells, we investigated the primary structures of opsins and other phototransduction proteins. Then, the molecular evolution of each vertebrate phototransduction protein was analyzed and compared. These studies led us to speculate that isoforms and their selective expression may generate the diversities of individual photoreceptors.

MATERIALS AND METHODS

Genomic DNAs and retinal mRNAs were isolated from various vertebrates. Retinal cDNAs were prepared according to an ordinary method and cDNA libraries were constructed as described previously [4]. Phylogenetically related sequences were obtained by BLAST searches in the databases. Molecular phylogenetic analyses were carried out by the NJ-method [5] from the amino acid sequences of opsins and other phototransduction proteins. *In situ* hybridization and immunohistochemistry were carried out as described previously [4,6].

RESULTS AND DISCUSSION

We prepared genomic DNAs and retinal mRNAs from more than 20 vertebrates, for example a diurnal gecko (*Phelsuma madagascariensis longinsulae*), bullfrog (*Rana catesbeiana*), medaka (*Oryzias latipes*) and lamprey (*Lampetra japonica*). About 50 opsin genes were isolated by polymerase chain reactions and library screenings. Phylogenetic analysis suggests that vertebrate opsin sequences fall into five fundamental subfamilies, designated as RH1, RH2, LWS, SWS1 and SWS2. Except for some special cases [6,7], RH1 opsins are expressed in rods, whereas opsins belonging to other subfamilies are generally expressed in cones.

Though the absorption maxima of vertebrate retinal-based pigments spreads out from 350 to 570 nm, the distribution of absorption maxima of the pigments belonging to the same subfamily is within a hundred nanometer. The clear relationship between the spectral sensitivity and these five subfamilies suggests that the presence of five fundamental opsin subfamilies is essential for the uptake of a wider range of wavelength of light, thus providing the vertebrates with the ability to discriminate color.

To investigate the existence and distribution of subfamilies in other phototransduction proteins, we searched retinal cDNAs encoding phototransduction proteins, such as opsin kinase, arrestin, guanylate cyclase (GC), GC-activating protein (GCAP), and phosducin. Each of these vertebrate phototransduction proteins forms a single cluster, even if other phylogenetically related sequences of non-visual proteins or invertebrate phototransduction proteins are taken into consideration. Sequences of these proteins can fundamentally be classified into a few subfamilies.

Then, expression of each protein isoform is investigated by *in situ* hybridization and immunohistochemistry. Our results suggest that except for opsins, cones generally share the phototransduction protein isoforms which are different from those found in rods. The spectral sensitivity of cones is likely determined only by the switching of opsin expression. Detailed analyses on phototransduction

proteins revealed the difference in molecular properties of these isoforms that may influence their physiological responses between rods and cones [8,9].

These subfamilies have apparently duplicated before the teleost-tetrapod divergence. The similarity of the dendrograms among the phototransduction proteins suggests that these subfamilies have arisen by a large gene duplication including whole phototransduction genes and/or by co-evolution of phototransduction proteins as a system. Isoforms and their selective expression may characterize individual photoreceptors, thus providing us with physiological functions such as color vision and daylight/twilight visions.

9. Matsuda, S., O. Hisatomi and F. Tokunaga (1999) Role of carboxyl-terminal charges on S-modulin membrane affinity and inhibition of rhodopsin phosphorylation. *Biochemistry* 38, 1310-1315.

REFERENCES

1. Hárosi, F. I. (1994) An analysis of two spectral properties of vertebrate visual pigments. *Vision Res.* 34, 1359-1367.
2. Yokoyama, S. (1995) Amino acid changes and wavelength absorption of visual pigments in vertebrates. *Mol. Biol. Evol.* 12, 53-61.
3. Ebrey, T. and Y. Koutalos (2001) Vertebrate photoreceptors. *Prog. Retin. Eye Res.* 20, 49-94.
4. Hisatomi, O., T. Satoh and F. Tokunaga (1997) The primary structure and distribution of killifish visual pigments. *Vision Res.* 37, 3089-3096.
5. Saitou, L. and M. Nei (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406-425.
6. Hisatomi, O., Y. Takahashi, Y. Taniguchi, Y. Tsukahara and F. Tokunaga (1999) Primary structure of a visual pigment in bullfrog green rods. *FEBS Lett.* 447, 44-48.
7. Taniguchi, Y., O. Hisatomi, M. Yoshida and F. Tokunaga (2001) Pinopsin expressed in retinal photoreceptors of a diurnal gecko. *FEBS Lett.* 496, 69-74.
8. Imai, H., D. Kojima, T. Oura, S. Tachibanaki, A. Terakita, Y. Shichida, (1997) Single amino acid residue as a functional determinant of rod and cone visual pigments. *Proc. Natl. Acad. Sci. USA* 94, 2322-2326.