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G-Proteins Expressed in the Ocellus of the Hydromedusan, *Spirocodon saltatrix*.

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We have cloned a hydromedusan opsin cDNA and showed that the deduced amino acid sequence of the cytoplasmic loop between helices 5 and 6 (loop 5-6) was clearly different from that reported so far. The amino acid sequence of the loop 5-6 is important on determination of the specificity for the coupled G-protein. To clarify which class of G-protein mediates the phototransduction system in the ocellus of the hydromedusan, we investigated G-proteins expressed in the ocellus. By PCR against the cDNA of the ocellus with primers designed according to the conserved amino acid sequence in G-protein α subunit, we obtained three kinds of cDNA fragments. Based on the sequence similarities, ttwo of them (JG1 and JG3) were classified as G_i and G_q, respectively. The other one (JG2) was a new subtype within G_i class. Electron microscopic immunocytochemistry with the antiserum against the C-terminal sequence of G_q or G_t revealed the presence of the both classes in the ocellus. The similarity of the C-terminal sequence of the JG2 with that of bovine G_t suggests that the anti-G_t antiserum would bind to JG2. These results suggest the possibility that the hydromedusan rhodopsin decides the specificity for the coupled G-protein by the other domain than the loop 5-6.

Key words: hydromedusan, phototransduction, G-protein, ocellus, rhodopsin, cDNA cloning, electron microscopic immunocytochemistry

INTRODUCTION

The phototransduction cascade in the animal photoreceptor cells is one of the most elucidated G-protein mediated signaling systems. In order to study the molecular evolution of phototransduction systems, we cloned jellyfish opsin cDNA expressed in the ocellus of hydromedusan, *Spirocodon saltatrix*. The deduced amino acid sequence was very characteristic. Especially that of the cytoplasmic loop between helices 5 and 6 (loop 5-6) was clearly different from that reported so far. The amino acid sequence of the loop 5-6 is important on determination of the specificity for the coupled G-protein [1] . To date, three kinds of phototransduction systems have been reported. They are different in the class of G-protein which couples with the photoactivated rhodopsin. One is the G_t-mediated system found in the

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vertebrate photoreceptor cells. The others are G_q-mediated system and G_o-mediated system found in the invertebrate visual cells [2] .

In this study, we investigated G-proteins expressed inthe ocellus to clarify which class of G-protein mediates the phototransduction system in the ocellus of the hydromedusan.

MATERIALS AND METHODS

Animals. Hydromedusae, *Spirocodon saltatrix*, were collected in the vicinity of Ushimado Marine Laboratory.

PCR amplification of Gα cDNA from the ocellus of hydromedusan. Two degenerated oligonucleotide primers were synthesized based on the conserved amino acid motifs of the α subunit of G-proteins [3] . The cDNA fragments of the α subunit of G-proteins were obtained by PCR of cDNA of the hydromedusan ocellus and sequenced as reported previously [3] . The 3'-terminal sequence was cloned by 3'-RACE method with the cDNA specific primer and ENT primer (5'-AACTGGAAGAATTCGCGGCCGCAGGAA(T)18-3').

Microscopy. The basal parts of several tentacles including the ocellus were dissected out and immediately fixed according to Karakisawa et al. [4] . For light-microscopical observation and *in situ* hybridization, the specimens were fixed, frozen and sectioned as described in [4] . The handling of the specimens for electron-microscopical observation and iummunocytochemistry was as described in [4] .

Electron microscopic immunocytochemistry. The antiserum against the C-terminal sequence of G_q or G_t (anti-G_q and anti-G_t) were kind gifts from Dr. T. Suzuki (Hyogo College of Medicine). Antibody binding was visualized with secondary goat-antirabbit antibodies conjugated to 10-nm gold particles.

In situ hybridization. Digoxigenin-labeled antisense RNA probes were synthesized from the cDNA fragments subcloned into pT7blue or pBluescript SK(+) plasmid, using the Dig RNA labeling kit (Roche). The procedure for the *in situ* hybridization and detection of digoxigenin-labeled probes were according to the supplier's protocol.

Molecular phylogenetic tree construction. The multiple alignment of amino acid sequences and the construction of the phylogenetic tee were done with the aid of the GENETYX-MAC ver 10.1 (Software Development). Sequences were obtained from GenBank™.

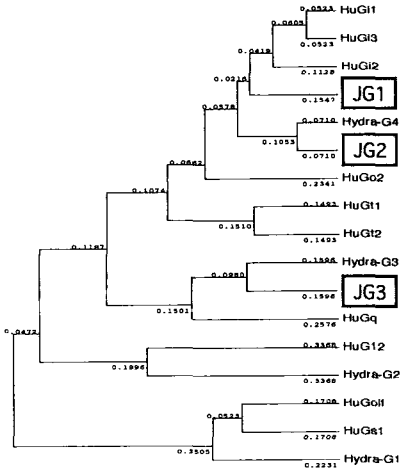

Figure 1. The molecular phylogenetic tree of G-protein α subunits. The tree was constructed on the basis of the deduced amino acid sequences of PCR fragments (JG1-3) and herical domains of human (Hu) and hydra (Hydra) with UPGMA methods of GENETYX MAC 10.1.

RESULTS AND DISCUSSION

Cloning of cDNA fragment encoding G-protein α subunit.

Three kinds of cDNA fragments encoding the G-protein α subunit were obtained by PCR against cDNA of the ocellus of the hydromedusan. On the basis of the sequence similarities, two of them (JG1 and JG3) were classified as G_i and G_q, respectively. The other one (JG2) was a new subtype within G_i class. As shown in the molecular phylogenetic tree (Fig. 1), JG2 was not clustered with the human $G\alpha_i$, but with hydra G4 and forms a new subclass.

Electron microscopic immunocytochemistry.

The anti-G_q and anti-G_t antiserums label membrane structures in the ocellar cavity. We observed the binding of anti-hydromedusan rhodopsin antibody to these membrane structures. A single sensory cilium projects into the ocellar cavity from the receptor cell and the cilium possess villi. The supporting cells also project microvilli in the ocellar cavity. The microvilli of supporting cell and villi originating ciliary shaft of the receptor cell could not be distinguished from each other. Thus, the results suggests the possibility of colocalization of both classes of G-protein with rhodopsin. Because we did not find G_t class of G-protein from cDNA of the ocellus, the sequence of antigenic peptide for anti-G_t

Table 1. The amino acid sequences of the loop 5-6.

Hydromedusan Rhodopsin	QKELRGMT-DRSKEIAGQESAITAATQK--
Gt-coupled Rh (Hum Rh.)	FTVKEAAA--QQQ-----ESA---TTQK--
Gq-coupled Rh (Squid Rh.)	MSVSNHEKEMAAMAKRLNAKELRKAQAGAN
Go-coupled Rh (SCOP2)	QEKVCKDS--RKNGIRAQQRYTPRFIQD--

antibody was compared with the corresponding part of JG2, The anti-G_t antibody was against the C-terminal peptide of bovine G_t. Only two amino acids out of 14 residues are different in the C-terminal sequence of JG2, it was likely that the anti-G_t antibody bound to the C-terminal of JG2.

In situ hybridization.

We formed digoxigenin-labeled antisense RNA probes for JG1 and JG2. Only the antisense probe for JG2 showed positive signals in the ocellus. No signals was observed in the ocellar cavity, indicating the presence of mRNA for JG2 in the cell body not in the villi. The absence of the positive signal for JG1 suggests a smaller amount of mRNA for JG1. The investigation with the antisense prove for JG3 is remained for the future studies.

The amino acid sequence of the cytoplasmic loop between helices 5 and 6 (loop 5-6) of the hydromedusan opsin was clearly different from that reported so far (Table 1). The amino acid sequence of the loop 5-6 is important on determination of the specificity for the coupled G-protein [1] . The above results suggest the possibility that the hydromedusan rhodopsin may couple with JG2 or JG3. Although JG3 belongs to G_q class, the loop 5-6 of the hydromedusan rhodopsin was different from that of G_q-coupled rhodopsin. The C-terminal sequence of JG2 was similar to that of bovine G_t, which is reported as the site of the G-protein-rhodopsin interaction. This suggests that the interaction with rhodopsin is similar in both bovine G_t and JG2. The amino acid sequence of loop 5-6 of the hydromedusan opsin, however, is different from that of G_t-coupled rhodopsin. These results suggest the possibility that the hydromedusan rhodopsin decides the specificity for the coupled G-protein by the other domain than the loop 5-6.

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