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G Protein α Subunit Genes in Octopus Photoreceptor Cells

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ABSTRACT—The G protein messages in *Octopus vulgaris* photoreceptor were isolated and characterized with molecular biological techniques. Four classes of G protein α subunit cDNA were isolated from an octopus eye cDNA library: OvGα_i, OvGα_o, OvGα_q and OvGα_s. Northern blot analysis of octopus tissues showed abundant expression of OvGα_q in the eye, and specific expression of OvGα_o in neural tissues. OvGα_i was expressed in all tissues studied. *In situ* hybridization revealed that OvGα_q message was expressed in almost all photoreceptor cells. Based on these results, a possible phototransduction pathway in the octopus visual cells is discussed.

INTRODUCTION

Phototransduction in the vertebrate photoreceptor cell is one of the most extensively studied G protein-coupled signal transduction systems: Photo-activated rhodopsin couples with a photoreceptor-specific G protein, transducin (G_t), leading to the activation of cGMP phosphodiesterase (PDE), which hydrolyzes cGMP in the cytoplasm. A reduction in the concentration of cGMP results in the closure of cGMP-gated channels and hyperpolarization of the photoreceptor cytoplasmic membrane (Stryer, 1986; Chabre and Deterre, 1989). Despite the established phototransduction pathway of vertebrate photoreceptor cells, there is no consensus about the molecular mechanism of phototransduction pathway(s) in invertebrates (Tsuda, 1987; Yarfitz and Hurley, 1994).

Whereas in vertebrate photoreceptors it has been accepted that there is only one class of G protein (transducin) involved, biochemical studies using invertebrate photoreceptors suggest the presence of several different types of G protein that can couple with light-activated rhodopsin. This has been demonstrated by ADP-ribosylation studies with bacterial toxins in squid (Vandenberg and Montal, 1984), octopus (Tsuda *et al*., 1986; Tsuda and Tsuda, 1990) and fly (Bentrop and Paulsen, 1986) photoreceptor membranes. There are two light-dependent ADP-ribosylated bands in octopus photoreceptor membranes: one catalyzed only by pertussis toxin (41KDa; G_{ip}), and the other by either pertussis or cholera toxin (34 KDa; G_{34}). Recently, light-activated G_q has been demonstrated in the photoreceptors of both *Drosophila* (Yarfitz and Hurley, 1994; Hardie and Minke, 1995; Zuker, 1996) and cephalopods (Pottinger *et al*., 1991; Ryba *et al*., 1993; Suzuki *et al*., 1993 and 1995; Kikkawa *et al*., 1996). Since the G_q α subunit is insensitive to bacterial toxins, these results suggest the existence of at least three kinds of G protein able to couple with photo-activated rhodopsin in invertebrate photoreceptors. The present study aims to clarify the presence of G proteins in octopus photoreceptor cells using molecular biological techniques.

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MATERIALS AND METHODS

Animals and cDNA library of an octopus hemisected eye

Living Japanese common octopuses (*Octopus vulgaris*; local common name 'madako') were purchased from a local fishery (Tokusui Shoji, Akashi, Japan). Total RNA was prepared from one hemisected eye by standard CsCl-ultracentrifugation methods (Maniatis *et al*., 1982). cDNA was synthesized from the total RNA with a random 9-mer as a primer with Moloney murine leukemia virus reverse transcriptase (Super Script RT, Life Technologies, Rockville, USA) and used as a template for polymerase chain reaction (PCR). A cDNA library of the octopus eye was then constructed with a λZap-II system (Stratagene, La Jolla, USA) and *in vitro*-packaged by Giga Pack Gold III (Stratagene, La Jolla, USA).

Polymerase chain reaction

Degenerated PCR primers were designed according to the conserved amino acid motifs of the α subunit of G protein. A forward primer corresponded to amino acid sequence KQM(K/R)IIH, and a reverse primer corresponded to amino acid sequence (K/R)KWI(H/Q)CF (F1 and R1, respectively; Fig. 1 and shaded boxes in Fig. 2). PCRs were performed with random-primed cDNA as a template, and the PCR products were then analyzed by agarose gel electrophoresis, recovered from the agarose gel (Band prep kit, Amersham

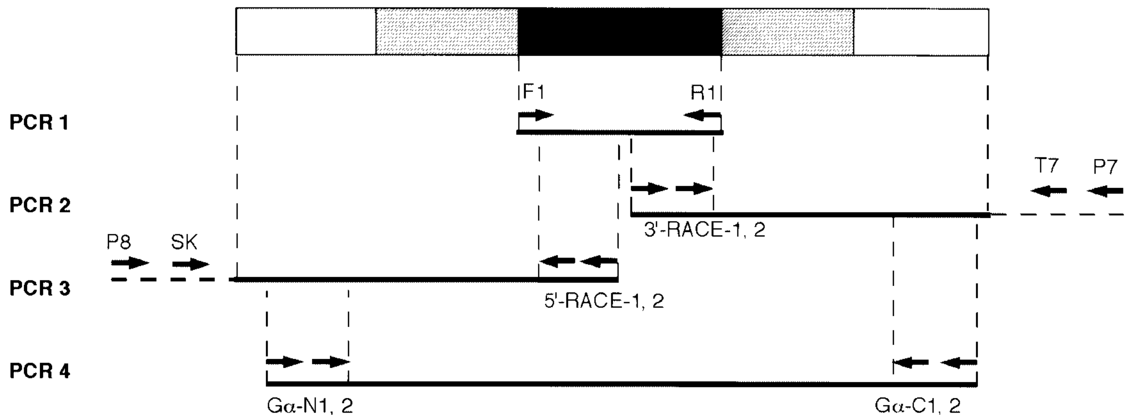

Fig. 1. Cloning strategy for octopus G protein α subunit genes. Degenerate oligonucleotides (F1 and R1) corresponding to conserved amino acid sequences were synthesized and used to amplify cDNA fragments of octopus G protein α subunits (PCR 1). Gene-specific primers were then synthesized (3'-RACE-1 and 2 , and 5'-RACE-1 and 2) and used with vector-specific primers (T7, P7, P8 and SK) to amplify 5' or 3'-cDNA fragments (PCR 2 and 3). Finally, gene-specific primers were made to amplify the whole coding sequence (Gα-N1 and 2, and Gα-C1 and 2; PCR 4).

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OvGi     1:MGC---AVS----SVDKAGAERSKAIDKSLRAEGERSAREVKLLLLGAGESGKSTIVKQMKIIH-EKGYTQEECLQYKPVVY-SNIMQSMMAIIRAMGLL 91
HsGi3    1:...---TL.----AE...AV....M..RN..ED..KA.K...........................-.D..SED..K...V...-..TI..II.......R. 91
OvGo     1:...---TM.----AEERLAL......E.N.KED.IQA.KDI.........................-.G.F.S.DNK.......-..TI..LV.......T. 91
HsGo     1:...---TL.----AEER.AL......E.N.KED.ISA.KD..........................-.D.FSG.DVK.......-..TI..LA..V...DT. 91
OvGq     1:-------MACCLSEEA.EQKRINQE.ERQ..RDKRDAR..L......T.......FI...R...-GA..SE.DRKGFEKI..QNIFSAIQTL.-A..ET. 91
OdGq     1:-------MACCLSEEA.EQKRINQE.ERQ..RDKRDAR..L......T.......FI...R...-GA..SE.DRKGFEKI..QNIFLAIQTL.-A..ET. 91
HsGq     1:.T-LESMMACCLSDEV.ESKRINAE.E.Q..RDKRDAR..L......T.......FI...R...-GA..SE.DKRGFTKL..QNIFTAMQAM.-...ET. 97
OvGs     1:...F--RRTRED-ED..LRK.QN.K.E.Q.QKDKQVYRSTHR.....................R.L.VDG.F-GP.ERKL.IEDIKR..RDAILT.TG..ST. 96
HsGs     1:...LGNSKTEDQRNEE..QR.AN.K.E.Q.QKDKQVYRATHR.....................R.L.VN-.FNGDSEKAT.VQDIKN.LKEAIET.VA..SN. 99
HsGt     1:-----------MGAGAS.EEKH.RELE.K.KEDA.KD..T..........................-QD.-YSL.ECLEFIAIIYG.TL..IL..V...TT. 87
                                                *              ***** *******     *** * *   *                        *  **  *

OvGi    92:KINFGSSEGENDAKTLFEISQNSDE-CDMTPELSALYMRLWSDPGVMSCVARSREYQLNDSAQYYLHALERISQPYYIPNVQDVLRTRVKTTGIVETHFT 190
HsGi3   92:..D..EAARAD..RQ..VLAGSAE.-GV.....AGVIK...R.G..QA.FS..........S...ND.D....SN...TQ.................. 190
OvGo    92:D.P..DN.R.C...MVLDVIGRMEDTEPFSD..LQAMK...A.T..QE.FG..N........K.F.DD.D.LGAKD.M.T...I............V..A 191
HsGo    92:G.EY.DK.RKA...MVCDVVSRMEDTEPFSA..LSAM....G.S.IQE.FN..........K...DS.D..GAAD.Q.TE..I................ 191
OvGq    92:--SLEYKTSG.NENAE.ID.IDA.SADTFDQSHVDAIKS..T.E.MQE.YD.R.....T...K...DDT..LHE.GHS.TL..I..V..P....I.YPPD 189
OdGq    92:--CLEYKTSG.NENAEYIN.IDA.SADTFEQSHVDAIKT..T.E.MQE.YD.R.....T...K...DDVD..HE.G...TL..I..V..P....I.YP.D 189
HsGq    98:--KILYKYEQ.K.NA.LIREVDVEKVTTFEHQYVSAIKT..E...IQE.YD.R.....S...K...TDVD..ATLG.L.TQ.....V..P....I.YP.D 195
OvGs    97:NPPVELDDSSLMHHVNYIQEIA.QQDF.YPA.FYEHTEI..R.K..QT.YE..N....I.C...F.DRVHIVKSAD.T.TE..I..C..L.S..F..R.I 196
HsGs   100:VPPVELANP..QFRVDYIL.VMNVPDF.FP..FYEHAKA..E.E..RA.YE..N....I.C...F.DKIDV.K.AD.V.SD..L..C..L.S..F..K.Q 199
HsGt    88:-NIQYGDSARQ.DARKLMHMADTI.EGT.PK.M.DIIQ...K.S.IQA.FE.AS........G...SD...LVT.G.V.TE....S.......I..Q.S 186
                                                                ** * *   *  *  **** * * * *              *  ** ** ** * ** *

OvGi   191:FKELRFKMFDVGGQRSERKKWIHCFEGVTAIIFIVAMSEYDLMLAEDQEMNRMMESMKLFDSICNNKWFLDTSIMLFLNKKDLF-E------DKI-K-NS 281
HsGi3  191:..D.Y.........................C..L.D...V....E.....H..............TE...I.........-.------E..-.-R. 281
OvGo   192:..T.N..L................D......C.......QV.H..ETT...Q..L............T....I.........-.------E..-.-K. 282
HsGo   192:..N.H.RL................D......C..L.G..QV.H..ETT...H..L............T....I........I.-.------E..-.-K. 282
OvGq   190:LDSTILR.A.........R.......N..S.M.L..L....QV.V.SDNE...E..KA..RT.ITYP..QNS.VI.........LE.KIMTSHL-AD-Y-- 285
OdGq   190:LDSII.R.V.........R.......N..S.M.L..L....QV.V.SDNE...E..KA..RT.ITYP..QNS.VI.........LE.KIMTSHL-AD-Y-- 285
HsGq   196:LENII.R.V.........R.......N..S.M.L..L....QV.V.SDNE...E..KA..RT.ITYP..QNS.VI.........LEDKILYSHL-VD-Y-- 291
OvGs   197:VDKVN.H..........D..R...Q..ND......VT.C.S.N.V.R..NTQ..LK..LE..Q..W..R.LRSI.VI......Q..LA.KVKAGKS..ETYFA 296
HsGs   200:VDKVN.H..........D..R...Q..ND......V..S.S.NMVIR..NQT..LQ.ALN..K..W..R.LRTI.VI......Q..LA.KVLAGKS..EDYFP 299
HsGt   187:..D.N.R.............P.........C....A.LTA..MV.V..D.V...H..LH..N....HRY.AT...V.........V.F.KVKKAHL-SICF-- 283
                    ******  *   ***  **  ** * *  *   *    *   **  *   **  *          *  ****** *

OvGi   282:PLT-TCFP-E--Y-EGGNTYEESAAY-IQLQ-FENLNKRKEEKEI-YTHFTCATDTNNVQFVFDVVTDVIIKNNLKDCGLF                     354
HsGi3  282:...-I.Y.-.--.-T.S.....A...-..C.-..D..R..DT...-..........K.......A...........E...Y                     354
OvGo   283:...-I...-.--.-T.RQ....A...-..A.-..-AKNKSAT...-.C.Q........I.....A......A...RG...Y                     354
HsGo   283:...-I...-.--.-T.PSAFT.AV..-..A.-Y.-SKNKSAH...-.S.V........I.....A......AK..RG...Y                     354
OvGq   286:------.---D.D-.PKKDAQA.R---EFILRMFVDLNPDPDK.I.S........E.IR...AA.K.T.LQL...EYN.V                     353
OdGq   286:------.---D.D-.PKCDYNA.R---EYMSDRY..LNEDKDKML.Y.Y......E.IR...AA.K.T.LQL...EYN.V                     353
HsGq   292:------.---EFD-.PQREPQA.R---EFILKMFVDLNPDSDK.I.S........E.IR...AA.K.T.LQL...EYN.V                     359
OvGs   297:DFERYVT.VDAHTEP.DDPEVVR.K.F.RDEFLRISTATRDGRHYC.P.....V..E.IRR..NDCR.I.QRMH.RQYE.L                     377
HsGs   300:EFARYTT.EDATPEP.EDPRVTR.K.F.RDEFLRISTASGDGRHYC.P.....V..E.IRR..NDCR.I.QRMH.RQYE.L                     380
HsGt   284:-PD-YDG.-NTYEDA.--------N.-.--KVQFLELNMRRDVKEI.S.M.....Q..K.C..A...I...E........                     350
                  *       *                           * * *** ** *    *    * *    *    *
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Fig. 2. Comparison of the amino acid sequences of octopus G protein α subunits. Dots represent identical amino acids; dashes are alignment gaps; asterisks mark conserved amino acid residues. Shaded boxes show the amino acid sequence used to design PCR primers (F1 and R1 in Fig. 1) to amplify a cDNA fragment of G protein α subunit. Open boxes represent A-, C- and G-boxes (in order from the N-terminal), which are conserved within all G protein α subunits. Sequence data used in the figure were taken from GenBank, EMBL and DDBJ data bases: Hs (*Homo sapiens*) Gi3 (M20604), HsGo (M60162), HsGq (M69013), HsGs (X04408) and HsGt (X15088). Sequence data for octopus are results of the present study, with accession numbers as follows: Ov (*Octopus vulgaris*) Gi (AB025780), OvGo (AB025781), OvGq (AB025782), and OvGs (AB025783).

Pharmacia Biotech, Uppsala, Sweden) and subcloned in a pT7blue vector (Stratagene, La Jolla, USA). The nucleotide sequence was determined in both directions from several independent clones, using the primer-labeled cycle sequence method according to the manufacturer's protocol (Amersham Pharmacia Biotech, Uppsala, Sweden). Sequences were analyzed with an automatic DNA sequencer (SQ3000, Hitachi Electronics Engineering, Tokyo, Japan or Shimadzu DSQ 1000L, Shimadzu, Kyoto, Japan). RACE-PCR, with a combination of a cDNA fragment specific primer (3'-RACE 1, 2, 5'-RACE 1 or 2 in Fig. 1) and a vector primer (P8, SK, P7 or T7 in Fig. 1), was performed to obtain a 3'- and 5'-sequence using 1μl aliquots of the *O. vulgaris* eye cDNA library as a template. Finally, the whole open reading frame (ORF) was PCR-amplified with a combination of specific primers based on the 3' and 5' non-coding sequence of each G protein α subunit (Gα -N1, -N2, -C1, or -C2 in Fig. 1).

Northern blot analysis

Total RNA from several tissues of *O. vulgaris* was prepared by the standard CsCl-ultracentrifugation method. This RNA (10 μg) was then separated on a 0.8% agarose-formamide gel and transferred to nylon membrane (Hybond N^+, Amersham Pharmacia Biotech, Uppsala, Sweden) and cross-linked with a UV cross-linker (CL-1000 Ultra Violet Cross Linker, UVP, San Gabriel, USA). Hybridization was carried out with random primed ^{32}P-labeled probes for each class of G protein α subunit (full length of ORF) (Megaprime DNA labeling system, Amersham Pharmacia Biotech, Uppsala, Sweden) in 50% formamide, 6 x SSC (0.9 M NaCl, 90 mM sodium citrate), 1% SDS, 5 x Denhardt's solution and 20 μg/ml denatured salmon sperm DNA at 42°C overnight. The filters were then washed twice in 2 x SSPE (360 mM NaCl, 20 mM sodium phosphate, 2 mM EDTA, pH 7.7) and 0.1% SDS for 10 minutes at room temperature, and twice in 1 x SSPE and 0.1% SDS for 15 minutes at 65°C. The washed membranes were then analyzed with an imaging plate (BAS2000, Fuji film, Tokyo, Japan).

***In situ* hybridization**

Digoxigenin-labeled anti-sense and sense riboprobes for each G protein α subunit message were made according to the manufacturer's protocol (DIG RNA labeling Kit, Boeringer-Mannheim, Mannheim, Germany). Octopus retinas were fixed with PLP (4% paraformaldehyde, 75 mM lysin, 37.5 mM phosphate buffer, pH 7.4, 10 mM sodium periodate) and washed in PBS (10 mM sodium phosphate, pH 7.5, 154 mM NaCl). The fixed tissues were then incubated in PBS containing 20% sucrose and 5% glycerol and embedded in OCT compound (Miles Inc., Indiana, USA). Cryostat sections (about 10 μm thickness) were dried in air, fixed again in 4% formaldehyde in PBS for 20 min at room temperature and washed in PBS (10 min, 3 times), followed by distilled water (1 min). The sections were then acetylated, briefly washed in 2x SSC, dehydrated in a graded EtOH series (50 –100%) and dried in air. They were then hybridized with each digoxigenin-labeled riboprobe at 50°C overnight. Unhybridized riboprobes were then digested with RNase at 37°C for 30 min, and washed in 2x SSC for 1 hr at room temperature, followed by 0.1x SSC for 1 hr at 60°C. The sections were then incubated with anti-DIG antibody - alkaline phosphatase conjugates at room temperature for 1 hr and the color was developed according to the manufacturer's protocol (Boehringer-Mannheim, Germany), for 4 hr - overnight at room temperature. After a wash with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH7.6), the sections were fixed with 1% glutaraldehyde in distilled water followed by a wash with distilled water. They were then dehydrated through EtOH, cleared in xylene, and embedded in Permount (Fisher Scientific, New Jersey, USA).

RESULTS

Molecular structure of cDNA clones of octopus G protein α subunits

Four different classes of cDNA clones were obtained by PCR amplification of octopus eye cDNA with primers designed for the conserved amino acid sequence of G protein α subunit. Full length cDNA clones were then obtained from a cDNA

Table 1. Percentage amino acid identity between octopus and human G protein α subunits.

	Amino acid identity				
	Human[a]	Gi	Go	Gs	Gt	Gq
Octopus						
OvGi	**74.9**	63.7	40.0	58.3	47.5
OvGo	71.0	**83.9**	44.0	56.1	48.3
OvGq	48.3	47.7	40.9	47.7	**79.6**
OvGs	39.5	42.5	**73.9**	41.1	40.1

[a]Amino acid sequences were obtained from GenBank.

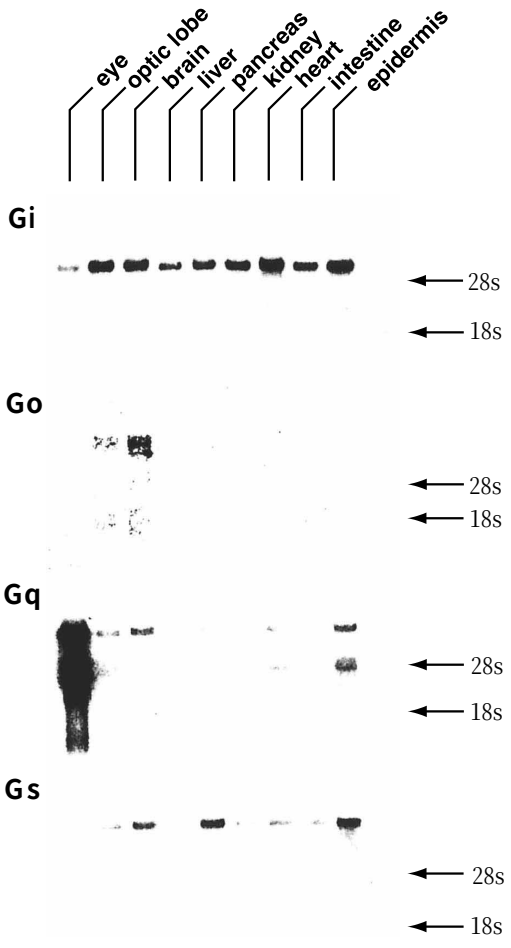

Fig. 3. Northern blot analysis of octopus G protein α subunit message in different tissues of *Octopus vulgaris*. Total RNA (10 μg) from each indicated tissue was analyzed with a specific probe for each class of octopus G protein α subunit message containing for entire coding and 3'-noncoding region. Arrows at right are size markers.

library of a hemisected eye of *O. vulgaris* with a series of runs of PCR with vector-specific and cDNA-specific primers. The nucleotide sequences will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases under Accession Nos. AB025780 – AB025783. The cDNA sequences contained a single open reading frame and the deduced amino acid sequences from the clones were 353 or 354 amino acids in length, except one of 377 amino acids. Based on comparison of the deduced amino acid sequences with those of human G protein α subunits (Table 1 and Fig. 2), they are classified as *O. vulgaris* G protein α subunits (OvGα_i, OvGα_o, OvGα_q and OvGα_s). They show high similarity (74 – 84%) to the corresponding subclasses of human G protein α subunit (Table 1, numbers in bold type). The well conserved amino acid motifs of G protein α subunits, domains A, C and G, were found in all the deduced sequences (open boxes in Fig. 2). Since these domains play an important role in the binding and catalysis of a guanine nucleotide, the octopus G protein α subunits obtained appear to be functional proteins in octopus eye.

Tissue distribution of G proteins

To determine the tissue distribution of messages corresponding to octopus G protein α subunits, Northern blot hybridization was performed with RNA purified from a variety of octopus tissues (Fig. 3). Two messages of different size were observed for OvGα_i in optic lobe, heart and epidermis; and three for OvGα_q in eye, intestine and epidermis. They were evident also in other RNA preparations, suggesting that they are not the products of a single degraded message.

As shown in Fig. 3, OvGα_i was expressed in the all tissues, with a weaker signal in the eye and liver than in the other tissues. The expression of OvGα_o was restricted to the neural tissues (brain and optic lobe) and was not observed in the eye. Although the cDNA clone of OvGα_o was obtained from the cDNA library of a hemisected eye, the absence of a signal indicates that the amount of OvGα_o in the eye is very low. These results agree well with our biochemical data, in that the amount of G_o class G protein α subunit was less than 10% of that of G_q class in the eye (unpublished results). The expression of OvGα_q was high in the eye, lower in the optic lobe, brain, pancreas, heart, intestine and epidermis, and negligible in the kidney, resembling the eye-specific expression of vertebrate transducin and photoreceptor protein (opsin). OvGα_s was present as a single band in the all tissues except

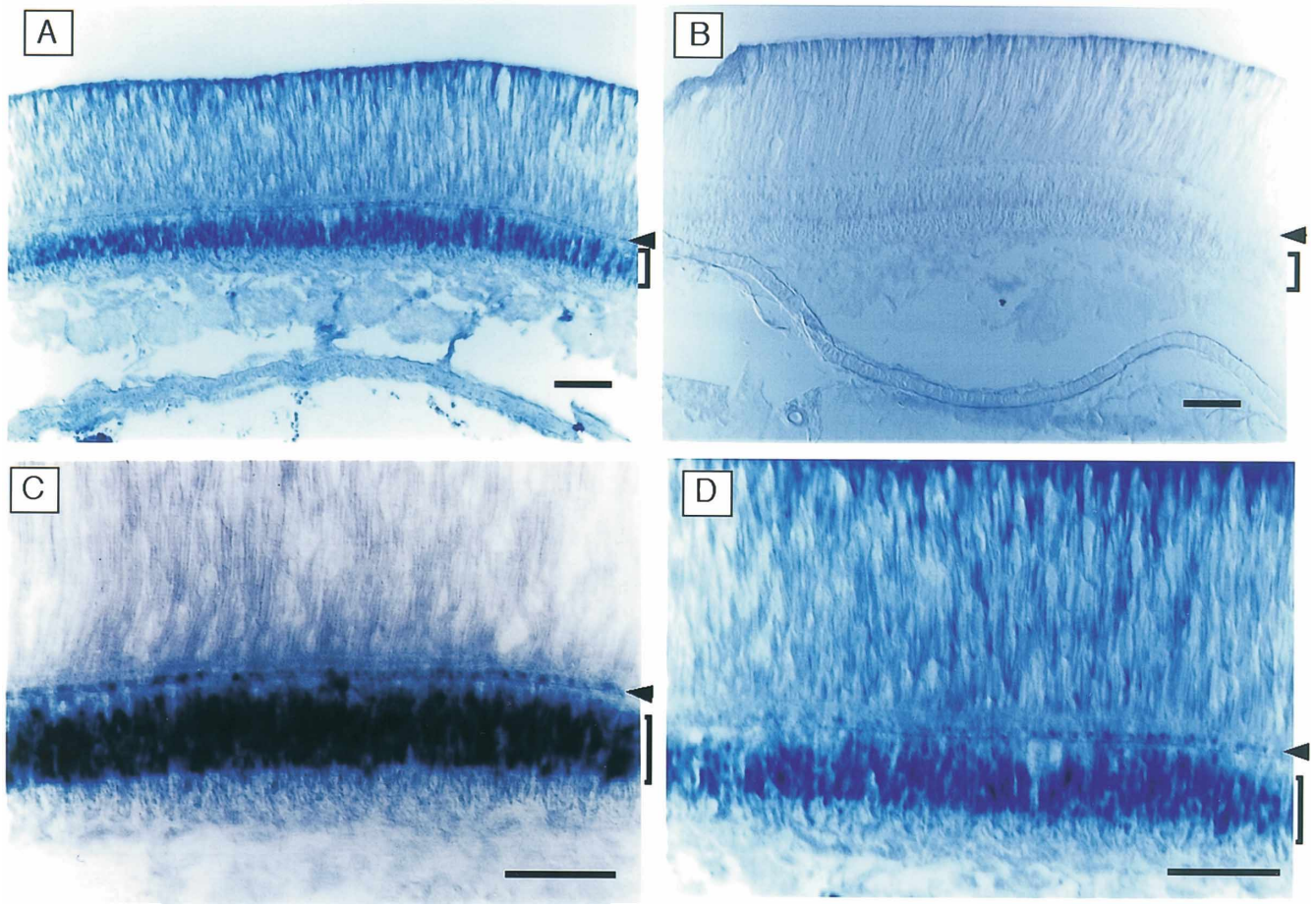

Fig. 4. *In situ* hybridization of octopus retina with digoxigenin-labeled probes for OvGα_q or octopus opsin message. A, Anti-sense probe for OvGα_q. The positive signal is observed in the nuclear layer of the photoreceptor cells. B, Sense probe for OvGα_q. C, Anti-sense probe for octopus opsin. D, Anti-sense probe for OvGα_q (cf. A), for comparison with C. Square brackets and arrowheads to the right of each figure indicate nuclear layers of the photoreceptor cells and supporting cells, respectively. Scale bars,100 μm.

the eye, with an intensity different for each tissue.

Distribution of OvGα_q in octopus retina

The spatial distribution of G protein messages in octopus photoreceptor cells was investigated by i*n situ* hybridization of retinal sections. Particular attention was paid to the expression of OvGα_q and OvGα_o in the photoreceptor cells, since both classes of G protein have been reported to couple with photo-activated rhodopsin in invertebrates (Lee *et al*., 1990; Suzuki *et al*., 1995; Kikkawa *et al*., 1996; Kojima *et al*., 1997). Unfortunately, no signal was obtained with the anti-sense RNA probe for OvGα_o, presumably because the amount of OvGα_o is very low in the eye, as indicated by the Northern blot analysis (Fig. 3).

The anti-sense probe for OvGα_q showed a signal in the inner segment layer around the nuclei of the photoreceptor cells (Fig. 4A), while the sense-probe did not (Fig. 4B). These results indicate that OvGα_q is expressed in the photoreceptor cells not in the supporting cells, because the nuclei of the supporting cells form a layer just between the inner segment and the outer segment (arrowhead in Fig 4A).

Opsin, the apoprotein of rhodopsin, is clearly expressed in photoreceptor cells (square bracket in Fig. 4C): all the photoreceptor cells showed an intense signal not only around the nuclei but also in the cytoplasm. This is consistent with the fact that only one opsin gene has been found in the octopus to date and that the photoreceptor cells of the octopus are morphologically similar. Under higher magnification, the expression of OvGα_q was observed around the photoreceptor nuclei (Fig. 4D) but with a signal intensity weaker than that of opsin (cf. Fig. 4C). Again almost all the photoreceptor cells expressed OvGα_q, suggesting that photoreceptor cells commonly co-express opsin and OvGα_q.

DISCUSSION

The present results demonstrate that there are four classes of G protein α subunit gene in the octopus eye. The existence of several different types of G protein in the cephalopod eye was suggested previously by biochemical studies with bacterial toxins (Vandenberg and Montal, 1984; Tsuda *et al*., 1986; Tsuda and Tsuda, 1990). In *Octopus dofleini* microvillar membranes, three (46, 41 and 34 kDa) G proteins were identified by ADP ribosylation (Tsuda *et al*., 1986; Tsuda and Tsuda, 1990). The cholera toxin substrate was a 46 kDa protein referred to as G_{sp}, which is in good agreement with the 44.6 kDa molecular mass of the amino acid sequence deduced for OvGα_s. The pertussis toxin substrate was a 41 kDa protein referred to as G_{ip}, close to the calculated molecular masses of OvGα_i and OvGα_o: 40.7 and 40.3 kDa, respectively. The 34 kDa protein was a substrate for both cholera and pertussis toxins and was smaller than any of the molecular masses estimated from the deduced amino acid sequences obtained in the present study.

Although four classes of G protein α subunit gene were obtained from the cDNA library of the eye, Northern blot analysis and *in situ* hybridization could not detect signals for G_o and G_s in the eye, presumably because they are expressed in only very small amounts in the eye. It was estimated that the amount of G_o present less than a tenth of that of G_q, based on the results of biochemical studies of the G proteins of octopus microvillar membrane (unpublished data). The signal for G_q was the most abundant of the G proteins found in the octopus eye, and its expression in the eye easily exceeded its expression among the other tissues tested. This is analogous to findings in vertebrates and *Drosophila*, where the G protein known to participate in phototransduction (transducin or Dgq, respectively) is expressed most abundantly in the eye. The presence of an eye-specific Gα_q protein aligns octopus visual transduction most closely with that of *Drosophila*.

However, biochemical studies with bacterial toxins (Tsuda and Tsuda, 1990) and our recent studies suggest the presence of bacterial toxin-sensitive G proteins which have the potential to couple with photo-activated rhodopsin: Partially purified octopus G_{ip} (a 41 kDa protein pertussis toxin substrate) cross-reacts with anti-Gα_o antibody and couples with photo-activated rhodopsin *in vitro* (unpublished data). This strongly suggests that each octopus photoreceptor cell contains several different types of G protein able to couple with photo-activated rhodopsin, providing the potential for more than one phototransduction pathway. This requires further investigation.

The observation that OvGα_o is abundantly expressed in neural tissues of octopus corresponds to similar observations in higher vertebrates (Worley *et al*., 1986; Terashima *et al*., 1987), suggesting a conserved role for G_o class G proteins in the neural systems of mammals and cephalopods. The physiological roles of OvGα_o, OvGα_l and OvGα_s in the octopus eye remain to be elucidated.

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