The molecular cloning of the squid (*Loligo forbesi*) visual Gq- α subunit and its expression in *Saccharomyces cerevisiae*

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The sequence of the α -subunit of the major G-protein from the squid (*Loligo forbesi*) retina was predicted from its cDNA to be a member of the Gq subclass. The abundance of the squid Gq- α in the squid photoreceptor membranes suggests that the protein functions in phototransduction; the sequence of this G-protein is consistent with it mediating the light-dependent

activation of a phospholipase C. The squid G- α was expressed in the yeast *Saccharomyces cerevisiae*, where it was unable to replace the function of GPA1, the yeast G- α homologue that regulates the mating response, suggesting that Gq- α was unable to interact with the endogenous G- $\beta\gamma$ (STE4-STE18).

INTRODUCTION

The squid visual system provides the biochemist with an unsurpassed opportunity to study a G-protein-coupled receptor-effector system. The specialization of the photosensitive region of squid photoreceptor membranes allows rapid isolation of the component parts of the light-sensitive transduction cascade in quantities suitable for biochemical analysis [1]. These membranes, like their vertebrate counterpart, the rod outer segment, essentially contain a single G-protein-coupled receptor pathway. However, whereas in vertebrate vision rhodopsin photolysis is coupled to a cyclic GMP phosphodiesterase, in cephalopods, as in arthropods and insects, visual transduction appears to involve G-protein-mediated inositol 1,4,5-trisphosphate (InsP₃) production as a result of activation of a phospholipase C (PLC) [2–5]. There have been many previous reports of α -subunits of G-proteins that may mediate phototransduction in the squid retina [6–11]. However, because of its concentration in the squid visual outer segments, only one now appears to be a likely candidate for this role [10,11]. We report the full coding sequence of this G- α and demonstrate that it belongs to the recently characterized Gq subclass [12,13]. There is growing evidence that this Gq class of G-protein is involved in pertussis-toxin-insensitive coupling of various receptors to β -PLCs [14–17]. The sequence of the squid visual G-protein supports the hypothesis that these G-proteins mediate toxin-insensitive coupling of receptors to β -PLC and that it is through this G-protein that rhodopsin activates a PLC in squid visual transduction.

The mating-response pathway of yeast has been demonstrated to involve a heterotrimeric G-protein encoded by the genes *GPA1* (G- α), *STE4* (G- β) and *STE18* (G- γ) [18–20]. Genetic evidence strongly suggests that the STE4-STE18 complex is the active unit in the mating-response pathway and that this component is regulated by GPA1 [21–23]. Complementation in yeast is a powerful technique that permits extension of molecular-genetic techniques to study proteins of higher organisms [24,25]. A recent report suggested that co-expression of mammalian Gs- α and the β -adrenergic receptor can result in functional coupling

of β -activation to the yeast mating response [26]. This indicates that co-expression in yeast of receptors and the G-proteins to which they are coupled may provide a very powerful system to study the structural basis of the processes involved in their coupling and prompted us to investigate the expression of Gq- α . Here the expression and function of the squid and mouse Gq- α in yeast are reported. Despite the expression of full-length Gq- α polypeptides, and their association with the cell membrane, genetic evidence suggested that Gq- α was unable to replace the function of the *GPA1*-gene product.

MATERIALS AND METHODS

Materials

Squid (*Loligo forbesi*) eyes were obtained from freshly killed squid at the Marine Biological Laboratory in Plymouth, U.K. Enzymes were from Boehringer-Mannheim, Pharmacia or from Bethesda Research Laboratories; chemicals were from Sigma, Aldrich, Fluka or BDH, and were AnalaR grade or equivalent; yeast media were from Difco.

RNA isolation and cDNA synthesis

RNA was isolated by acid phenol extraction from homogenates of tissue in 4 M guanidine thiocyanate containing 2% β -mercaptoethanol and 1% laurylsarcosine [27]. Single-stranded DNA was prepared from mouse brain total RNA using MuMLV-reverse transcriptase and an oligonucleotide specific for the 3' end of the coding sequence of mouse Gq- α [12,28] as a primer (Oligo-1). A second specific oligonucleotide primer (Oligo-2) was used to PCR-amplify the whole of the coding region of mouse Gq- α . In order to aid subsequent cloning procedures, the oligonucleotides used for this amplification introduced specific restriction-endonuclease sites in the non-coding region of mouse Gq- α .

Oligo-1: CTTCGGATCCATGACTCTGAGTC

Oligo-2: GCGAGGCAAGCTTAGACCAGATT

Abbreviations used: G- α , G- β , G- γ and G- $\beta\gamma$, α -, β -, γ -subunits and $\beta\gamma$ -heterodimeric subunits of heterotrimeric G-proteins; PLC, phospholipase C; InsP₃, inositol 1,4,5-trisphosphate; 1 $\times$ SSC, 0.15 M NaCl/0.015 M sodium citrate; Denhardt's solution, 0.02% BSA/0.02% poly(vinylpyrrolidone)/0.02% Ficoll 400.

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The sequence depicted in Figure 2 has been submitted to the GenBank Nucleotide Sequence Database under the accession number L10289.

Figure 1 Construction of the yeast expression plasmids

The coding region of squid Gq- α was excised from λ gt10 using *EcoRI*. The two fragments encoding the protein were subcloned into pBluescript to produce pB-sGqI and pB-sGqII. The 3'-*EcoRI* site was removed from pB-sGqII by removal of an *EcoRV* fragment from the vector to generate pB-sGqC. The *XmnI*-*EcoRI* fragment of pB-sGqI encoding the N-terminus of squid Gq- α was ligated into *SmaI*-*EcoRI*-cut pB-sGqC to regenerate the full coding region of the squid Gq- α in the plasmid pB-sGq. The *SphI*-*KpnI* fragment containing the *GAL10/1* promoter of YCpGT5 was inserted into the polylinker of YEpLac112 to generate YEpG68. The *XbaI*-*KpnI* fragment of pB-sGq encoding squid Gq- α was ligated to suitably prepared YEpG68 DNA to generate YEpG68-sGq. Restriction sites have been abbreviated as follows: R, *EcoRI*; V, *EcoRV*; Xmn, *XmnI*; Sm, *SmaI*; K, *KpnI*; Xba, *XbaI*; Sp, *SphI*. They are shown in parentheses where destroyed during ligation.

Amplified product was cleaved with *Bam*HI and *Hind*III and cloned into pBluescript to produce pB-mGq for sequence analysis and further amplification. The mGq-insert was used to generate a probe to screen a squid-eye library in λ gt10 [29,30], using random priming [28]. Approx. 10^3 recombinants of the library were transferred to nitrocellulose and were hybridized with this

probe at 42 °C in 6 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl/0.015 M sodium citrate)/30 % formamide/12.5 mM sodium pyrophosphate (pH 6.8)/0.5 % SDS/1 $\times$ Denhardt's solution [0.02 % BSA/0.02 % poly(vinylpyrrolidone)/0.02 % Ficoll 400] containing 100 μ g/ml denatured salmon sperm DNA. The filters were washed three times for 10 min at room temperature in

2 $\times$ SSC/0.1 % SDS and once in 0.5 $\times$ SSC. λ -DNA was isolated from all primary positives that were identified and was incubated with *EcoRI* to excise inserts. Southern analysis of recombinant λ -DNA was performed using standard methods [28], and in each case two fragments that hybridized at 70 % stringency were identified. One was common to all positives (approx. 600 bp) and one had variable length (approx. 500–900 bp). The primary positive with the longest hybridizing insert (the insert generated by hybridizing *EcoRI* fragments of 600 bp and 900 bp) was purified by a second round of screening. DNA isolated from this purified λ was restricted with *EcoRI*. The 600 and 900 bp fragments identified by Southern analysis of the primary positive were subcloned into suitably prepared pBluescript. The complete sequence (in both directions) of the two fragments was determined by double-stranded sequencing of a series of nested deletions generated by Exo-III and SI-nuclease, and from subcloning of restriction-enzyme-generated fragments [28]. The sequence at the internal *EcoRI* site was confirmed by sequencing a fragment derived by *DraI* and *EcoRV* digestion of the original λ -clone.

Plasmids

The galactose-inducible yeast-expression vector YCpGT5 contains *CEN3*, *URA3* (the selection marker), the *GAL10/1* promoter modified with unique *XbaI* and *KpnI* sites and the *TRP5* terminator [18]. Plasmid YEpG68 was constructed by insertion of the *GAL* promoter, isolated on an 840 bp *SphI*–*KpnI* fragment from YCpGT5, into the polylinker site of YEpLac 112 [31] (Figure 1).

Squid Gq- α contained an internal *EcoRI* site within the coding region. Therefore it was necessary to reconstruct the cDNA from the *EcoRI* fragments that had been subcloned into pBluescript. The 3'–*EcoRI* site was removed from the ~ 600 bp fragment by cleavage of pB-sGqII with *EcoRV* and re-ligation of the plasmid to generate pB-sGqC (Figure 1). The 5' coding region was excised from pB-sGqI using the *XmnI* site incorporated in the adapter during cloning [30] and the 3' *EcoRI* site. This fragment was ligated into pB-sGqC that had been prepared by treatment with *SmaI* and *EcoRI* to generate pB-sGq. An *XbaI*–*KpnI* fragment of pB-sGq that contained the coding region of squid Gq- α was then subcloned into appropriately prepared YCpGT5 and YEpG68 to generate the plasmids YCpGT-sGq and YEpG68-sGq. YCpGT-mGq and YEpG68-mGq were prepared by inserting a (filled-in *BamHI*)–*KpnI* fragment of pB-mGq that encoded mouse Gq- α into the (filled-in *XbaI*)–*KpnI* sites of YCpGT5 and YEpG68; YCpG1501 is a derivative of YCpGT5 containing the *GPA1* gene fused to the *GAL1* promoter [18]; YEpG68-GPA1 was constructed by insertion of the 1.7 kb *XbaI*–*KpnI* fragment encoding GPA1 isolated from YCpG1501 into appropriately prepared YEpG68.

Yeast strains and culture techniques

Saccharomyces cerevisiae strains used were JRY90 (*MAT α /MAT α* , *ade1 ade2/ade1 ade2*, *leu2-3/leu2-3*, *trp1-289/trp1-289*, *ura3-52/ura3-52*, *his3/his3*, *GPA1/gpa1::HIS3*) and, JRY64, a *FUS1-LacZ::LEU2* derivative of KMY1098C (*MAT α* , *ura3*, *his3*, *trp1*, *leu2*, *gpa1::HIS3*) obtained from Dr. K. Matsumoto, Department of Molecular Biology, Nagoya University, Nagoya, Japan.

Media used were as follows: YEPD, 1 % yeast extract/2 % peptone/2 % glucose; YPGal, 1 % yeast extract/2 % peptone/4 % galactose/0.2 % sucrose; SDCAA, 0.7 % yeast nitrogen base without amino acids/2 % glucose/0.5 % casamino acids;

SSGCAA, 0.7 % yeast nitrogen base without amino acids/4 % galactose/0.2 % sucrose/0.5 % casamino acids.

Standard methods were used for cell growth, transformation, sporulation and tetrad dissection [24]. Yeast cells were grown at 30 °C. Cells transformed with the plasmids described here were selected on SDCAA agar medium containing appropriate supplements. The various G- α genes under the *GAL1* promoter were expressed using SSGCAA medium containing appropriate supplements to maintain prototrophic selection of the plasmid.

Membrane preparation

Squid photoreceptor membranes and rat brain membranes (donated by Dr. R. Murray-Whelan, University of Geneva, Geneva, Switzerland) were prepared as previously described [29,32]. A crude membrane fraction from yeast was prepared by differential centrifugation: JRY64 cells carrying both YEp1301 and YEpG68, or its derivatives, grown to stationary phase ($A_{600} > 2$) in SSGCAA medium (50 ml), were harvested by centrifugation. All further treatments were performed at 4 °C. The cell pellet was washed in PBS (25 ml) and resuspended (1:4, w/v) in cell-breakage buffer [20 mM Tris/HCl (pH 8.0), 2 mM EDTA/1 mM phenylmethanesulphonyl fluoride/200 units of aprotinin, containing an equal volume of 0.5 mm glass beads]. This mixture was vortex-mixed until approx. 75 % of the cells were broken. The lysate was diluted with an equal volume of 10 mM Tris/HCl (pH 8.0)/10 % sucrose/1 mM EDTA/1 mM dithiothreitol. The large particles, unbroken cells and glass beads were pelleted (700 g, 10 min) and discarded. The supernatant was centrifuged (20000 g, 30 min) to pellet the cell membranes. This crude membrane fraction was resuspended in cell-breakage buffer.

Western blotting

The antiserum CQ-2 was raised against a synthetic decapeptide which corresponded to the predicted C-terminus (residues 350–359) of mouse Gq [33]. Membrane proteins were fractionated by SDS/12.5 % PAGE and were transferred to nitrocellulose membranes. Polypeptides that were recognized by the CQ-2 antiserum were detected using a goat anti-rabbit secondary antibody conjugated to alkaline phosphatase and revealed by colour development on incubation with the substrate Nitroblue Tetrazolium.

RESULTS AND DISCUSSION

Squid visual G- α

There have been several reports of G-proteins in the squid retina that may play a role in visual transduction [6–11]. The G-proteins that have been identified in these previous studies have generally been substrates for ADP-ribosylation by pertussis or cholera toxin [6–9]. Moreover, in several cases light-stimulated GTPase activity has been reported to be inhibited by pertussis-toxin-catalysed ADP-ribosylation [7,9]. However, screening of 30000 recombinants of the same library with cDNA encoding rat Gs- α or *Drosophila* Gi- α or Go- α at 60 % stringency revealed no hybridizing plaques. In contrast, of approx. 1000 squid eye library recombinants, 12 hybridized with DNA encoding the full protein sequence of mouse Gq- α [12]. DNA was prepared from recombinant λ that were identified from this primary screening. Southern-blot analysis of λ -DNA, cleaved by incubation with the restriction endonuclease *EcoRI*, demonstrated that all the clones contained a fragment of approx. 600 bp and a second fragment that varied in length between

Figure 2 Sequence of squid Gq- α

The nucleotide sequence of the full coding region of squid Gq- α was determined by double-stranded sequencing of pB-sGqI and pB-sGqII and of nested deletions produced by Exo-III and S1-nuclease digestion of these plasmids. The region of the internal *EcoRI* site was confirmed by determination of the sequence of the *DraI*–*EcoRV* fragment of the λ gt10 squid Gq- α clone subcloned into pBluescript. The amino acid sequence of the peptides from α -iodosobenzoic acid cleavage of the M_r 44000 polypeptide reported previously are underlined.

approx. 500 and 900 bp. Both of these fragments hybridized with mouse Gq- α DNA at 70% stringency. Thus it appears that a single Gq- α cDNA accounts for approx. 1% of the squid-eye cDNA library. This reflects the very high concentration (approx. 10% that of rhodopsin) of its protein transcript that is present in the retinal outer segments [11] and strongly indicates that Gq- α mediates phototransduction in cephalopods. A similar level of expression of the squid visual G- β has also been reported [29]. Based on protein sequence analysis and the insensitivity of Gq- α s to pertussis-toxin modification, we have suggested that many of the G-proteins that have been reported previously are either very minor components of the visual membranes or more likely result from contamination with other membranes [11]. This conclusion is strongly supported by the sequence of the squid visual G-protein which does not possess the consensus sequence

for pertussis-toxin-catalysed modification found in other G- α s (see below).

Sequence of squid visual G- α

The complete nucleotide sequence of the coding region of squid visual G- α was determined in both directions from the sequence of the approx. 600 bp and 900 bp *EcoRI* fragments which were subcloned from λ gt10 into pBluescript. To rule out the possibility that there was a short region of sequence that was lost by *EcoRI* digestion, an overlapping *DraI*–*EcoRV* fragment of squid visual G- α was also subcloned for sequence analysis. The sequence of squid visual G- α (Figure 2) shows that this protein is a member of the recently described Gq subclass of G-protein α -subunits. The amino acid sequence of squid visual G- α shows 77%

identity with that of mouse Gq- α , 77% with that of mouse G₁₁- α , 75% with that of *Drosophila* Gq- α , but only approx. 45% with that of Go- α , Gi- α , Gz- α , G₁₂- α , Gs- α or GPA1 (Figure 3). The predicted polypeptide contains the sequences of the *o*-iodosobenzoic acid-generated peptides which we have previously reported [11].

The 'GAGE-box' is a highly conserved region amongst G-proteins (Figure 3). This region of sequence has been implicated as forming part of the GTP-binding site and appears to play a role in GTP hydrolysis [34]. In the predicted squid sequence, this region is identical with that of other Gq- α s, but differs from the sequence of other G-proteins. The modifications in this region of mouse Gq- α have been suggested to be responsible for altered nucleotide-binding kinetics [16]. If the nucleotide-binding characteristics of the squid visual G- α are also unusual, this may explain the failure to detect light-dependent GTP-binding associated with this major component of the visual membranes in a number of previous studies [7–9]. However, it is also possible that the conditions used in the purification of squid outer-segment membranes have inactivated the G-protein in many of these previous studies.

Neither the squid nor the *Drosophila* visual G- α s share the five-amino-acid extension, N-terminal to the conserved start site of Go and Gi- α , which has been predicted for both mouse Gq- α and G₁₁- α . Gi and Go- α s are modified by myristoylation of the glycine immediately following the starting methionine. Myristoylation is required for their localization at the membrane surface [34]. However, in squid Gq- α , as also in both mouse Gq- α s and *Drosophila* Gq- α , the second amino acid is not glycine. It therefore remains to be determined which parts of the sequence and what post-translational modifications, if any, are involved in attaching Gq- α to the membrane. Very recently it has been proposed that the short sequence Val-Asp-Thr-Glu-Asn-Ile-Arg-Arg-Val-Phe (residues 367–376) of Gs- α is sufficient to direct membrane attachment of otherwise soluble G- α derivatives [35]. This sequence is more closely conserved in G- α s, which, like Gs- α , lack the myristoylation sequence (Figure 3), than in members of the myristoylated class of G-proteins. Charged amino acids at the positions highlighted by boldfacing above are found in the non-myristoylated G-proteins, but are not conserved among those modified by myristoylation. Therefore it is tempting to speculate that these two charged residues may have a role in the localization of squid Gq- α to the photoreceptor membrane (and the heterologously expressed protein to the yeast plasma membrane).

Four residues from the C-terminus, the cysteine that is the site of pertussis-toxin ADP-ribosylation in G-proteins that are sensitive to this modification [36,37] is absent. This explains our inability to observe pertussis-toxin-dependent modification of squid visual G- α [11]. [We have also found that the protein did not undergo cholera-toxin-catalysed ADP-ribosylation [11], despite the presence of the conserved arginine that is modified in Gs- α and Gt- α (Figure 3). This residue is conserved in all G-proteins, including Gi and Go- α s, which are not normally sensitive to cholera-toxin-catalysed modification [36,37].] It is interesting to note that though it is likely that squid and *Drosophila* Gq- α s are functionally closely related, their sequences are no more related than either is to that of the mouse Gq- α s. This holds equally well for the variable regions as it does overall (Figure 3). Indeed, there are regions where the mouse proteins are significantly more similar to the squid visual G- α than is the *Drosophila* protein.

The squid and mouse Gq- α subunits are identical over the C-terminal 31 amino acids, and have very high sequence identity over the whole C-terminal half (approx. 5% non-conservative

amino acid substitutions; see Figure 3). This region of G-proteins has been implicated in effector interaction [36,38]. Therefore it seems likely that the mouse and squid proteins will be coupled to the activation of related proteins. There are now strong indications that the mouse Gq- α s can interact with PLC- β [14–17], and the *Drosophila* protein most probably couples rhodopsin photolysis to PLC- β (*norpA*) activation [2]. In squid visual membranes there is evidence that the photoreceptor rhodopsin is coupled to a PLC [3–5]. Therefore it is highly likely that this coupling is mediated by the protein whose sequence we report here. We have previously reported the complete sequence of squid rhodopsin, and have noted that there is more similarity in the cytoplasmic loops of the protein to those of *Drosophila* rhodopsins than to those of vertebrate opsins [39]. Thus it will be of great interest to examine to what extent the various Gq- α s can cross-react between receptor systems. The membranes of the squid eye can be selectively depleted of Gq- α by extraction with urea [11]. Therefore, the reconstitution of the yeast-expressed Gq- α subunits with G- α -depleted squid membranes may provide a very versatile system for the investigation of functionally critical determinants of this and other classes of G- α .

There are only a few regions of sequence where the sequence similarity within the Gq- α subclass is relatively low (Figure 3). These regions may represent sites of interaction of the α -subunit with the receptors or with the G- $\beta\gamma$ complex. We have also reported the complete sequence of the β -subunit of the squid visual G-protein and have demonstrated that it very closely resembles the three classes of mammalian G- β for which the sequence has been reported [29]. A similar β -subunit has also been identified in *Drosophila* [40]. However, it has been reported that the *Drosophila* retinal β -subunit has a considerably lower sequence identity [41]. Bearing this in mind, comparison of the sequences of the G1- α s provides few clues as to regions of interaction with G- β (i.e. regions where the squid and mammalian G- α s are more closely related than either is to the *Drosophila* protein). At the extreme C-terminus the squid and mouse Gq- α s are identical with one another, and the *Drosophila* protein shows some variation. However, comparison with other mammalian G- α s (which interact with G- β s closely related in sequence to the squid G- β [29]) suggest that differences in this region of the sequence are not caused by interaction with the less conserved *Drosophila* visual G- β . The other regions of sequence variation between squid and *Drosophila* Gq- α are regions where all the G- α s differ from one another. Thus it may be that the interaction of Gq- α with G- $\beta\gamma$ is determined more by interaction of G- α with G- γ than by interaction with G- β .

Expression of Gq- α genes in yeast

The Gq- α genes from mouse and squid were subcloned into two galactose-inducible yeast expression plasmids YEpG68, a high-copy-number plasmid with *TRP1* as its selection marker; and the low-copy-number YCpGT5 that carries the *URA3* gene as a selection marker. These plasmids were transferred to the yeast strain JRY64 for studies of heterologous expression and function. This haploid yeast strain was chosen because it has a *gpa1*-null mutation that is complemented by expression of the *GPA1* gene of the plasmid YEp1301 (carrying the *URA3* gene) or the plasmid YCp1302 (carrying the *TRP1* gene) [42]. Since JRY64 carrying only YEp1301 or YCp1302 still required tryptophan or uracil respectively for growth, nutritional selection of both the high- and low-copy-number recombinant Gq- α expression plasmids could be maintained. JRY64 carries the *GPA1* gene on a plasmid, therefore the relative rate of plasmid loss was also studied to determine if expression of squid or mouse Gq- α could

myristoylation
 (GxxxS) region: 'A (GAGGS-box)'
 MACCLSEEAKQKRINQIEKQLRRDKRDARRELKLLLOTGSGKSTFIKQMRIHSGSYSEDRKGFKEIVYQ -squid
 .E.....d..Kr.yI.l.f. -Drosophila Gq
 *.....Ar...d...rhv.....d..Kr..T.l... -mouse Gq
 *.....d.V..s...A.....a.....kr..T.l... -mouse G11
 .g.Tv.A.D.AAAERskM.d.n..E.GEK.A..v.....a.....iv...k...ED....eCrQyRAV..S -human G12
 .gAGA.A.E.HsrEl-eKKL.eDA-e-...T-v.....a.....iv...k...QD....L.eCLE.IA.i.G -bovine Gt
 .g.RQ.S.E..AA.RsRR.drh..SeSqRQ...i.....sn.....iv...k...sg.fnL.AC.EyKPlIY -human Gz
 .g.T..A.ErAALERskA...n.kE.GIS.Akdv.....a.....iv...k...ED.f.G..V.QyKpV..S -human-Go
 *tEDQRn.E.A.reA.kk....qk..qVY.ATHr.....a.....iv...l.Vn.fn*GekATKVqDiKn -human-Gs
 *GAARdAer.Ar.Rsrd.dAL.A..r.AV..Lv.i....a.....REFDqKALLE.RDTifd -mouse G12
 *IGDE.dPFLqn..a.dv..qS.qLe.qRDkN.i.....a.....Vl..lklI.Qg.f.hqe.LQyAqviWA -yeast

NIFSAIQTLIAAMETLSLEYKDPSNNEHAFLNSIDADSADIFEDGHVTAIKGCWTDPGMQECYDRRREYQLTDS -squid
 ...M.m.sm.K..dm.KiS.GqGEhs.L.dlvM...YetVTT...PYln...TL.D.a.i..... -Drosophila Gq
 ...t.m.am.R..d..KiP..YEH.KA..qlvREv.VeKVSA..nPY.D...sL.N...i.....s... -mouse Gq
 ...t.m.amvR....KiL..YEQ.kAn.LliREv.VeKVTT..hQY.n...TL.s...v.....f..s... -mouse G11
 .TiQs.MaivK..GN.QidfA...Radd.*.AL.CT.eEQgvlp.dLSGV.rRL.a.H.v.A.fg.S....N... -human G12
 .TLQs.LaivR..T..niq.G.SaRqdd.*lmHMA.TIEEGTMPKEMSDI.QRL.K.S.i.A.f..AS....N... -bovine Gt
 .AidslTRI.R.lAa.RidfHn.DRAYd.*.AltGP.e.KgEiTPELlGVmrRL.a...A.A.fS.SS..h.E.n -human Gz
 .TiQslAaivR..d..gv..G.KERKAds*CDVVSrMDeTp.SAELLS.mMRL.G.s.i...fn.S....N... -human-Go
 .lKE..e.iv...SN.*v.LAn.E.qFrVdyil.vMNVpDFD.PPEFYEHA.aL.E.E.vrA..e.SN...I.C -human-Gs
 .lKgSrv.vD.RdK.giPWqhsE.eK.gM..Ma*glPVEPaTqLY.P.lSaL.R.s.ir.AfS...S.f.ge -mouse G12
 dAIQsmki..IQARK.*.dCD..I..KdL*****lihedIAK...QL.*.K.ikq.fA.SN.f..EG. -yeast

cholera-toxin-modification
 * region: 'C'
 AKYYLDDVERIHPEGYIPTLQDILRVVPTTGIIEYPFDLYSIIFRMVDVGGQRSEERRKWIHCFENVTSIMFLVA -squid
S.lA..eqad.l..E....A.....l.....Dg.v.....i.... -Drosophila Gq
 t....n.ld.vAd.s.l..Q..v.....Q.v..... -mouse Gq
 ...T..d..ATV..l..Q..v.....En..... -mouse G11
 .A..n.l..Aqsd...Q..v..T..K...v.TH.TfkdLh.k.F.....k.....G..a.i.C... -human G12
 .G...S.l..lVT...v..E..v..S..K...TQ.SfkdLn...F.....k.....G..C.i.i.a... -bovine Gt
 .A..n.l..AAad...ve...S.DM...v.NK.TfKElt.k.....k.....G..a.i.C.E -human Gz
 ...Sld..GAad.Q.LE....T..K...v.TH.TfKnLh..lF.....k.....e..a.i.C... -human-Go
 .q.f..KidV.Kqad.v.sD..l..C..L.s..f.TK.qvDKvN.h.F.....D.....q.nd..a.i.v... -human-Gs
 V.....nld..GqLn.f.sK...LA.Ka.K..v.HD.V.KK.P.k.....q.q..fq..dGi...l.m.s -mouse G12
 .A..f.ni.kfAS.N.vC.De...kG.iK...T.TE.niG.SK.kvl.a.....k.....Gi.avl.vl. -yeast

region: 'G'
 LSEYDQVLVESDNEENRMEESKALFRITIIYPWFQNSSVILFLMKLLLEEKIMTSHLADYFPDYDGPKCDYEA -squid
i.F.....Y...V...e....Q.HA.. -Drosophila Gq
Y...V...e....qR.Aq.. -mouse Gq
d..lH...V...ef...qR.Aq.. -mouse G11
 .A..L..A..-e.M...h..MK..Ds.CnNK..Tdt.i.....f...TH.P.tIC..e.T.an-K.dE. -human G12
 .A..M...-d.V...h..LH..Ns.CnHRY.ATt.iv.....vIs...Kka..sIC....n..N-T..D. -bovine Gt
 .G..LK.Y...qTs..A..LR..Ds.CnNN..I.t.l.....A...RRIP.tIC..e.K.Qn-T..E. -human Gz
 .G...H...-eTT..h..LM..Ds.CnNKF.Idt.i.....fG...KK.P.tIC..e.T..n-T..D. -human-Go
 S.S.nm.iR..-qT..lq.aLN..ks.WnNR.LrTI.....q...A..vl*.KiE...efARYTTPed*Y -human-Gs
 S.....DRRT..RV..MN..E..vnNKL.F.V.i.....M...V..vKsVSiKKH...fK.DPHRL.DV -mouse G12
 m.....M.F..-eRV...h..IM..D.llnSK..kdtPF.....I..f...vKsMPiRK....q.RVG.A..g -yeast

Gs-attachment
 region: 'I'
 REFMMDSYMDLNEDEKMLYYHYHCTATDTENIRFVFAAVKDTILQNLKEYNLV -squid
 kq.vlkK.lAC.P.P.rQC.S.f.T.....kl...C.....m.NA...f..G -Drosophila Gq
 ...ilkMfv...P.Sd.ii.S.f..... -mouse Gq
 ...ilkMfv...P.Sd.ii.S.f..... -mouse G11
 ASyIQSKfE...KR.*.Et.T.f.....K.vg...D..T.V.ikN...dCG.F -human G12
 GnyiKVQfle..MRr*.Et.S.M...g.vk...D..T.I.ike...dCG.F -bovine Gt
 AVyIQRFle...Rn.*.Et.S.f.....S.g...D..T.V.i.N...YIG.C -human Gz
 AAYIQAQfESK.RSPn.Ei.C.M...n..qV...D..T.I.iaN..rGCG.Y -human-Go
 FIRDEFLRISTASGD*HYC.P.f..Y.....R..NDcR.I.Qrmh.rq.e.l -human-Gs
 qRylvqCfDRKRRnrS.P.fh.f.T.I.....H.....E...DIM.Q -mouse G12
 LKyFEKiflS.-Ktn.Pi.VkR...gTmk..Ls..T.L.i.Q...KIGii -yeast

Figure 3 Comparison of the sequence of squid Gq- α with those of other G- α s

The protein sequence of squid Gq- α was aligned with those of *Drosophila* Gq- α [12,13], mouse Gq- α , G11- α [12], human Gi $_2$ - α [43], bovine Gt- α [44], Gz- α [45], Go- α [46], Gs- α [47], mouse G12- α [48] and yeast GPA1 [19]. Identical residues in the sequences of the proteins compared with squid Gq- α have been indicated by '.'; regions where a few amino acids have been deleted to make the alignment have been indicated by an asterisk (*); in the yeast GPA1 the position of the long non-conserved insertion has been depicted by multiple asterisks; inserted gaps are represented by a '-'; extra amino acids found at the N-terminus of mouse Gq- α , G11- α , human Gs- α , mouse G12- α and yeast GPA1 have been omitted. Non-conservative [49] amino acid substitutions have been indicated by capitalization of the amino acid single-letter code, whereas conservative replacements have been shown in lower case. Also indicated are regions of the sequence that are highly conserved and have been suggested to interact with bound guanine nucleotide [36]; the sites modified in susceptible G-proteins by cholera- and pertussis-toxin-catalysed ADP-ribosylation [37]; the site and consensus sequence for N-terminal myristoylation of Gi [34] (the N-termini of several of the G-proteins are not shown, including that of yeast GPA1, where this consensus sequence is found); and the peptide proposed to direct Gs- α to the plasma membrane [35].

Figure 4 Expression of Gq- α genes in *S. cerevisiae*

JRY64 (YEp1301) cells carrying YEpG68, YEpG68-sGq or YEpG68-mGq were grown in SSGCAA medium to induce expression of Gq- α . A crude membrane fraction was prepared after cell lysis, followed by differential centrifugation. Membrane proteins from yeast (200 μ g of protein; YEpG68, lanes 3 and 6; YEpG68-sGq, lanes 4 and 7; YEpG68-mGq, lanes 5 and 8), squid retina (5 μ g of protein, lanes 1 and 9) and rat brain (10 μ g of protein; lanes 2 and 10) were resolved by SDS/PAGE and transferred to nitrocellulose. Gq- α subunits in samples on the left half of the membrane (lanes 1–5) were identified by immunoblotting with the CQ-2 antiserum (1:200 dilution). The specificity of CQ-2 for Gq- α was demonstrated by probing the right half of the membrane (lanes 6–10) with antiserum mixed with 50 μ g of the Gq- α C-terminal peptide. Abbreviation: M, molecular mass.

substitute for that of GPA1 for complementation of the growth defect caused by the chromosomal *gpa*-null mutation (see below).

Galactose-dependent expression of the Gq- α mRNAs was verified by Northern analysis. The steady-state level of mouse Gq- α mRNA was estimated to be 10-fold higher in transformants carrying YEpG68-mGq than in transformants carrying YCpGT5-mGq (results not shown). To demonstrate that the presence of Gq- α mRNA corresponded with protein expression, cell membrane preparations were fractionated by SDS/PAGE, transferred to nitrocellulose, and immunoblotted with antiserum CQ-2 (Figure 4). The CQ-2 antibody detected a single polypeptide

Figure 5 Tetrad analysis to examine complementation of the disrupted GPA1

Diploid JRY90 (*GPA1/gpa1::HIS3*) cells were transformed with the plasmids indicated. After sporulation, 20 tetrad spores were dissected from each transformant strain. The spores were germinated by incubation on galactose-containing medium for 48 h. The segregation patterns of representative tetrads are shown with the four spores from each ascus aligned vertically.

of M_r 42000 in a preparation of rat brain membranes. A protein of identical mobility was detected in membranes from JRY64 cells carrying the YEpG68-mGq plasmid, but was absent from cells harbouring YEpG68 or YEpG68-sGq. In membranes prepared from squid eye outer segments there was a very strong signal from what appeared to be a doublet of similarly sized polypeptides of approx. M_r 45000 and M_r 46000. The lower-mobility band (M_r 46000) was a modified form of squid Gq- α which we have not previously observed on Coomassie Blue-stained gels [11]. It may have been generated by boiling the squid photoreceptor membrane sample that contained the detergent sucrose monolaurate in gel sample buffer containing urea. A polypeptide of similar mobility to squid Gq- α (M_r 45000) was detected in the membrane-proteins of yeast cells carrying

Table 1 Complementation of the *gpa1*-null mutation using plasmid segregation

The stability of plasmid YEp1301 (*GPA1, URA*) or YCp1302 (*GPA1, TRP*) [36] in the *gpa1::HIS3* (JRY64) was determined after transformation with the indicated plasmids. The transformants were grown (30 °C, 2 days) without nutritional selection in the presence of galactose (YPGal medium) to induce expression of squid Gq- α , mouse Gq- α or GPA1. Serial dilutions were plated on YPGal agar and were incubated (30 °C, 2 days) until large colonies had grown. The presence of the *TRP* or *URA* selective marker was determined by replica plating onto an SSGCAA *Trp*⁻ plate and an SSGCAA *Ura*⁻ plate. Cells that originally carried both YCp1302 and YCpGT5 or its derivatives and could subsequently grow on an SSGCAA *Ura*⁻ plate but not on an SSGCAA *Trp*⁻ plate (*trp*⁻, *URA*⁺) represent those cells which can grow without YCp1302. Cells which originally carried both YEp1301 and YEpG68 or its derivatives and could subsequently grow on an SSGCAA *Trp*⁻ plate but not on an SSGCAA *Ura*⁻ plate (*TRP*⁺, *ura*⁻) represent cells which can grow without YEp1301.

Strain and plasmid	Phenotypes ...	Number of colonies			
		TRP ⁺ ,URA ⁺	TRP ⁺ ,ura ⁻	trp ⁻ ,URA ⁺	trp ⁻ ,ura ⁻
JRY64 <i>gpa1::HIS3</i> [YCp1302 <i>GPA1, TRP</i>]					
pGTsGq, <i>URA</i>		900	1800	0	0
pGTmGq, <i>URA</i>		130	280	0	0
pGTGolf, <i>URA</i>		190	340	0	0
pGTGs, <i>URA</i>		430	670	11	0
pGTGPA1, <i>URA</i>		460	520	9	0
pGT5, <i>URA</i>		500	1200	0	0
JRY64 <i>gpa1::HIS3</i> [YEp1301 <i>GPA1, URA</i>]					
pG68sGq, <i>TRP</i>		1300	0	2800	0
pG68mGq, <i>TRP</i>		1950	0	5700	0
pG68Golf, <i>TRP</i>		160	0	360	0
pG68Gs, <i>TRP</i>		240	5	390	0
pG68GPA1, <i>TRP</i>		130	3	180	0
pG68, <i>TRP</i>		560	0	990	0

YEpG68-sGq. A component of the same size was also visible in the immunoblot of membranes prepared from JRY64 cells carrying either YEpG68 or YEpG68-mGq. However, the quantity of reactive M_r -45000 protein was substantially increased by the presence of YEpG68-sGq in the cells. To examine the specificity of binding, the antiserum CQ-2 was incubated with excess of the decapeptide against which it had been raised. Pretreatment resulted in reduced binding of CQ-2 to proteins, squid and rat Gq- α and to the polypeptides of corresponding size that were expressed in yeast. This competitive reduction in binding of CQ-2 to the yeast membrane components strongly suggested that they were recombinant squid and mouse Gq- α , as only the detection of these components by the antiserum was immunogen-specific. However, the non-specific M_r -45000 component recognized by the CQ-2 antiserum in all JRY64 cells remained unaltered by competition with the peptide immunogen. It is therefore unlikely that this component represents a yeast Gq- α homologue. As CQ-2 was raised against the extreme C-terminus of Gq- α , the detection of polypeptides in transformed yeast indicated that both the full-length squid and mouse Gq- α were expressed.

Complementation of the *gpa1*-null mutation

The ability of Gq- α to function in yeast was tested by complementation of a *gpa1*-null mutation. Loss of GPA1 function in haploid cells results in cell-cycle arrest [18,19]. In a previous study, the efficacy of mammalian G- α to complement *gpa1* paralleled their level of expression to some extent [25]. For this reason, complementation by Gq- α was tested following transformation of *GPA1/gpa1* diploid cells with constructs in the high-copy-number plasmid YEpG68.

Following sporulation of the diploids, viability of the spores was analysed on galactose-containing medium. Representative tetrads are shown in Figure 5. Whereas four viable spores were recovered from the tetrads of diploids transformed with YEpG68-GPA1, the segregation pattern of the asci from cells transformed with YEpG68, YEpG68-sGq or YEpG68-mGq was two viable colonies: two microcolonies. This was not due to an abnormally long lag or a low growth rate of cells expressing Gq- α , since cells from the microcolonies failed to grow after transfer to fresh plates.

To eliminate the possibility that defects in the mating-response pathway could affect sporulation, the loss of the plasmid YEp1301 [42] from JRY64 carrying a second plasmid (YEpG68, YEpG68-GPA1, YEpG68-sGq or YEpG68-mGq) was investigated (Table 1). After several generations of growth on non-selective YPGal media, cells carrying YEpG68-GPA1 could grow without YEp1301 (and therefore required uracil). However, no loss of YEp1301 was detected when cells were transformed with YEpG68, YEpG68-sGq or YEpG68-mGq. Similar results were obtained for the loss of YCp1302 [42] from JRY64 cells carrying a second YCpGT5-based expression plasmid (Table 1). Therefore expression of Gq- α is unable to replace the essential function of GPA1, probably because Gq- α binds poorly to STE4-STE18 (G- $\beta\gamma$). In this respect, Gq- α resembles Go- α and Gi- α rather than Gs- α [25].

Conclusions

It appears that the squid eye contains the transcript for a single major G-protein which is localized in the light-sensitive outer-segment membranes. This squid G-protein is a member of the recently characterized Gq subclass. The predicted protein had

75–80 % sequence identity with the G- α s of this subclass, whereas the identity with other G-proteins was significantly lower (40–50 %). The deduced protein sequence lacked the consensus sequences for pertussis-toxin-catalysed ADP-ribosylation and for N-terminal myristoylation. Therefore the protein is not likely to be inhibited by pertussis-toxin modification and probably possesses as yet unidentified regions of sequence that direct membrane attachment. The squid photoreceptor G-protein was expressed in *S. cerevisiae*, where it was also associated with the cell membrane, but where it failed to complement deletion of the gene for GPA1, the G- α involved in the yeast mating response. The characterization of the squid visual G-protein combined with the evidence of a role for a light-activated PLC- β in visual transduction [2–5] strongly suggests that cephalopod visual transduction is mediated by pertussis-toxin-insensitive activation of PLC through light-dependent activation of Gq- α .

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