Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 5535–5539, June 1995
Neurobiology

Cloning and expression of a second photoreceptor-specific membrane retina guanylyl cyclase (RetGC), RetGC-2

(cGMP/calcium/phototransduction)

DAVID G. LOWE*†, ALEXANDER M. DIZHOOR‡, KATHLEEN LIU*, QIMIN GU*, MARIBETH SPENCER‡§, RICHARD LAURA‡, LUCY LU*, AND JAMES B. HURLEY‡§

*Genentech, Inc. South San Francisco, CA 94080; and §Howard Hughes Medical Institute and ‡Department of Biochemistry, University of Washington, Seattle, WA 98195

Communicated by David L. Garbers, The University of Texas Southwestern Medical Center, Dallas, TX, February 16, 1995 (received for review January 20, 1995)

ABSTRACT **One of the membrane guanylyl cyclases (GCs), RetGC, is expressed predominantly in photoreceptors. No extracellular ligand has been described for RetGC, but it is sensitive to activation by a soluble 24-kDa protein (p24) and is inhibited by Ca^{2+}. This enzyme is, therefore, thought to play a role in resynthesizing cGMP for photoreceptor recovery or adaptation. By screening a human retinal cDNA library at low stringency with the cytoplasmic domains from four cyclases, we cloned cDNAs encoding a membrane CG that is most closely related to RetGC. We have named this GC RetGC-2, and now term the initially described RetGC RetGC-1. By *in situ* hybridization, mRNA encoding RetGC-2 is found only in the outer nuclear layer and inner segments of photoreceptor cells. By using synthetic peptide antiserum specific for each RetGC subtype, RetGC-2 can be distinguished from RetGC-1 as a slightly smaller protein in immunoblots of bovine rod outer segments. Membrane GC activity of recombinant RetGC-2 expressed in human embryonic kidney 293 cells is stimulated by the activator p24 and is inhibited by Ca^{2+} with an EC_{50} value of 50–100 nM. Our data reveal a previously unappreciated diversity of photoreceptor GCs.**

Light activation of the visual signal transduction cascade results in transient membrane hyperpolarization of photoreceptors. This process is initiated by a heterotrimeric G-protein-coupled pathway that activates phosphodiesterase hydrolysis of cGMP, resulting in closure of the cGMP-gated cation channel in the plasma membrane. Resynthesis of cGMP by guanylyl cyclase (GC) results in channel opening and recovery of the dark state (1, 2). Early studies with amphibian photoreceptors suggested an inverse feedback relation between cGMP levels and Ca^{2+} (3, 4). Subsequent work with bovine rod outer segments (ROS) established the presence of a soluble regulator of ROS GC that imparted Ca^{2+} sensitivity to the active enzyme (5). Two candidates for regulators of retinal cyclase have recently been described, the Ca^{2+} binding protein p21 also called GCAP (6, 7) and p24, a distinct retina-specific molecule (8).

Efforts to purify photoreceptor GC have yielded a 110- to 112-kDa protein (9, 10). Amino acid sequencing of the purified enzyme (11, 12) showed identity to the photoreceptor-specific membrane GC RetGC cloned from a human retina cDNA library (13). Recombinant RetGC behaves very similarly to GC in bovine ROS with respect to p24 activation, Ca^{2+} inhibition, and K_m value for GTP (8). These results demonstrate that RetGC is probably the photoreceptor enzyme responsible for resynthesis of cGMP. Other members of the membrane GC family (14, 15) and soluble GC (16) are also thought to be expressed in photoreceptors. However, the role of these enzymes in phototransduction or neuromodulation is not known.

In this report we described the cDNA cloning and expression of another membrane of the membrane GC family. The encoded protein is most closely related to RetGC by amino acid sequence similarity, is specifically expressed in photoreceptors, and displays regulation of enzyme activity very similar to RetGC. We have therefore termed this protein RetGC-2¶ and designated the previously described RetGC (8, 13) as RetGC-1.

MATERIALS AND METHODS

cDNA Cloning. DNA fragments encoding the kinase homology domain and GC domain were prepared from human NPR-A (17), NPR-B (18), STaR (19), and RetGC-1 (13) by PCR amplification from cloned cDNAs. Primers were as follows: NPR-A, 5′-GTTGAGCCCAGTAGCCTTGAG-3′ and 5′-CCCAAGGAGCCAGTAGGTCCG-3′; NPR-B, 5′-CTGCAGTTTGGCAACTCAGAG-3′ and 5′-TCCTAAGAGCCAGTATGTTCG-3′; STaR, 5′-AATATCTTTCCTCTGGAGACC-3′ and 5′-CCCAGTCAGCCAGTAGGTAGT; RetGC-1, 5′-TGGACGACATCACCTTTCTCC-3′ and 5′-GCCGCGTCTGCCCACTAGCCA-3′. PCR products were purified after electrophoresis in a 0.8% agarose gel by using Geneclean II (Bio101) and ^{32}P-labeled by random priming (Boehringer Mannheim). Approximately 2×10^6 clones from a human retina cDNA library in λgt10 (20) were screened first at high stringency (13) with the four pooled probes and then filters were reprobed at low stringency (17). Approximately 60 low-stringency specific positive plaques were purified, and cDNA inserts were amplified by PCR from intact phage with the primers 5′-TGTAAAACGACGGCCAGTAGCAAGTTCAGCCTGGTTAAGT-3′ and 5′-CAGGAAACAGCTATGACCCTTATGAGTATTTCTTCCAGGG-3′, incorporating M13 sequencing primer sites (underlined). Fragments were gel-purified as described above and sequenced with M13 forward or reverse primers by using dye terminators and an Applied Biosystems 370A DNA sequencer. Sequences were compared against the GenBank data base to identify potential GCs. Additional RetGC-2 cDNA clones were obtained by rescreening the cDNA library with 5′ probes. Both strands of the RetGC-2 cDNA were sequenced.

***In Situ* Hybridization.** Frozen sections (8 μm) were prepared from rhesus monkey tissues and hybridized to RetGC-2 sense and antisense probes as described for RetGC-1 (13). A fragment for *in vitro* transcription was prepared by PCR amplifi-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GC, guanylyl cyclase; RetGC, retina GC; ROS, rod outer segment.

†To whom reprint requests should be addressed.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. L37378).

cation of a 693-bp piece from the RetGC-2 cDNA by using the primers 5′-TCTAATACGACTCACTATAGTCTTGCTGGATCTCAT-3′, and 5′-TACATTAACCCTCACTAAAGTCAAAGCCCTCAG-3′, incorporating T3 and T7 RNA polymerase-specific promoters (underlined), respectively. The PCR product was gel-purified and transcribed *in vitro* by using [^{33}P]ATP to label the RNA as described by Lu and Gillett (21).

Synthetic Peptide Antisera. A 12-residue peptide corresponding to the sequence from Val-169 to Arg-180 of the predicted RetGC-2 amino acid sequence (peptide 8, CVLTTGQDSQSMR) was synthesized by solid-phase methods and incorporated an N-terminal Cys residue for heterobifunctional cross-linking to soybean trypsin inhibitor (22). New Zealand White rabbits were immunized with the peptide carrier and sera were titared as described (22).

RetGC Expression. The RetGC-2 cDNA was cloned into the vector pRC and transfected into the human embryonic kidney cell line 293 by using calcium phosphate (23). Membranes were prepared as described by Dizhoor *et al.* (8).

Immunoblot Analysis. The 293 cell membrane protein (80 μg) or the ROS protein (40 μg) was fractionated by SDS/PAGE in a 7.5% gel and transferred to nitrocellulose by electroblotting. Filters were probed with RetGC-2 peptide 8 antisera at a 1:500 dilution or with affinity-purified antibodies from RetGC-1 peptide 4 antisera (8).

GC Assay. The GC activator was purified and assayed by stimulation of GC activity as described by Dizhoor *et al.* (8). Membranes were prepared from transiently transfected human embryonic kidney 293 cells or bovine ROS as described (8). Reverse-phase chromatography fractions were assayed for activator in a 25-μl reaction volume with 293-cell RetGC-2 membrane protein (38 μg) or ROS protein (8.4 μg). Ca^{2+} inhibition experiments were carried out with 27 μg of 293-cell RetGC-1 or 293-cell RetGC-2 membrane protein. Ca^{2+}/EGTA buffers were prepared as described (24). Free Ca^{2+} concentrations in GC reaction mixtures containing Ca^{2+}/EGTA were calculated by using a multiparameter computer program that corrects for the effects of reaction components on Ca^{2+}/EGTA interaction (25). Free Ca^{2+} concentrations were also confirmed by a Ca^{2+} electrode and titration of the Ca^{2+}-sensitive fluorescent dye Rhod-2 (Calbiochem).

RESULTS

Cloning of Human RetGC-2 cDNA. To identify additional members of the membrane GC family we screened a human retina cDNA library with a pool of four probes derived from the cytoplasmic domains of NPR-A, NPR-B, STaR, and RetGC (also known as GC-A, GC-B, GC-C, and GC-E, respectively). To eliminate known sequences, hybridization was first performed at high stringency (≈100 positive clones), followed by a low-stringency screen to identify more distantly related sequences (≈60 additional positive clones). In addition to members of the soluble GC family, the low stringency-specific set of positive clones contained several partial cDNA clones encoding a protein with both a kinase homology domain and a GC domain. The presence of the two membrane GC signature domains (26) and a GenBank screen identifying RetGC as the most closely related sequence suggested that we had identified a membrane GC. Rescreening the cDNA library twice with progressively more 5′ probes resulted in the identification of a single long open reading frame of 1108 codons contained within 3723 bp of cloned cDNA.

This clone has been designated RetGC-2 due to its expression pattern, biochemical regulation (see below), and similarity to the previously identified RetGC (13), which we now term RetGC-1. The alignment of RetGC-2 and RetGC-1 predicted amino acid sequences is shown in Fig. 1. RetGC-2 has a 50-amino acid signal sequence preceding a 1058-residue mature protein of M_r 119,089. The 417-amino acid mature extracellular domain has no N-linked glycosylation sites and 8 Cys residues. The two proteins share 36% identity in the extracellular portion, 55% identity in the kinase homology domain (amino acids 475–764 of RetGC-2), and 88% identity in the cyclase domain (amino acids 779–1015 of RetGC-2). Both cyclases have C-terminal extensions of 44 amino acids beyond the cyclase domain, in analogy to the 69-amino acid extension in the heat-stable enterotoxin receptor, STaR.

Localization of RetGC-2 mRNA by *in Situ* Hybridization. ^{33}P-labeled sense and antisense transcripts corresponding to codons 601–830 of the RetGC-2 cDNA were hybridized to cryosections from rhesus monkey tissues and exposed for 6 weeks. Hybridization of the antisense probe was observed only in the photoreceptor layer of the retina (Fig. 2*A*); no signal was observed in this layer with the sense probe (Fig. 2*B*). Specific labeling was localized to the outer nuclear layer (photoreceptor cell bodies) and inner segments. Hybridization was not observed for either probe in a variety of other tissues: colon, jejunum, duodenum, ileum, lung, kidney, distal and intermediate lobes of the pituitary, adrenal, cerebral cortex, midbrain, and cerebellum. The hybridization pattern of RetGC-2 in the retina is indistinguishable from that of RetGC-1.

Immunoblot Analysis of RetGC-2 Expression. Affinity-purified antisera specific to the RetGC-2 sequence from Val-169 to Arg-180 (peptide 8) was used to demonstrate expression of recombinant protein in 293 cells and to detect the presence of RetGC-2 in ROS (Fig. 3). Transient expression in 293 cells followed by SDS/PAGE and immunoblot analysis of membrane proteins specially detected an ≈115-kDa protein in expression vector-transfected cells that is absent in membranes from control cells (Fig. 3*A*). Probing ROS for RetGC-2 with peptide 8 antibodies and with RetGC-1 antibodies (8), either alone or in combination, demonstrates that RetGC-2 is present in ROS and is slightly smaller than RetGC-1 (Fig. 3*B*).

Regulation of RetGC-2 GC Activity. We examined RetGC-2 for sensitivity to p24, the RetGC-1 activator (8). Lyophilized fractions from the final step of the activator purification were reconstituted with membranes from 293 cells transiently expressing RetGC-1 or RetGC-2 or with ROS membranes (Fig. 4). For all three sources of GC, peak stimulation of cGMP production was in C_4 reverse-phase-column fraction 47 (Fig. 4*A*), corresponding to the fraction most enriched in p24 (Fig. 4*B*). In separate experiments, we observed that RetGC-1 and RetGC-2 stimulating activity copurified at all steps of the activator purification, including heat treatment, phenyl-Sepharose chromatography, nondenaturing gel electrophoresis, and C_4 reverse-phase chromatography.

We also examined the Ca^{2+} sensitivity of RetGC-2 in the presence of p24 (Fig. 5). In the absence of p24, the RetGC-2 basal GC activity did not vary from a free Ca^{2+} concentration of 7 nM to 17 μM (Fig. 5*A*). The activation of RetGC-2 by p24-containing fractions was sharply inhibited by Ca^{2+}, with an EC_{50} value of 50–100 nM. Comparison of Ca^{2+} sensitivity between membranes from RetGC-2 transfected and untransfected cells shows that Ca^{2+}-sensitive p24-stimulated cGMP production is extremely dependent on expression of RetGC-2. These results demonstrate that in a reconstituted system, RetGC-2 can be activated by the retinal cyclase regulator, presumably p24 itself, in a Ca^{2+}-sensitive manner. These data strongly suggest a role for RetGC-2 in phototransduction and/or adaptation.

DISCUSSION

In a general screen for members of the membrane GC family, we employed a low-stringency hybridization strategy on a human retinal cDNA library. We chose the retina as a target to search for membrane GCs due to the complexity of the tissue, which is reflected in the expression of several known members of this family, NPR-A, NPR-B, and RetGC-1 (13–

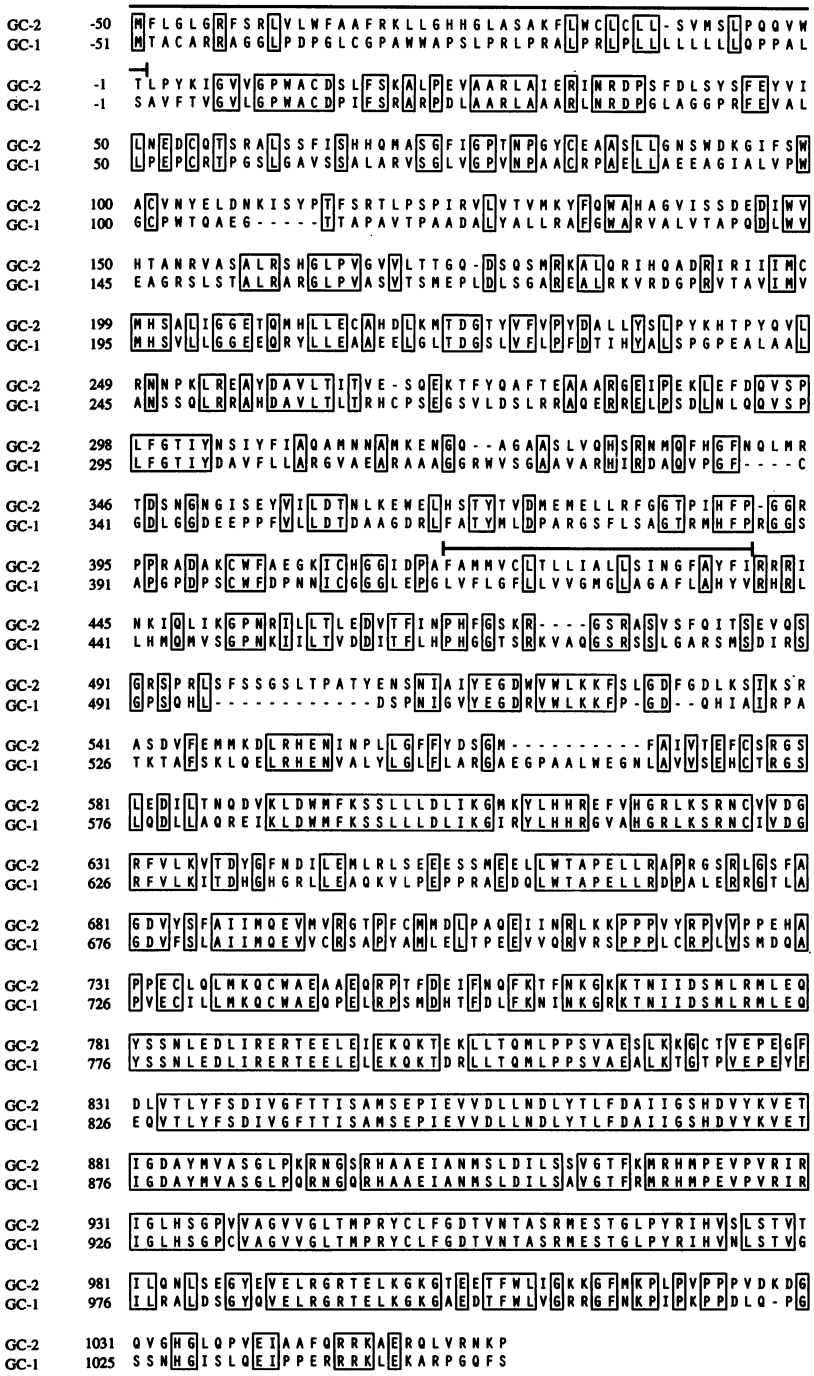

FIG. 1. Amino acid sequence homology between RetGC-2 (GC-2) and RetGC-1 (GC-1) is shown by boxing identical amino acids. The corrected sequence of RetGC-1 is used in this alignment (GenBank accession no. M92432). The deduced amino acid sequences for the precursors of the two human cyclases are shown in single letter code and numbered according to the predicted first residue of the mature proteins (+1). The signal sequence (positions −50 to −1) and the transmembrane domain (positions 418–440) are indicated by solid overlines.

15). This screen resulted in the cloning of cDNAs encoding a photoreceptor-specific membrane GC, RetGC-2. The expression pattern of RetGC-2 mRNA is indistinguishable from RetGC-1 by *in situ* hybridization, with regional localization of the photoreceptor cell bodies and inner segments. Although the antibodies we have to RetGC-2 recognize the protein on immunoblots, they have not proved suitable for immunocytochemical analysis. More detailed studies employing additional antibodies and higher-resolution techniques, such as immunoelectron microscopy or nonisotopic *in situ* hybridization, are required to determine whether these two cyclases are coexpressed or differentially expressed in rods and cones.

Two other groups have reported bovine and rat homologues of the human RetGCs. The sequence of bovine ROS GC (27) is 87% identical to the corrected human RetGC-1 sequence (GenBank accession no. M92432). The similarity is evenly distributed along the sequence, suggesting that these are orthologous proteins. In addition, two recently described membrane GCs from rat eye, termed GC-E and GC-F (28), are probably orthologous to human RetGC-1 and RetGC-2, respectively.

The hallmark of RetGC activity is Ca^{2+} inhibition (1, 2). Activation of photoreceptors results in cGMP hydrolysis, closure of the cGMP-gated cation channel, and inhibition of

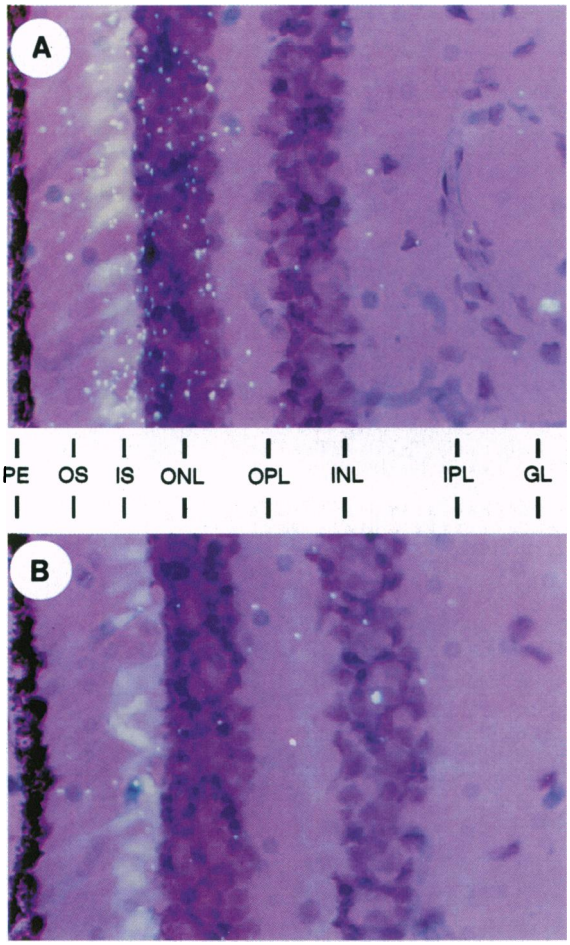

FIG. 2. Sections of rhesus monkey eye were probed with ^{33}P-labeled antisense (*A*) or sense (*B*) strand probes specific for RetGC-2. The retinal layers are pigmented epithelium (PE), outer segment (OS), inner segment (IS), outer nuclear layer/photoreceptor cells (ONL), outer plexiform layer (OPL), inner nuclear layer/bipolar cells (INL), inner plexiform layer (IPL), and ganglion cell layer (GL).

the dark current. A potential feedback relationship between Ca^{2+} and cGMP suggested that recovery of the dark state occurs as cGMP is resynthesized by activation of GC at low Ca^{2+} concentrations (3, 4). Restoration of the dark current results in elevated free Ca^{2+} levels, which then mediate cooperative inhibition of GC. The observation that cGMP synthesis at low Ca^{2+} is dependent on a soluble GC regulator set off a search to identify the soluble factors (5). To date there are two candidates for regulators of photoreceptors GC, p21 or GCAP, a recently described Ca^{2+} binding protein (6, 7), and p24 (8). The biochemical properties of these two proteins indicate that they are distinct, and recent data on the protein sequence of p24 indicates that it is also a Ca^{2+} binding protein (A.M.D., W. Henzel, E. Olshevskaya, and J.B.H., unpublished data) distinct from, but homologous to, p21.

RetGC-2 displays the biochemical properties expected of a photoreceptor GC involved in the recovery phase of the phototransduction cycle. Activation by p24 and inhibition by Ca^{2+} demonstrate that RetGC-2 is similar to RetGC-1 in its regulation. Previous work on the purification and amino acid sequencing of the 110- to 112-kDa GC from ROS demonstrated identity with RetGC-1 and did not suggest heterogeneity of GC (11, 12). Although DEAE-cellulose chromatography gave two peaks of GC activity from rod disk membranes (29), heterogeneity of Asn-linked carbohydrate on photoreceptor GC could account for this (12). In addition, comparison of RetGC-1 and ROS GC kinetic properties does not suggest

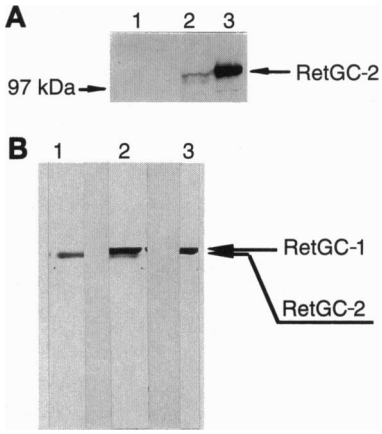

FIG. 3. Immunoblot detection of RetGC-2 transiently expressed in 293 cells (*A*) and detection of RetGC-1 and -2 in ROS (*B*). Recombinant RetGC-2 is visible as an ≈115-kDa band from both transfected cells (*A*, lane 2) and ROSs (*A*, lane 3) compared to control cells (*A*, lane 1). ROS membranes were fractionated by SDS/PAGE, blotted to nitrocellulose, and cut into strips that were probed with RetGC-2 antisera (*B*, lane 1), RetGC-1 peptide antisera (*B*, lane 3), or both antisera (*B*, lane 2).

functional heterogeneity (8) of photoreceptor GC. These observations suggest that RetGC-2 may be relatively less abundant than RetGC-1 in the retina.

The presence of two GC isoforms in the photoreceptor layer of the retina is not surprising but reveals a hitherto unappreciated complexity. Other components of the phototransduction cascade are present as rod- or cone-specific isoforms [including opsins (30); transducin α, β, and γ subunits (31–33); phosphodiesterase catalytic (34) and inhibitory (35) subunits; and the cGMP-gated cation channel (36–38)]. Currently we do not know the extent to which RetGC-1 or RetGC-2 may be

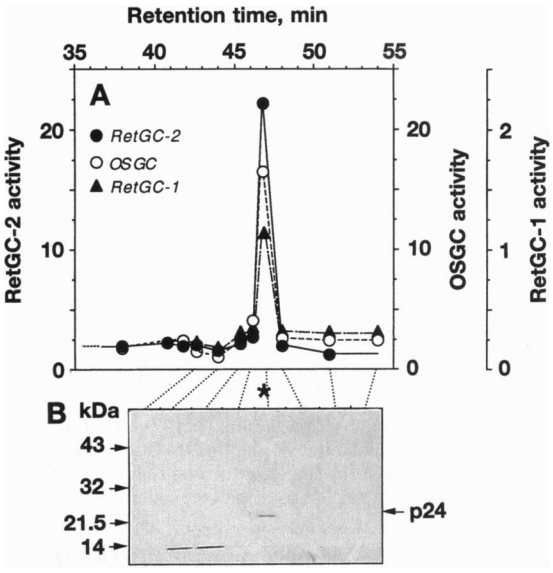

FIG. 4. RetGC-2 and RetGC-1 stimulating activity copurifies with GC activator p24. Heat-treated bovine retinal extracts were purified by phenyl-Sepharose chromatography and nondenaturing gel electrophoresis and then fractionated by HPLC on a C_4 reverse-phase column (*A*). GC activity was measured by reconstituting lyophilized HPLC fractions with membranes from 293 cells expressing RetGC-2, RetGC-1, or membranes from bovine ROSs (OSGC). Coincidence of p24 and GC stimulating activity is indicated by SDS/PAGE analysis of HPLC column fractions (*B*). Units: RetGC-2 activity, pmol of cGMP per min per mg of protein; OSGC activity, nmol of cGMP per min per mg of protein; RetGC-1 activity, nmol of cGMP per min per mg of protein.

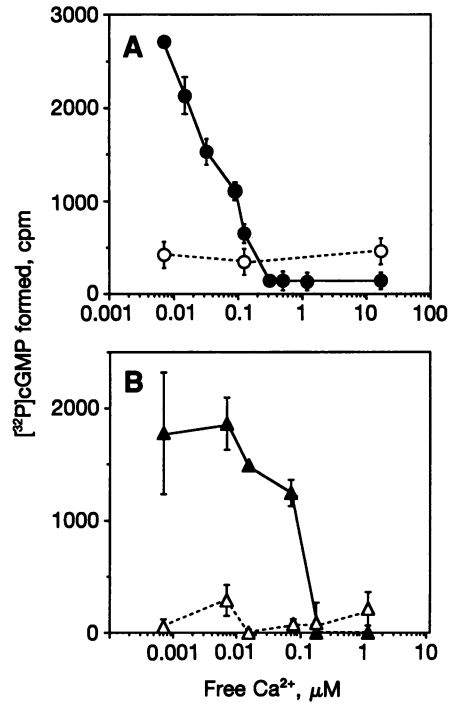

FIG. 5. (*A*) Ca^{2+} sensitivity of RetGC-2 was measured on membranes from 293 cells transiently expressing RetGC-2 reconstituted with (●) or without (○) electrophoretically purified activator. (*B*) Membranes from untransfected (△) or RetGC-2-transfected (▲) 293 cells were reconstituted with activator and GC activity was measured as a function of free Ca. Data are the average ± SD of three measurements.

expressed in a cell-type-specific manner. In fact, RetGC-1 is present in both rods and cones, with apparently higher levels in cones (8, 39). These two enzymes may also hold a preference for either of the activators p21 (7) or p24 (8) and/or have different kinetics of activation and Ca^{2+} inhibition. Addressing these questions should help to define the mechanistic role of both the cyclases and their regulators in photoreceptor recovery and adaptation.

We thank the Genentech peptide synthesis group for synthetic peptides, Dr. David L. Garbers for communication of results before publication, Dr. Nancy Gillett for advice on *in situ* hybridization, Greg Bennett and Jill Schoenfeld for technical assistance, Allison Bruce for figures, and Kathie Lewis for secretarial assistance. This work was supported in part by a grant to A.M.D. and J.B.H. from the Human Frontiers Science Program Organization, and by National Institutes of Health Grant EYO 6441 to J.B.H.

1. Lagnado, L. & Baylor, D. (1992) *Neuron* **8,** 995–1002.
2. Yarfitz, S. & Hurley, J. B. (1994) *J. Biol. Chem.* **269,** 14329–14332.
3. Pepe, I. M., Panfoli, I. & Cugnoli, C. (1986) *FEBS Lett.* **203,** 73–76.
4. Pepe, I. M., Boero, A., Vergani, L., Panfoli, I. & Cugnoli, C. (1986) *Biochim. Biophys. Acta* **889,** 271–276.
5. Koch, K. W. & Stryer, L. (1988) *Nature (London)* **334,** 64–66.
6. Gorczyca, W. A., Gray-Keller, M. P., Detwiler, P. B. & Palczewski, K. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4014–4018.
7. Palczewski, K., Subbaraya, I., Gorczyca, W. A., Helekar, B. S., Ruiz, C. C., Ohguro, H., Huang, J., Zhao, X., Crabb, J. W. Johnson, R. S., Walsh, K. A., Gray-Keller, M. P., Detwiler, P. B. & Baehr, W. (194) *Neuron* **13,** 395–404.
8. Dizhoor, A. M., Lowe, D. G., Olshevskaya, E. V., Laura, R. P. & Hurley, J. B. (1994) *Neuron* **12,** 1345–1352.
9. Hayashi, F. & Yamazaki, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4746–4750.
10. Koch, K. W. (1991) *J. Biol. Chem.* **266,** 8634–8637.
11. Margulis, A., Gorazniak, R. M., Duda, T., Sharma, R. K. & Sitaramayya, A. (1993) *Biochem. Biophys. Res. Commun.* **194,** 855–861.
12. Koch, W.-W., Stecher, P. & Kellner, R. (1994) *Eur. J. Biochem.* **222,** 589–595.
13. Shyjan, A. W., de Sauvage, F. J. Gillett, N. A., Goeddel, D. V. & Lowe, D. G. (1992) *Neuron* **9,** 727–737.
14. Ahmad, I. & Barnstable, C. H. (1993) *Exp. Eye Res.* **56,** 51–62.
15. Duda, T., Goracznaiak, R. M., Sitaramayya, A. & Sharma, R. K. (1993) *Biochemistry* **32,** 1391–1395.
16. Koch, K.-W., Lambrecht, H.-G., Haberecht, M., Redburn, D. & Schmidt, H. H. H. W. (1994) *EMBO J.* **13,** 3312–3320.
17. Lowe, D. G., Chang, M. S., Hellmiss, R., Chen, E., Singh, S., Garbers, D. L. & Goeddel, D. V. (1989) *EMBO J.* **8,** 1377–1384.
18. Chang, M. S., Lowe, D. G., Lewis, M., Hellmiss, R., Chan, E. & Goeddel, D. V. (1989) *Nature (London)* **341,** 68–72.
19. de Sauvage, F. J., Camerato, T. R. & Goeddel, D. V. (1991) *J. Biol. Chem.* **266,** 17912–17918.
20. Nathans, J., Thomas, D. & Hogness, D. S. (1986) *Science* **232,** 193–201.
21. Lu, L. H. & Gillett, N. A. (1994) *Cell Vision* **1,** 169–176.
22. Bennett, B. D., Bennett, G. L., Vitangcol, R. V., Jewett, J. R. S., Burnier, J., Henzel, W. & Lowe, D. G. (1991) *J. Biol. Chem.* **266,** 23060–23067.
23. Gorman, C. M., Gies, D. R. & McCray, G. (1990) *DNA Protein Eng. Technol.* **2,** 3–10.
24. Tsien, R. & Pozzan, T. (1989) *Methods Enzymol.* **172,** 230–262.
25. Marks, P. W. & Maxfield, F. R. (1991) *Anal. Biochem.* **193,** 61–71.
26. Garbers, D. L. & Lowe, D. G. (1994) *J. Biol. Chem.* **269,** 30741–30744.
27. Goraczniak, R. M., Duda, T., Sitaramayya, A. & Sharma, R. (1994) *Biochem. J.* **302,** 455–461.
28. Yang, R.-B., Foster, D. C., Garbers, D. L. & Fulle, H.-J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 602–606.
29. Hakki, S. & Sitaramayya, A. (1990) *Biochemistry* **29,** 1088–1094.
30. Jackson, T. (1991) *Pharmacol. Ther.* **50,** 425–442.
31. Lerea, C. L., Somers, D. E., Hurley, J. B., Klock, I. B. & Bunt-Milam, A. H. (1986) *Science* **234,** 77–80.
32. Lee, R. H., Lieberman, B. S., Yamane, H. K., Bok, D. & Fung, B. K. (1992) *J. Biol. Chem.* **267,** 24776–24781.
33. Peng, Y. W., Robishaw, J. D., Levine, M. A. & Yau, K. W., (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10882–10886.
34. Hurwitz, R. L., Bunt-Milam, A. H., Chang, M. L. & Beavo, J. A. (1985) *J. Biol. Chem.* **260,** 568–573.
35. Hamilton, S. E. & Hurley, J. B. (1990) *J. Biol. Chem.* **265,** 11259–11264.
36. Kaupp, U. B., Niidome, T., Tanabe, T., Terada, S., Boenigk, W., Stuehmer, W., Cook, N. J., Kangawa, K., Matsuo, H., Hirose, T., Miyata, T. & Numa, S. (1989) *Nature (London)* **342,** 762–766.
37. Bonigk, W., Altenhofen, W., Muller, F., Dose, A., Illing, M. Molday, R. S. & Kaupp, U. B. (1993) *Neuron* **10,** 865–877.
38. Chen, T., Peng, Y. W., Dhallan, R. S., Ahamed, B., Reed, R. R. & Yau, K. W. (1993) *Nature (London)* **362,** 764–767.
39. Liu, X., Seno, K., Nishizawa, Y., Hayashi, F., Yamazaki, A., Matsumoto, H., Wakabayashi, T. & Usukura, J. (1995) *Exp. Eye Res.* **59,** 761–768.