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Unique transducins expressed in long and short photoreceptors of lamprey *Petromyzon marinus* ☆,☆☆

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ABSTRACT

Lampreys represent the most primitive vertebrate class of jawless fish and serve as an evolutionary model of the vertebrate visual system. Transducin-α ($G\alpha_t$) subunits were investigated in lamprey *Petromyzon marinus* in order to understand the molecular origins of rod and cone photoreceptor G proteins. Two $G\alpha_t$ subunits, $G\alpha_{tL}$ and $G\alpha_{tS}$, were identified in the *P. marinus* retina. $G\alpha_{tL}$ is equally distant from cone and rod G proteins and is expressed in the lamprey's long photoreceptors. The short photoreceptor $G\alpha_{tS}$ is a rod-like transducin-α that retains several unique features of cone transducins. Thus, the duplication of the ancestral transducin gene giving rise to rod transducins has already occurred in the last common ancestor of the jawed and jawless vertebrates.

1. Introduction

Vertebrate vision is based on two physiologically different photoreceptor cell types: rods and cones. The phototransduction cascades in rods and cones are principally similar. In rods, photoexcited rhodopsin (R^*) stimulates GTP–GDP exchange on the rod G protein, transducin (Gt_1). Activated transducin-α molecules, $G\alpha_{t1}$GTP, dissociate from the $G\beta_1\gamma_1$-subunits and R^*, and stimulate the effector enzyme, cGMP phosphodiesterase (PDE6)[1], by displacing the inhibitory γ-subunits (Pγ) from the PDE6 catalytic core. cGMP hydrolysis by activated PDE6 leads to a closure of cGMP-gated channels in the plasma membrane and generates an electrical signal (Burns & Arshavsky, 2005; Fu & Yau, 2007; Lamb & Pugh, 2006). The turn-off and recovery of the visual signal are achieved by reactions inactivating R^* and the $G\alpha_{t1}$GTP/PDE6 complex, and restoration of cGMP levels through Ca^{2+}-dependent activation of retinal guanylate cyclases. A photoreceptor-specific member of the RGS (regulators of *G* protein *s*ignaling) family, RGS9-1, in the complex with $G\beta_{5L}$ and the anchoring protein R9AP acts as a GTPase-activating protein for $G\alpha_{t1}$ catalyzing a rate limiting step in rod recovery (Krispel et al., 2006). Cone signaling proteins are highly homologous to their rod counterparts. Yet, the physiology of rods and cones is remarkably distinct. Rods are exceptionally sensitive to light and provide for nighttime (scotopic) vision, whereas cones are markedly less sensitive and signal during daytime (photopic receptors). Cone electrical responses to light are smaller in amplitude with much faster kinetics than rod responses. Furthermore, cones adapt to a much broader range of illumination conditions than rods and can function in intensely bright light (Burns & Arshavsky, 2005; Burns & Baylor, 2001; Fu & Yau, 2007; Lamb et al., 2006) A novel distinction between rods and cones has been recently recognized. In rods, but not in cones, transducin translocates in response to light from the outer segment (OS) to the inner segment (IS) and other photoreceptor compartments in what is believed to be an adaptive and/or protective mechanism (Calvert, Strissel, Schiesser, Pugh, & Arshavsky, 2006; Coleman & Semple-Rowland, 2005; Kennedy, Dunn, & Hurley, 2004; Rosenzweig et al., 2007).

However narrow, sequence differences in rod and cone transduction components appear to be well conserved in vertebrate evolution, and thus may underlie differences in the physiology of the two types of photoreceptors. With few exceptions, links between structural variations in rod and cone proteins and specific aspects of photoreceptor signaling are unknown. A high rate of spontaneous thermal isomerization, as well as dissociation of 11-cis retinal without isomerization in cone pigments, activate signal transduction in the dark and contribute to cone desensitization

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[1] *Abbreviations used:* $G\alpha_t$, transducin α subunit; R, rhodopsin; PDE6, cone or rod outer segment GMP phosphodiesterases; Pγ, inhibitory subunit of PDE6; OS/IS, outer segment(s)/inner segment(s) of photoreceptor cells; LP(s) and SP(s), long and short photoreceptors of sea lamprey *Petromyzon marinus*; LWS, long-wavelength sensitive.

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(Kefalov et al., 2005; Kefalov, Fu, Marsh-Armstrong, & Yau, 2003). Nonetheless, human rhodopsin and red cone pigment expressed in *Xenopus* cones and rods, respectively, produced responses identical to native responses of *Xenopus* photoreceptors, suggesting that the rod and cone pigment signaling is not different (Kefalov et al., 2003). Cones have been shown to express much higher levels of the RGS9 GAP complex than rods, leading to a hypothesis that RGS9-1 abundance controls rapid response kinetics in cones (Zhang, Wensel, & Kraft, 2003). Overexpression of the GAP complex in mouse rods accelerated the recovery kinetics, but the activation phase and the sensitivity of flash responses were unchanged (Krispel et al., 2006). Thus, high GAP complex concentrations most likely contribute to the faster recovery in cones compared to rods, whereas additional mechanisms are required to explain the outstanding key differences.

Certain lower vertebrate species with uniquely evolved photoreceptor cells provide an opportunity to pinpoint potential significance of specific sequence variations between rod and cone components. One such example is Tokay gecko photoreceptors. These photoreceptors are rods in terms of their morphology and physiology, but utilize cone-like components, including pigments, $G\alpha_t$, PDE6, arrestin, and cGMP-gated channel subunits (Zhang, Wensel, & Yuan, 2006). Therefore, critical sequences might be confined to a limited number of rod-only specific residues conserved in cone-like phototransduction molecules of the Tokay gecko (Zhang et al., 2006). As representatives of the earliest known vertebrate class of jawless fish, lampreys constitute a unique model to study the evolution of the vertebrate visual systems (Lamb, Collin, & Pugh, 2007; Walls, 1942). Two morphologically distinct types of photoreceptor cells, short (SPs) and long photoreceptors (LPs), are described in the retina of sea lamprey *Petromyzon marinus* (Dickson & Graves, 1979). Classification of SPs and LPs as cones or rods had long been debated (Dickson & Graves, 1979; Govardovskii & Lychakov, 1984; Ishikawa et al., 1987; Ohman, 1976). The controversy has not been clarified with the identification of the *P. marinus* rhodopsin gene apparently expressed in SPs (Zhang & Yokoyama, 1997). This pigment was initially categorized as an Rh1 opsin, indicative of rod function (Zhang & Yokoyama, 1997). A competing viewpoint emerged later suggesting that the lamprey's Rh-like opsin gene diverged from an ancestral Rh-gene prior to its duplication into the Rh1 and Rh2 lineages (Collin et al., 2003; Collin & Trezise, 2004). Even so, by most morphological and electrophysiological criteria LPs are cones, whereas SPs are mixed cone/rod photoreceptors or unusual rods that operate under scotopic and photopic conditions (Govardovskii & Lychakov, 1984). We recently demonstrated expression of a single type of PDE6 catalytic subunit in *P. marinus* with nearly equivalent relations to cone and rod PDE6s (Muradov, Boyd, Kerov, & Artemyev, 2007). The PDE6 holoenzyme incorporates a cone-type Pγ-subunit in LPs and a distinct mixed cone/rod-type Pγ-subunit in SPs (Muradov et al., 2007). These findings indicated that lampreys represent an interesting model of evolution of cone and rod phototransduction components. Here, we investigated transducins in *P. marinus* and examined the identity of the visual pigment expressed in LPs.

2. Materials and methods

2.1. Materials

All restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Ipswich, MA). AmpliTaq DNA polymerase was a product of Applied Biosystems (Foster City, CA), and cloned *Pfu* DNA polymerase was a product of Stratagene (La Jolla, CA). TRI Reagent and oligo(dT) column were purchased from Molecular Research Center (Cincinnati, OH). All other reagents were purchased from Sigma–Aldrich (St. Louis, MO). Oligonucleotides were synthesized by IDT, Inc. (Coralville, IA).

2.2. Cloning of the lamprey's transducin-α subunits and red-sensitive pigment

Procedures for isolation of total RNA and mRNA from *P. marinus* retina, preparation of cDNA, and generation of the phage cDNA library were described previously (Muradov et al., 2007). Lamprey retina cDNA was PCR amplified with *Pfu* DNA polymerase using a forward primer CAGATCCGGGCGGTGTCGGCGACCTCGGCGAAG corresponding to the $G\alpha_t$ 5′ untranslated region from GENSCAN00000139905 (contig5512, *P. marinus* genomic database, PreEnsemble release) and a reverse primer CTAGAAGAGGCC GCAGTCCTTGAGGTTTTC corresponding to GENSCAN00000017638 from contig11495. Sequencing of the PCR product yielded the full-length sequence of $G\alpha_{tS}$. A partial sequence of $G\alpha_{tL}$ (aa 110–354) was established following PCR amplification of cDNA with *Pfu* DNA polymerase using a forward primer CTGGCCGACTCACTG GAGGAGGGATCCATGCCC and a reverse primer GCGTTTTGAAAT GAACCGTGTCTTCC corresponding to the database sequences (GENSCAN00000064894, contig4334). The missing N-terminal sequence of $G\alpha_{tL}$ was obtained by 5′RACE analysis using Clontech SMART™ RACE cDNA Amplification Kit and a reverse primer ggtgtccttccacaggcggatgatgatggcgg corresponding to $G\alpha_{tL}$ aa sequence 124–134. The sequence of $G\alpha_{tL}$ was confirmed by sequencing of the full-length PCR product amplified from *P. marinus* cDNA with *Pfu* DNA polymerase.

A partial sequence of the *P. marinus* red-sensitive pigment (aa 76–333) was obtained using PCR and two primers designed utilizing river lamprey *Lethenteron japonicum* mRNA sequence (GI:46917272). The opsin C-terminal sequence was identified through PCR amplification of the bacteriophage λ *P. marinus* cDNA library (Muradov et al., 2007) using a 5′primer specific to the lamprey opsin and a 3′primer specific to the λSCREEN-1 vector. The opsin N-terminal sequence was obtained by 5′RACE analysis using a Clontech SMART™ RACE cDNA Amplification Kit and an opsin-specific reverse primer. The sequence of the red-sensitive opsin was confirmed by sequencing of the full-length PCR product amplified from *P. marinus* cDNA with *Pfu* DNA polymerase.

2.3. Phylogenetic analysis

The rooted phylogenetic trees of transducin-α subunits and the long-wavelength sensitive (LWS) opsins were obtained using multiple alignments of the full-length protein sequences with (1) Clustal X 2.0 and the Gonnet matrix (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997), or (2) with a PHYLIP-NEIGHBOR program in a Neighbor-Joining format and the Jones–Taylor–Thornton matrix through the web-based Max-Planck Institute's Bioinformatics Toolkit. Human Gα-subunits ($G\alpha_{i1-3}$, $G\alpha_o$, $G\alpha_s$, $G\alpha_q$) and invertebrate opsins were used as the outgroups for the $G\alpha_t$-tree and the LWS tree, respectively. The clustering probabilities were generated by bootstrap resampling: 1000 replicates using Clustal X 2.0 and 200 replicates using PHYLIP-NEIGHBOR. The two procedures yielded similar phylogenies and clustering probabilities. The trees were plotted using NJPlot (v.2.1) (Perrière & Gouy, 1996).

2.4. Expression of the lamprey transducin-α subunits in E. coli

The DNA sequences for the lamprey's $G\alpha_t$-subunits were amplified using lamprey retina cDNA as the template and subcloned into the modified pET15b vector using the XhoI and SpeI