A Novel Gα-mediated Phototransduction Cascade in Scallop Visual Cells*

(Received for publication, May 28, 1997, and in revised form, July 12, 1997)

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Scallop retinas contain ciliary photoreceptor cells that respond to light by hyperpolarization like vertebrate rods and cones, but the response is generated by a different phototransduction cascade from those of rods and cones. To elucidate the cascade, we investigated a visual pigment and a G-protein functioning in the hyperpolarizing cell. Sequencing of cDNAs and in situ hybridization experiments showed that the hyperpolarizing cells express a novel subtype of visual pigment, which showed significant differences in amino acid sequence from other visual pigments. Cloning cDNA genes of G-protein and immunohistochemical analysis revealed the presence of an alpha subunit of a Gα type G-protein, 83% identical in amino acid sequence to mammalian Gαs, in the nervous system, in the photoreceptive region of the cells. The results demonstrate that a novel, Gα-mediated, phototransduction cascade is present in the hyperpolarizing cells. The phototransduction cascade in the scallop hyperpolarizing cell provides an alternative system to investigate Gα-mediated transduction pathways in the nervous system. Molecular phylogenetic analysis strongly suggests that the Gα-mediated phototransduction system emerged before the divergence of animals into vertebrate and invertebrate in the course of evolution.

In the photoreceptor cells of animals’ eyes, visual pigments trigger a G-protein-mediated phototransduction cascade, which eventually generates an electrical response of the cells.

Two kinds of the phototransduction systems have been reported. One is the Gq1-mediated system of vertebrate hyperpolarizing photoreceptor cells in which the visual pigment activates a cGMP-specific phosphodiesterase via a heterotrimeric G-protein, transducin (Gt) (1–3). The other is the Gα-mediated system of invertebrate depolarizing cells, such as cephalopod’s and arthropod’s, where phospholipase C is activated via a Gq-type G-protein (4–7). The visual pigments of these two systems show sequence homology, but clearly split into two subtypes (Gα and Gα2-coupled ones) in a molecular phylogenetic tree (8).

In addition to the depolarizing rhodopsinergic photoreceptor cells present in invertebrates, scallop retinas contain ciliary photoreceptor cells that hyperpolarize in response to light (9). After the first electrophysiological recordings of Hartline (10), the mechanism of the hyperpolarizing response as well as its evolution have been discussed in comparison with vertebrate hyperpolarizing ciliary photoreceptor cells, rods and cones (11–14). It has been reported, however, that the hyperpolarizing response in the scallop cell is due to opening of a cGMP-sensitive potassium channel (11–14), which is different from that of the vertebrate cells (closing of a cGMP-sensitive cationic channel) (15). Our immunohistochemical experiments showed that an antibody to frog Gt did not cross-react to the scallop hyperpolarizing cell. Therefore we expected the presence of an unknown G-protein-mediated phototransduction cascade other than a Gα-mediated one in the scallop hyperpolarizing cells, while the depolarizing photoreceptor cells contain a Gq-mediated cascade. Here, we show evidence that the phototransduction system in the invertebrate hyperpolarizing photoreceptor cells is not mediated by Gα or Gαq, but rather uses a novel, Gα-mediated cascade.

EXPERIMENTAL PROCEDURES

Visual Pigment cDNA of Scallop Eyes—Two gene fragments encoding a partial peptide of visual pigment homologues (SCOP1 and SCOP2) were obtained by PCR of scallop (Patinopecten yessoensis) genomic DNA with degenerated oligonucleotide primers: 5′-TTTHTHTHGTHRC-ICITAYRC-3′ and 5′-AYISCRTAIAYDIIGRRTT-3′, where “I” stands for inosine, “W” for A/G, “H” for T/A, “D” for T/G/A, “V” for T/C, and “S” for G/C. The primers were designed on the basis of comparison of the known amino acid sequences of visual pigments. As for each of the two genes, 5′- and 3′-rapid amplification of cDNA ends with the gene-specific oligonucleotide primers were accomplished with RNA prepared from scallop mantle eyes, to obtain the whole sequence of the protein cDNA. To confirm that the sequence thus obtained comes from a single species of mRNA, scallop eye cDNA was subjected to PCR to amplify the entire coding region of the proteins (1497 base pair (bp) for SCOP1 and 1197 bp for SCOP2). The cDNA sequence was confirmed at least twice for each gene.

Gαm cDNA of Scallop Eyes—To obtain cDNA fragments encoding a region containing “helical domain” of Gαm, where the primary sequence is characteristic of each Gα subtype, PCR of scallop eye cDNA was carried out with a set of the degenerated oligonucleotide primers: 5′-GGIAAR-WSIACWHTHTIAAARCARTG-3′ and 5′-AARCMTIDGDACCCAYTT-3′, where “W” stands for T/A. The primers were designed on the basis of comparison of the known amino acid sequences of Gαm. The entire coding region for either Gαm (1058 bp) or Gαm (1071 bp), which localized in the photoreceptor cells (see “Results and Discussion”), was sequenced as described above for the visual pigment genes.

* The abbreviations used are: Gt, transducin; Gαm, α-subunit of heterotrimeric G-protein; Gα, α-type G-protein; Gαm2, α2-subunit of Gαm Gα, type G-protein; Gαm, α-subunit of Gαm; PCR, polymerase chain reaction; bp, base pair; SCOP1, Scallop opsin 1; SCOP2, Scallop opsin 2.
** A. Terakita and H. Ohtsuki, unpublished observation.
The amino acid sequence of a scallop visual pigment, SCOP2. The sequence is aligned along a heptahelical model (lower panel), which includes seven transmembrane regions (helicles I–VII). The SCOP2 sequence of loop domain between helices V and VI (loop V–VI) is aligned with those of vertebrate and invertebrate groups of visual pigments, with the aid of the Clustal W program (16) (upper panel). Among each of the groups, the amino acid residues conserved (A = G = P = S = T, I = L = M = V, D = E = N = Q, H = K = R, F = W = Y) among more than half of the members are shown with white characters with black background. Note that the amino acid sequences in this domain are similar to each other in each of the Gq and Go-coupled groups and quite different in SCOP2.

In situ Hybridization—The ~0.2-kilobase pair DNA fragments were subcloned from the cDNA of SCOP2, Gq, and Go, using PCR. Digoxigenin-labeled antisense RNA probes were synthesized from these subclones, using the Dig RNA labeling kit (Boehringer Mannheim). The RNA probe for SCOP2 was a mixture of antisense RNAs from six clones, using the Dig RNA labeling kit (Boehringer Mannheim). The genin-labeled antisense RNA probes were synthesized from these subclones, using the Dig RNA labeling kit (Boehringer Mannheim). The genin-labeled antisense RNA probes were synthesized from these subclones, using the Dig RNA labeling kit (Boehringer Mannheim).

Molecular Phylogenetic Tree Construction—The multiple alignment of amino acid sequences of visual pigments was done with the aid of the Clustal W 1.4 program (16). Sequences were obtained from GenBank™ and PIR data bases. The aligned sequences (235 amino acid residues for each sequence) included all the residues except for the N- and C-terminal fragments and some of the loop domains (loops IV–V, V–VI, and VI–VII as shown in Fig. 1). A tentative phylogenetic tree for the alignment was calculated with the Neighbor-Joining method by use of the PHYLIP 3.572 software package (17). The tree topology thus obtained was subjected to Maximum Likelihood analysis (ProtML) using the MOLPHY 2.3 software package (18) with the local rearrangement option and with the JTT-F option of amino acid substitution model. The bootstrap probabilities (percent) of local topologies were also estimated for the final tree with the maximum likelihood.

RESULTS AND DISCUSSION
Since phylogenetic analysis of visual pigments can roughly suggest the specific subclass of G-protein to which they couple (8), we examined into which subtypes the scallop visual pigments are classified. From the scallop eyes’ cDNA, two kinds of cDNAs of visual pigments were obtained by several steps of PCR, and tentatively designated as SCOP1 and SCOP2. The amino acid sequence of SCOP1 was highly similar to those of squid and octopus Gq-coupled rhodopsins (hereafter referred to as Gq-rhodopsins). Since an antibody against the squid Gq-rhodopsin cross-reacted to the scallop depolarizing photoreceptor cells but not to the hyperpolarizing cells (data not shown),
SCOP1 could be the cDNA of G_q-rhodopsin in the depolarizing cells. On the other hand, the amino acid sequence of SCOP2 (Fig. 1) did not show significant similarities to those of any other visual pigments, although SCOP2 had the functional amino acid residues conserved among all the known visual pigments. The molecular phylogenetic tree (Fig. 2) clearly indicated that SCOP2 was not a member of the G_t- nor G_q-coupled group of visual pigments. Furthermore, in the loop region between helices V and VI (Fig. 1), which is one of the G-protein interaction sites in bovine rhodopsin (19), SCOP2 was quite different in amino acid sequence from either G_t- or G_q-coupled visual pigments. These data strongly suggest that SCOP2 is a new subtype of visual pigment that couples with a G-protein other than G_t and G_q.

To test this prediction, we next investigated which subtype of G-protein was colocalized with SCOP2 in the hyperpolarizing cells. The cDNA fragments of five kinds of G_a encoding their helical domains were obtained by PCR against the scallop eyes’ cDNA. On the basis of sequence similarities, four of them were classified as G_q, G_s, G_i, and G_o, respectively, while the other one was a new subtype (G?).

Table I

| Percentage of amino acid identity between scallop and other G_a | G_s | G_q | G_o | G_i | G_t |
|---------------------------------------------------------------|-----|-----|-----|-----|-----|
| Drosophila                                                   | 58  | 39  | 37  | 32  | 30  |
| G_s                                                          | 36  | 69  | 45  | 44  | 34  |
| G_q                                                          | 42  | 41  | 87  | 54  | 36  |
| G_o                                                          | 35  | 45  | 55  | 58  | 41  |
| G_i                                                          | 36  | 34  | 34  | 34  |     |
| Human                                                         |     |     |     |     |     |
| G_s                                                          | 52  | 30  | 33  | 37  | 29  |
| G_q                                                          | 40  | 66  | 44  | 46  | 36  |
| G_o                                                          | 40  | 46  | 80  | 60  | 39  |
| G_i                                                          | 39  | 45  | 61  | 63  | 39  |
| G_t                                                          | 36  | 44  | 53  | 53  | 41  |
| G_o                                                          | 35  | 38  | 50  | 50  | 37  |
| G_12                                                         | 30  | 37  | 36  | 36  | 31  |

a Five kinds of cDNA fragments of G_a encoding their helical domains were obtained by PCR against the scallop eye cDNA. Based on the deduced amino acid sequence identities, they were classified as G_q, G_s, G_i, and G_o, respectively, while the other one was a new subtype (G?).

To test this prediction, we next investigated which subtype of G-protein was colocalized with SCOP2 in the hyperpolarizing cells. The cDNA fragments of five kinds of G_a encoding their helical domains were obtained by PCR against the scallop eyes’ cDNA. Based on the deduced amino acid sequence identities, they were classified as G_q, G_s, G_i, and G_o, respectively, while the other one was a new subtype (G?). Then we prepared the specific antibody against each of the peptides encoded by these cDNA fragments. Among these antibodies, anti-G_q and anti-G_o antibodies strongly reacted with the scallop photoreceptor cells by immunohistochemical experiments (Fig. 3). The scallop retina has two layers of photoreceptor cells: the hyperpolarizing cell layer with its photoreceptive region adjacent to the lens and the depolarizing cell layer distant from the lens (20). As expected, anti-G_q antibody clearly stained the rhabdomeric depolarizing cells. Interestingly, anti-G_o antibody specifically stained the hyperpolarizing cells. The specificity of anti-G_q and G_o antibodies were confirmed by immunoblot analysis; they specifically reacted with 42- and 41-kDa peptides in ocular proteins, respectively (Fig. 4). The molecular weights were consistent with those calculated based on the whole amino acid sequences deduced from the cDNAs of the scallop G_q and G_o. The scallop G_q shows 83% identity in amino acid sequence to mammalian G_q, and 90% to Drosophila G_o, and they show complete identical sequences in C terminus region, which is characteristic of each G_a subtype.

To further confirm the colocalization of SCOP2 with the G_o subtype of G-protein, we performed in situ hybridization experiments (Fig. 5). The results show that SCOP2 coexpresses with G_o, but not with G_q, in the hyperpolarizing cells. It should be
noted that the Go is localized only in the outer segment (photoreceptive region) of the cells in the immunohistochemical experiments (Fig. 3). Taken together, these results indicate that SCOP2 triggers a Go-mediated phototransduction cascade in the hyperpolarizing cell. Therefore, we propose SCOP2 be named scallop Go-rhodopsin.

Our results indicated that the phototransduction system leading to the scalp hyperpolarizing response is different from that in the vertebrate hyperpolarizing cells (Go-mediated system). This indication is consistent with recent electrophysiological findings on their channels; the scallop hyperpolarizing cells respond with closing of the cationic channel resulting from decrease of cGMP (15). Thus, it is most likely that the scallop Go-mediated phototransduction cascade couples with an effector enzyme, probably a guanylyl cyclase, to elevate cytosolic cGMP concentration.

The scallop Go shows high similarity in amino acid sequence to mammalian Gα, which is localized mainly to brain and nervous cells (21, 22). The sequence similarities suggest that the Go phototransduction cascade is similar to a Gα cascade in the nervous system. However, little is known about a specific effector enzyme(s) directly coupling with Gα, although it has been reported that mammalian Gα, especially its βγ subunit, is involved in regulating voltage-sensitive calcium channels in the synaptic region of neuronal cells (23–26). The phototransduction in the scalp hyperpolarizing cells can be an alternative system to identify the Gα-coupled effector enzyme because of the highly specific expression of Gα with photoactivable receptor proteins (Go-rhodopsin) in the cells.

The molecular phylogenetic tree of visual pigments (Fig. 2) strongly suggests that Go-rhodopsin diverged from an ancestral visual pigment before the divergence of animals (700 million years ago) into higher invertebrates (deuterostomia) and vertebrates (protostomia). This was also supported by the fact that visual pigment-like proteins (RGR), recently found in mammals (27), clustered with Go-rhodopsin (at the point of the closed triangle in Fig. 2) with relatively high bootstrap probability (89%) when these extra members were added to the tree. The deepest root of the tree (closed circle in Fig. 2) corresponds to the generation of three different genes for visual pigments and not to the divergence of animal species. Thus the multiple phototransduction systems of vision may have emerged before the divergence of animals into vertebrates and invertebrates in the course of evolution. It is likely that for some time following this divergence, both vertebrates and invertebrates kept each of the multiple phototransduction systems. The Go-mediated phototransduction system might spread over a wide variety of animal species, since the mechanism of photoreponse in the scalp hyperpolarizing cells appears to be shared by some other species; the ciliary photoreceptor cells of a lizard “parietal eye” respond to light by increasing cytosolic cGMP and opening channels (28), suggesting the presence of a similar Go-mediated phototransduction system.

Acknowledgments—We thank Dr. T. Harioyama for help with preparing scallop eyes and Prof. T. Miyata and Dr. N. Iwabe for helpful discussions about phylogenetic analysis.

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FIG. 5. In situ hybridization against scallop retina. Both the Go (A) and SCOP2 (B) antisense RNA probes show positive signals only in the hyperpolarizing cell layer (h), while the Go (C) probe does in the depolarizing cell layer (d). L, lens. Scale bar = 100 μm.