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Molecular cloning and primary structure of squid (*Loligo forbesi*) rhodopsin, a phospholipase C-directed G-protein-linked receptor

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The sequence of squid (*Loligo forbesi*) rhodopsin was determined by protein and cDNA sequencing. The protein has close similarity to octopus rhodopsin, having an *N*-terminal region (residues 1–340) which resembles other guanine-nucleotide-binding protein (G-protein)-linked receptors and a repetitive proline-rich *C*-terminus (residues 340–452). Comparison of the sequence of squid rhodopsin with those of other members of the G-protein-linked receptor superfamily reveals features which we predict to have both structural and functional importance.

INTRODUCTION

The rhodopsins form a functionally related sub-group within the superfamily of guanine-nucleotide-binding protein (G-protein)-linked receptors [1–3]. Like other visual pigments, squid (*Loligo forbesi*) rhodopsin contains an 11-*cis*-retinal prosthetic group which is attached to the protein by a protonated Schiff's base [4]. The retinal chromophore absorbs visible light and isomerization of the 11-*cis* double bond results in activation of a G-protein [5,6]. However, whereas in vertebrate vision light absorption is coupled to a cyclic GMP-phosphodiesterase [5], invertebrate phototransduction results in the activation of a PtdIns(4,5)P_2-directed phospholipase C [7,8]. This paper reports the primary structure of squid rhodopsin. Comparison of its sequence with those of other rhodopsins and G-protein-linked receptors has enabled us to make predictions of the regions of the protein with critical structural and functional roles.

MATERIALS AND METHODS

Materials

All enzymes were purchased from Boehringer Mannheim, Pharmacia or New England Biolabs. pBluescript was from Stratagene. Radiochemicals (3000 Ci/mmol) were obtained from New England Nuclear. Oligonucleotide probes were made in the Biotechnology Unit, University of Leeds. Reagents were of the highest grade available.

Loligo forbesi were obtained from the Marine Biological Association, Plymouth, U.K. Eyes from freshly killed animals were dissected and washed with saline buffer [600 mM-NaCl/5 mM-Hepes (pH 7.4)/5 mM-EDTA/1 mM-EGTA] and were rapidly frozen in isopentane cooled with liquid N_2 or were homogenized immediately for RNA isolation.

Protein isolation and sequencing

Squid photoreceptor membranes were prepared essentially as previously described [9]. Briefly, frozen eye-cups were defrosted in saline buffer. The outer segments were detached by gentle shaking in fresh buffer and purified by flotation on sucrose (42%, w/v). Membranes were resuspended three times in 50 mM-Tris/HCl, pH 7.0, then three times in 2 M-urea/50 mM-Tris/HCl, pH 7.0, with recovery between washes by centrifugation (9000 *g*, 5 min). Urea was removed by a final wash in 50 mM-Tris/HCl, pH 7.0. The depleted photoreceptor membrane preparation was solubilized in 2% (w/v) sucrose monolaurate for 1 h at 4 °C. Centrifugation (9000 *g*, 5 min) removed insoluble components of the microvillar cytoskeleton. Solubilized proteins were resolved by SDS/PAGE and rhodopsin was electroeluted. Peptides were generated by digestion of rhodopsin using *Staphylococcus aureus* V8 endopeptidase in 50 mM-$NaHCO_3$/0.1% SDS [2% (w/w) protease/rhodopsin; 37 °C for 30 min] and were purified by electroelution after SDS/PAGE [10]. CNBr cleavage was performed by dissolving 1 mg of freeze-dried rhodopsin in 70% (v/v) formic acid, adding 5 mg of CNBr and incubating at room temperature in the dark under N_2 for 24 h; the peptides were then isolated by h.p.l.c. Peptides were attached to a solid-phase glass support for protein sequencing [11].

cDNA cloning and sequencing

Eyes were homogenized immediately in 4 M-guanidinium thiocyanate/25 mM-sodium citrate/0.1 M-2-mercaptoethanol, pH 7.0, and RNA was purified by ultracentrifugation through a caesium chloride cushion [12].

RNA was separated by formaldehyde/gel electrophoresis (1% agarose) and transferred to nitrocellulose for Northern analysis. Nitrocellulose filters were prehybridized in 6 × NET [1 × NET = 150 mM-NaCl/15 mM-Tris/HCl (pH 8.3)/1 mM-EDTA]/0.1% SDS/5 × Denhardt's reagent/denatured sheared salmon-sperm DNA (100 μg/ml) for 2 h at 65 °C. Hybridization was performed in 6 × NET/0.1% SDS/5 × Denhardt's reagent for 6 h at 35 °C with 25 ng of the 5′ phosphorylated (~0.5 μCi/ng) oligonucleotide dY-T-G-C-A-T-C-A-T-N-G-C-C-A-T (probe 1). Stringent washes were performed in 6 × SSC (1 × SSC = 150 mM-NaCl/15 mM-sodium citrate, pH 7.0)/0.1% SDS (2 × 10 min, 42 °C) [13].

Polyadenylated [poly(A)$^+$] RNA was purified by two rounds of oligo(dT)–cellulose chromatography [14] and was used as a template for the oligo(dT)-primed synthesis of cDNA [15]. A cDNA library was constructed in pUC13 using *Eco*RI linkers and was screened using probe 1 (hybridization as for Northern

Abbreviations used: G-protein, guanine-nucleotide-binding protein; 1 × NET, 150 mM-NaCl/15 mM-Tris/HCl (pH 8.3)/1 mM-EDTA; 1 × SSC, 150 mM-NaCl/15 mM-sodium citrate, pH 7.0; poly(A)$^+$ RNA, polyadenylated RNA.

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The nucleotide sequence data reported here will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X56788.

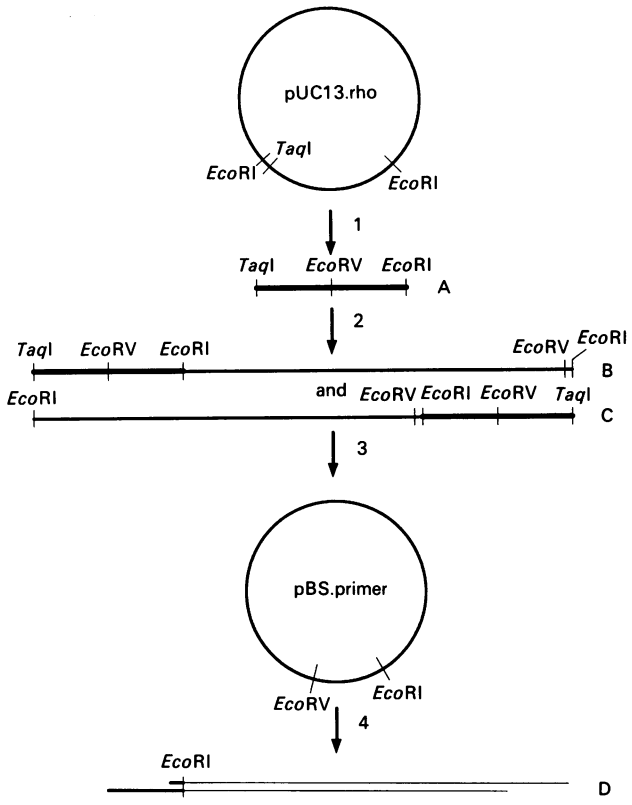

Fig. 1. Generation of the vector prime

(1) The *Taq*I–*Eco*RI fragment (A) was excised from the pUC13.-rhodopsin-3′-clone (Fig. 2). (2) This fragment was ligated into the *Eco*RI site of pBluescript to generate B and C. (3) Cleavage of B with *Eco*RV allowed circularization to yield pBluescript containing the *Eco*RV–*Eco*RI fragment of the pUC13.rhodopsin-3′-clone (pBS.primer); cleavage of C with *Eco*RV generated fragments unable to circularize to form functional plasmids. After *Eco*RV digestion of B and C the products were diluted, ligated and used to transform competent *E. coli* [13]. (4) Linearization of the pBS.primer with *Eco*RV followed by digestion with λ-exonuclease generated the vector-primer D which was used exactly as described previously [40]. During second-strand synthesis an oligonucleotide encoding the *Eco*RV–*Xho*I region of pBluescript was added to prevent deletion of vector sequence [40].

analysis). Extended cDNA clones were produced using a vector primer constructed from a 3′-fragment of rhodopsin cDNA that had been isolated from the pUC13 library (Fig. 1). A specific oligonucleotide, dC-T-C-T-T-A-G-C-C-A-T-G-G-C-T-G-C-C (probe 2), was used to screen a second library in λgt10 (10^4 plaques, hybridization at 42 °C, stringent washes at 42 °C). Positive inserts were sub-cloned into pBluescript and nested deletions were generated using *Escherichia coli* exonuclease-III and S1-nuclease digestion [13]. Double-stranded sequencing was carried out using T7 DNA polymerase [16].

RESULTS AND DISCUSSION

Protein isolation and sequencing

Squid rhodopsin is probably *N*-terminally acetylated, as it is resistant to Edman degradation. Sequence information obtained from polypeptide fragments produced by limited proteolysis with *S. aureus* V8 endopeptidase and from CNBr-cleaved peptides is shown, together with the protein sequence predicted from the cDNA (Fig. 2).

Isolation and sequencing of cDNA clones

Probe 1 hybridized with a single major mRNA transcript of 2 kbp (results not shown). However, when a pUC13 library was screened, only a clone encoding the *C*-terminus of rhodopsin could be isolated. Therefore a vector-primer was constructed and used to prime cDNA synthesis (Fig. 1). This approach generated longer but incomplete clones, from the sequence of which probe 2 was constructed. More than 2% of a λgt10 library hybridized strongly with this specific oligonucleotide, highlighting the abundance of rhodopsin mRNA in the squid eye. A clone (1809 bp) representing the entire coding region of rhodopsin was identified [the different 3′-termini of the full-length and pUC13 clones (Fig. 2) probably result from cDNA synthesis rather than use of alternative sites of polyadenylation]. Overlapping cDNA sequence information for both strands predicts that squid rhodopsin is 452 amino acids long, with a molecular mass of 51 kDa.

Post-translational modification

The peptide sequence data are in agreement with those predicted from the cDNA, apart from at position 8. Codon 8 encodes Asn, but protein sequencing revealed a non-standard amino acid (a very low yield of Asp was also detected at this position). Squid rhodopsin binds quantitatively to concanavalin A [17]; therefore it appears that carbohydrate is attached to Asn-8 but not to Asn-14, which was recovered quantitatively on protein sequencing. Lys-305 is likely to be the site of attachment of retinal to squid rhodopsin. The sequence around Lys-305 is identical to that of peptides containing the retinal Schiff's base attachment site in other squid rhodopsins (*Todarodes pacificus* and *Watasenia scintillans*) [18]. The two cysteine residues which have been shown to be essential for the expression of functional bovine rhodopsin, and which have been proposed to form a structurally important disulphide bond [19], have equivalents (Cys-108 and Cys-187) in squid rhodopsin. In addition, *C*-terminal to the membrane-spanning region, the squid photopigment contains a double cysteine, which, by analogy with bovine rhodopsin, may be palmitoylated [20].

Sequence alignment

The topology of the membrane-spanning region of squid rhodopsin was predicted by alignment of its sequence with those of other members of the G-protein-linked receptor superfamily (Fig. 3). It contains many of the features which are common to this class of receptor [21]. These include the two cysteines, Cys-108 and Cys-187, the conserved Asp-Arg-Tyr at the cytoplasmic end of helix-3, the intramembraneous prolines at residues 140, 170, 212 and 312, and charged and polar residues Asp-80, Asn-53 and Asn-311, as well as several aromatic residues (Table 1).

Buried charged residues

In squid rhodopsin the only charged residues found within the membrane domain appear to be Lys-305, the retinal attachment site, Asp-80, which is highly conserved throughout the superfamily, and His-310, which is only found in cephalopod rhodopsins. Modelling (results not shown) suggests that His-310 is internally oriented and spatially close to Asp-80. Thus a salt-bridge may add stability to this region of the protein fold. A direct role for Asp-80 in the G-protein activation mechanism appears unlikely, as it is absent from the human blue-cone pigment with no obvious compensatory mutation [22]. No influence on absorption spectra has been detected on mutation of bovine Asp-83, the residue equivalent to squid Asp-80 [23,24]. However, the presence of an Asp residue at this position throughout the superfamily (with the exception of the human

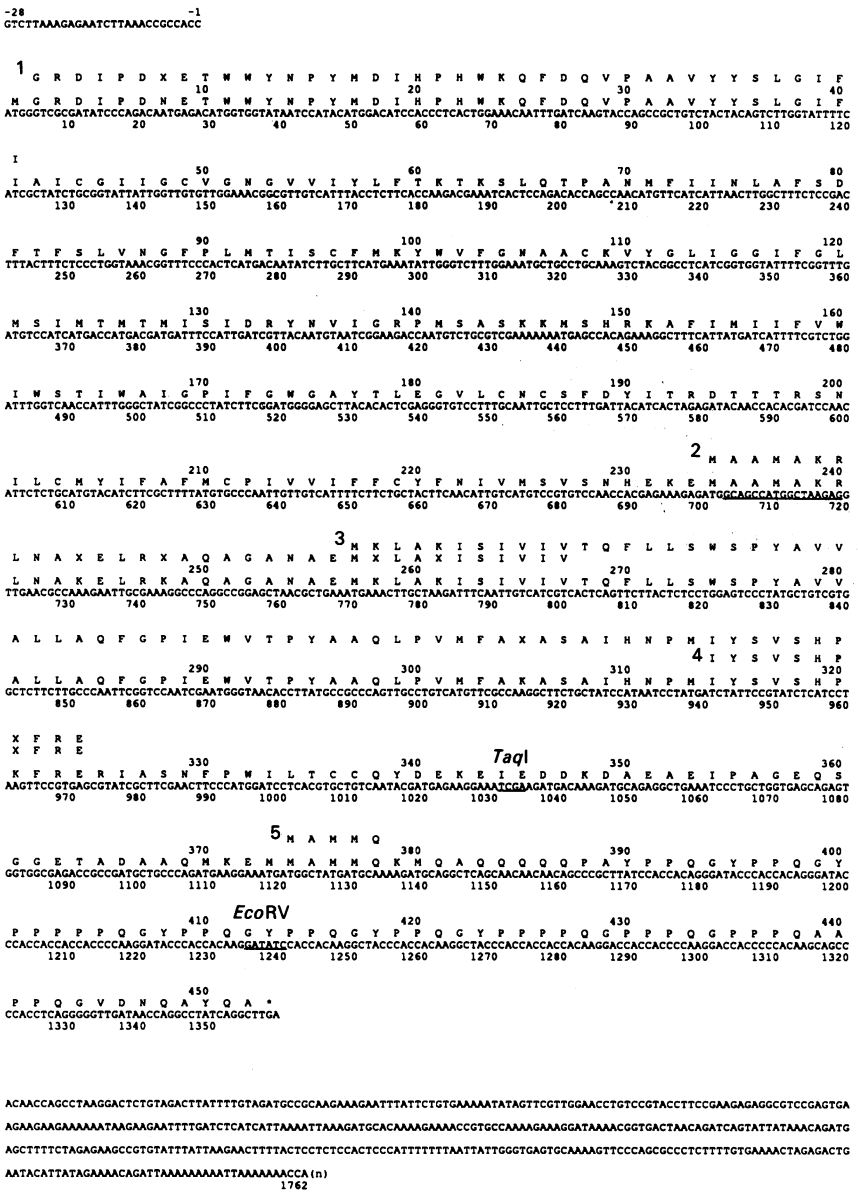

Fig. 2. Full-length clone of squid rhodopsin

The predicted protein sequence of squid rhodopsin is shown above its cDNA. Protein sequencing of polypeptides from *S. aureus* V8 protease cleavage of squid rhodopsin yielded sequences 1, 2 and 3. CNBr-cleaved peptides produced sequences 4 and 5. Probe 1 was based on peptide 5; the sequence used for the construction of probe 2 is underlined. The pUC13.rhodopsin-3′-clone contained residues 1020–1484; the extended clone contained residues 589–1484. The *Eco*RV and *Taq*I sites used in the construction of the vector-primer (Fig. 1) are also indicated.

blue pigment) suggests that it plays more than a passive role in structural determination. Therefore we would suggest an indirect role for this residue in G-protein activation, namely in stabilizing the positioning of groups involved in the process.

A Schiff's base counterion?

The possibility that Glu-113 could act as a counterion to the protonated retinal Schiff's base in bovine rhodopsin has provoked considerable interest [23–25]. Whereas most models would place this residue slightly outside the membrane core at the extracellular end of helix-3, mutation of it has been reported to alter both pigment absorption and the photocycle. This has been interpreted as resulting from a direct interaction between Glu-113 and the protonated retinal Schiff's base [23,25]. Other investigators have observed little effect of mutation at this position [24], although it seems that the differences in ionic strength of measuring buffers may be responsible. The residue in squid rhodopsin corresponding to bovine Glu-113 is Tyr-111, a conserved aromatic amino acid in the other invertebrate rhodopsins and indeed in the whole superfamily. The absence of negatively charged residues within the membrane region of squid rhodopsin suggests that Schiff's base nitrogen may hydrogen-bond to a polar but neutral group, and that a group of dipoles or even one end of a helix stabilizes the positive charge. As replacement of retinal with dihydro derivatives [26] suggests similarity between the vertebrate and invertebrate binding sites, it is unlikely that there is direct interaction of the chromophore with either Tyr-111 in squid or Glu-113 in bovine rhodopsin. The sensitivity of the absorption spectrum of bovine rhodopsin to mutation of Glu-113 may be explained if it and residues with which it interacts seal

Table 1. Proposed roles of various amino acid residues in squid rhodopsin

Possible roles have been predicted both from structure/function studies of G-protein-linked receptors and from sequence conservation between them [1–3,18–25,28,32]. Abbreviations used: H1–H7, helix-1 to helix-7; CL1–CL3, cytoplasmic loops 1–3; EL1 and EL2, extracellular loops 1 and 2 (Fig. 3).

Residue	Location	Proposed role	Comments
Asn-53	H1	Structural/activation mechanism	Identity, possible interaction with Asp-80
Val-55, Ile-57	H1	Structural	Conserved
Leu-65	H1/CL1	G-protein interaction	Identity
Pro-68	CL1	Structural	Rhodopsins only
Asn-70	CL1/H2	Structural/G-protein interaction	Identity
Ile-73, Asn-75, Leu-78	H2	Structural	Conserved
Asp-80	H2	Structural/activation mechanism	Identity, missing from human blue
Tyr-100, Trp-101, Val-102, Phe-103, Pro-104	EL1	Structural/chromophore binding	Rhodopsins
Cys-108	EL1/H3	Structural	Probably disulphide-bonded to Cys-187
Tyr-111	H3	Structural	Conserved except is Glu in mammalian opsins
Ile-129, Asp-131, Arg-132, Tyr-133, Ile-137	H3	G-protein binding	Very tightly conserved motif at membrane interface
Pro-140	CL2	Structural	Conserved
Ala-152, Thr-156	H4	Structural	Conserved
Phe-158	H4	Chromophore binding/wavelength	Rhodopsins only (not blue/insect u.v.)
Trp-160, Pro-170	H4	Structural	Identity
Trp-174	H4/EL2	Structural	Conserved
Cys-187	EL2	Structural	Probably disulphide-bonded to Cys-108
Phe-209, Pro-212, Val-215, Ile-216, Tyr-220, Ile-223	H5	Structural	Conserved or identical throughout
His-Glu-Lys...Ala-Lys-Arg-Leu-Leu-Arg...Asn-Ala-Glu-Met-Lys-Leu-Ala-Lys-Ile	H5/CL3/H6	G-protein selection/activation	Conserved in invertebrate rhodopsins
Glu-256, Lys-258, Leu-259	CL3/H6	G-protein interaction	Conserved
Phe-270, Trp-274, Pro-276, Tyr-277	H6	Structural	Very tightly conserved motif
Lys-305	H7	Retinal binding	Rhodopsins only
Asn-311	H7	Structural/activation mechanism	Tightly conserved
Pro-312, Tyr-316	H7	Structural	Identity
Phe-322	H7	G-protein interaction	Conserved
Val-Ser-His-Pro-Lys-Phe-Arg	H7	G-protein selection/activation	Conserved in invertebrate rhodopsins
Cys-336, Cys-337	*C*-Terminus	Palmitoylation sites	Some conservation

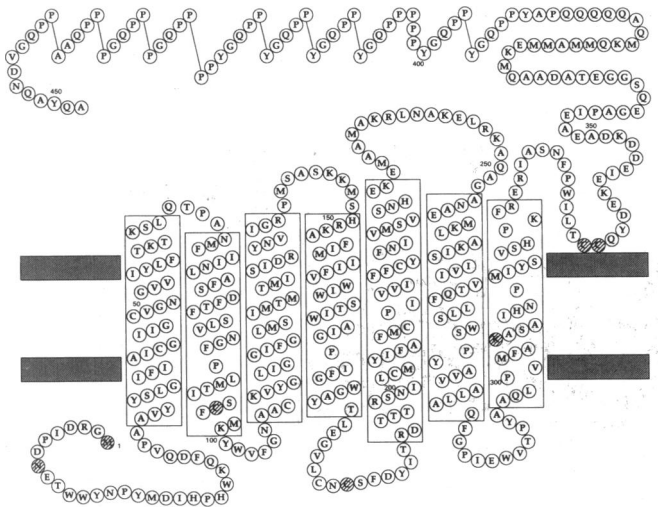

Fig. 3. Transmembrane topology of squid rhodopsin

The transmembrane topology was predicted after alignment of the sequence of squid rhodopsin with the sequences of other members of the G-protein receptor superfamily. The helices are predicted to extend beyond the membrane surface to varying extents. Beyond helix-7, the polypeptide chain is depicted as looping back to the membrane surface at Cys-336 and Cys-337 which may be palmitoylated. Residues identified in the text as sites of potential post-translational modification are indicated by shading. The proline-rich domain has a schematic representation which emphasizes the repeated sequence rather than any higher-order structure.

the chromophore binding site. We would suggest that Tyr-111 plays a similar role in squid rhodopsin. Further support for this proposal comes from comparison of the pH-sensitive spectrum of cephalopod *meta*-rhodopsin [4] with that of Glu-113→Gln mutants of bovine rhodopsin. The light-induced (and ionic-strength-dependent) decrease in the pK_a of the retinal Schiff's base of octopus rhodopsin has been related to increased conformational flexibility of this region of the protein rather than to changes in direct bonding interactions [27]. Mutation of bovine rhodopsin Glu-113 to Gln lowers the pK_a of the retinal Schiff's base in a similar fashion [23]. In addition to the influencing the environment of the Schiff's base, Glu-113 may catalyse its hydrolysis in vertebrate rhodopsins. The Glu-113→Gln mutant [23] and all invertebrate rhodopsins (where this residue is aromatic) have more stable photoproducts than does bovine rhodopsin.

G-protein binding and activation

Another region of squid rhodopsin with functional significance is the cytoplasmic surface. At the cytoplasmic end of helix-3, squid rhodopsin contains the consensus sequence Asp-Arg-Tyr that seems to be essential for G-protein binding [23,28]. The third cytoplasmic loop and the *C*-terminal regions adjacent to the membrane are the determinants of G-protein specificity of chimaeric receptors [29]. The similarity of these regions in invertebrate rhodopsins supports the involvement of closely related G-proteins throughout invertebrate phototransduction. Significantly, this surface of invertebrate photoreceptors is more similar to that of other G-protein-linked receptors than to that of vertebrate rhodopsins.

The *C*-terminus

The unusual *C*-terminus residues 340–452 of cephalopod rhodopsins can be loosely divided into two, a negatively charged region and a proline-rich region. In membrane preparations, the former is highly susceptible to protease digestion, indicating that it forms a surface loop. Its charge distribution resembles that of binding sites for Ca^{2+} [30], an ion with a key role in both visual transduction and adaptation [31]. However, as yet we have no indication whether squid rhodopsin binds Ca^{2+} with a physiologically significant affinity. The charged region corresponds to the serine- and threonine-rich stretch of vertebrate and insect rhodopsins [1,32] (in vertebrate rhodopsin, the sites of phoshorylation by rhodopsin kinase [33]). We have not been able to demonstrate light-dependent phosphorylation, and although past reports suggest that it occurs [34,35], squid rhodopsin contains few *C*-terminal serine and threonine residues that could be phosphorylated. It is therefore possible that the negatively charged region somehow substitutes for phospho-serine and -threonine in deactivation.

C-Terminal to the negatively charged region there is a multiple tandem repeat of a pentapeptide with a Pro-Pro-Gln-Gly-Tyr consensus sequence [repeated approx 10 times (Fig. 3)]. This type of repeat has been observed in octopus rhodopsin and in a range of proteins associated with the membrane surface [3,36,37]. The synaptic vesicle proteins synapsin and synaptophysin, a salivary protein VAMP1 and an actin-binding protein from *Dictyostelium* have related repeated structures, which also occur in several other uncharacterized *Dictyostelium* gene products [36,37]. The role played by this structure in squid rhodopsin is not yet clear, although our preliminary studies suggest that it influences rhodopsin–rhodopsin interactions. We suggest that it may be involved in the maintenance of the highly organized structure of the visual microvilli [38]. Again, it is not known that role (if any) the high degree of organization plays in visual transduction, but light-dependent alterations of cytoskeleton structure have been reported [39].

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REFERENCES

1. Findlay, J. B. C. & Pappin, D. J. C. (1986) Biochem. J. **238**, 625–642
2. Dixon, R. A. F., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohlman, H. G., Frielle, T., Bolanowski, M. A., Bennett, C. D., Rands, E., Diehl, R. E., Mumford, R. A., Slater, E. E., Sigal, I. S., Caron, M. G., Lefkowitz, R. J. & Strader, C. D. (1986) Nature (London) **321**, 75–79
3. Ovchinikov, Yu, A., Abdulaev, N. G., Zolotarev, A. S., Artamonov, I. D., Bespalov, I. A., Dergachev, A. E. & Tsuda, M. (1988) FEBS Lett. **232**, 69–72
4. Hubbard, R. & St. George, R. C. C. (1958) J. Gen. Physiol. **41**, 501–528
5. Fung, B. B. K. & Stryer, L. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 2500–2504
6. Ryba, N. J. P., Pottinger, J. D. D., Keen, J. N. & Findlay, J. B. C. (1990) Biochem. J. **273**, 225–228
7. Baer, K. M. & Saibil, H. R. (1988) J. Biol. Chem. **263**, 17–20
8. Bloomquist, B. T., Shortridge, R. D., Schneuwly, S., Perdew, M., Montell, C., Steller, J., Rubin, G. & Pak, W. L. (1988) Cell **54**, 723–733
9. Saibil, H. R. & Michel-Villaz, M. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 5111–5115
10. Aitkin, A., Geisow, M. J., Findlay, J. B. C., Holmes, C. & Yarwood, A. (1989) in Protein Sequencing: A Practical Approach (Findlay, J. B. C. & Geisow, M. J., eds.), pp. 43–68, IRL Press, Oxford
11. Findlay, J. B. C., Pappin, D. J. C. & Keen, J. N. (1989) in Protein Sequencing: A Practical Approach (Findlay, J. B. C. & Geisow, M. J., eds.), pp. 69–84, IRL Press, Oxford
12. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) Biochemistry **18**, 5294–5299

13. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
14. Aviv, H. & Leder, P. (1972) Proc. Natl. Acad. Sci. U.S.A. **69**, 1408–1412
15. Gubler, U. & Hoffman, B. J. (1983) Gene **25**, 263–269
16. Tabor, S. & Richardson, C. C. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 4767–4771
17. Nashima, K., Mitsudo, M. & Kito, Y. (1978) Biochim. Biophys. Acta **536**, 78–87
18. Seidou, M., Kubaota, I., Hiraki, K. & Kito, Y. (1988) Biochim. Biophys. Acta **957**, 318–321
19. Karnik, S. S., Sakmar, T. P., Chen, H.-B. & Khorana, H. G. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 8459–8463
20. Ovchinikov, Yu. A., Abdulaev, N. G. & Bogachuk, A. S. (1988) FEBS Lett. **230**, 1–5
21. Attwood, T. K., Eliopoulos, E. E. & Finlay, J. B. C. (1991) Gene, in the press
22. Nathans, J., Thomas, D. & Hogness, D. S. (1986) Science **232**, 193–202
23. Sakmar, T. P., Franke, R. R. & Khorana, H. G. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 8309–8313
24. Janssen, J. J. M., De Caluwe, G. L. J. & de Grip, W. J. (1990) FEBS Lett. **260**, 113–118
25. Zhukovsky, E. A. & Oprian, D. D. (1989) Science **246**, 928–930
26. Koutalos, Y., Ebrey, T. G., Tsuda, M., Odashima, K., Lien, T., Park, N. H., Shimizu, N., Derguini, F., Nakanishi, K., Gilson, H. R. & Honig, B. (1989) Biochemistry **28**, 2732–2739
27. Koutalos, Y., Ebrey, T. G., Gilson, H. R. & Honig, B. (1990) Biophys. J. **58**, 493–501
28. Franke, R. R., König, B., Sakmar, T. P., Khorana, H. G. & Hofmann, K. P. (1990) Science **250**, 123–125
29. Kobilka, B. K., Kobilka, T. S., Daniel, K., Regan, J. W., Caron, M. G. & Lefkowitz, R. L. (1988) Science **240**, 1310–1316
30. Szebenyi, D. M. E. & Moffat, K. (1986) J. Biol. Chem. **261**, 8761–8767
31. Payne, R., Corson, D. W. & Fein, A. (1986) J. Gen. Physiol. **88**, 107–126
32. O'Tousa, J. E., Baehr, W., Martin, R. L., Hirsch, J., Pak, W. L. & Applebury, M. L. (1985) Cell **40**, 839–850
33. Findlay, J. B. C., Brett, M. & Pappin, D. J. C. (1981) Nature (London) **293**, 314–316
34. Paulson, R. & Hoppe, I. (1978) FEBS Lett. **96**, 55–58
35. Vandenberg, C. A. & Montal, M. (1984) Biochemistry **23**, 2347–2352
36. Trimble, W. S. & Scheller, R. H. (1988) Trends Neurochem. Sci. **11**, 241–242
37. Noegel, A. A., Gerisch, G., Lottspeich, F. & Schleicher, M. (1990) FEBS Lett. **266**, 118–122
38. Saibil, H. R. & Hewat, E. A. (1987) J. Cell Biol. **105**, 19–28
39. Tsukita, S., Tsukita, S. & Matsumoto, G. (1988) J. Cell Biol. **106**, 1151–1160
40. Chirala, S. S., Kasturi, R., Pazirandeh, M., Stolow, D. T., Huang, W.-Y. & Wakil, S. J. (1989) J. Biol. Chem. **264**, 3750–3757

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