

Bovine cone photoreceptor cGMP phosphodiesterase structure deduced from a cDNA clone

(visual transduction/cyclic nucleotide-binding sites/CAAX motif)

TIANSEN LI, KRISTI VOLPP, AND MEREDITH L. APPLEBURY*

Departments of Ophthalmology and Visual Sciences; and Pharmacological and Physiological Sciences, The University of Chicago, Eye Research Laboratories, 939 E. 57th Street, Chicago, IL 60637

Communicated by Aron A. Moscona, September 20, 1989

ABSTRACT A full-length cDNA clone encoding the α' subunit of cGMP phosphodiesterase (PDE) from bovine cone photoreceptors was selected by probing a retinal library with a DNA fragment encoding the catalytic core of the rod cGMP PDE α subunit. Identity of the clone was confirmed by comparing its deduced sequence with cone PDE peptide sequences determined by Charbonneau *et al.* [Charbonneau, H., Prusti, R. K., LeTrong, H., Sonnenburg, W. K., Mullaney, P. J., Walsh, K. A. & Beavo, J. A. (1990) *Proc. Natl. Acad. Sci. USA*, pp. 288–292]. The cone PDE α' and the rod PDE α and β subunits are encoded by distinct genes. cGMP PDE subunits share a common ancestry with cAMP PDEs and cyclic nucleotide-binding proteins. Sequence comparisons predict the presence of a catalytic core and possible secondary sites for noncatalytic cGMP binding. The presence of a C-terminal CAAX (Cys-aliphatic-aliphatic-Xaa) motif suggests the cone enzyme may be posttranslationally modified by proteolysis, methylation, and isoprenylation.

cGMP phosphodiesterase (PDE) is the pivotal enzyme that elicits visual signaling in vertebrate photoreceptor cells. The enzyme is the target of G protein activation in the cGMP cascade. Its substrate cGMP is a second messenger that allosterically affects the conductance of the photoreceptor plasma membrane ion channels. Briefly, upon absorption of light the visual pigment receptors catalyze nucleotide exchange by the G protein G_t (transducin). The active form of transducin, GTP- $G_t\alpha$, disinhibits cGMP PDE activity. Increased hydrolysis reduces the cytoplasmic concentration of cGMP, leads to channel closure, hyperpolarization of the plasma membrane, and, therefore, visual signaling (1).

Biochemically distinct forms of PDEs are found in rod and cone photoreceptors, indicating that these cells use similar, but functionally different, pathways of signaling. In bovine rods, the holoenzyme $\alpha\beta\gamma_2$ is composed of two similar large subunits, α (88 kDa) and β (84 kDa), and two small γ subunits (13 kDa) (2, 3). The bovine cone enzyme is composed of two identical α' subunits (90 kDa) and is associated with smaller subunits of 15, 13, and 11 kDa (4). A bovine cDNA has been cloned that encodes the rod α subunit (5). We report here the isolation of cDNA clones encoding the bovine cone α' subunit[‡] and compare properties of the rod and cone transcripts and primary amino acid sequences.

METHODS AND MATERIALS

Materials and General Methods. mRNA and DNA were prepared from fresh adult or calf bovine eyes obtained from local abattoirs. Enzymes used for recombinant DNA manipulations were obtained from Boehringer Mannheim. ³²P- and ³⁵S-labeled nucleotides were purchased from Amersham.

RNA size markers were purchased from BRL. General techniques of recombinant DNA fragment preparation, probe labeling, Southern and Northern (RNA) blotting, end-labeling, etc., were carried out according to Maniatis *et al.* (6) or as described in Ausubel *et al.* (7). The 5' primer extension was performed (8, 9) with 3 μ g of bovine retina poly(A)⁺ RNA using a synthetic oligomer complementary to nucleotides 55–84 of the cone sequence (Fig. 2). The size was estimated from a known DNA sequencing ladder; brain poly(A)⁺ RNA was used as a control. As shown in Fig. 1, double-stranded DNA fragments were sequenced using the dideoxynucleotide chain-termination method of Sanger *et al.* (10). Progressive unidirectional deletion clones of p21-1 were prepared using exonuclease III and mung bean nuclease according to the supplier's protocols (Stratagene). Sequencing was completed for both strands of DNA fragments, and data were aligned, decoded, and examined using MICROGENIE (Beckman).

RNA and DNA Preparation and Hybridization. Total retinal or brain RNA was prepared according to Chirgwin *et al.* (11). Poly(A)⁺ RNA was selected with oligo(dT)-cellulose (P-L Biochemicals) according to Aviv and Leder (12). DNA was prepared from bovine spleen according to Frischauf *et al.* (13). Southern and Northern blot hybridizations, as shown in Figs. 3 and 4, were carried out at high stringency, 6 \times SSC (1 \times SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7), 0.5% SDS, 5 \times Denhardt's solution (1 \times Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin) at 68°C and washed with 0.2 \times SSC at 68°C. The following probes were used for hybridization: PDE α subunit (p13), a 3.0-kilobase (kb) insert containing the complete coding region plus 70 base pairs (bp) of 5'-untranslated regions and 326 bp of 3'-untranslated regions; PDE β subunit (p21-22), a 2.0-kb insert including the C-terminal half of the coding and the complete 3'-untranslated regions; cone PDE (p21-1), the 2.9-kb insert including the complete coding, 54 bp of 5'-untranslated and complete 3'-untranslated regions (see Fig. 2); opsin (SP1116), a 1.1-kb probe described by Martin *et al.* (14); and a 140-bp *Pst* I-*Nco* I N-terminal fragment prepared from SP1116 that includes 30 bp of 5'-untranslated and 110 bp of the N-terminal coding region of opsin cDNA used for Northern blotting (see Fig. 4).

Retinal cDNA Libraries, Probes, and Screening. To initiate this work, a rod PDE α subunit cDNA clone was obtained from a bovine retinal cDNA phage library (15) by probing with two synthetic 30-nucleotide oligomers corresponding to bases 91–120 and 2518–2547 of the PDE α subunit sequence (5). Partial sequencing of the clones and extensive restriction mapping indicated that the clones were identical to the PDE α subunit previously described (5). For subsequent screening under reduced stringency, a 657-bp *Eco*RI fragment (bases 1655–2311) was prepared from the PDE α subunit clone as a

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.

Abbreviation: PDE, phosphodiesterase.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M29465).

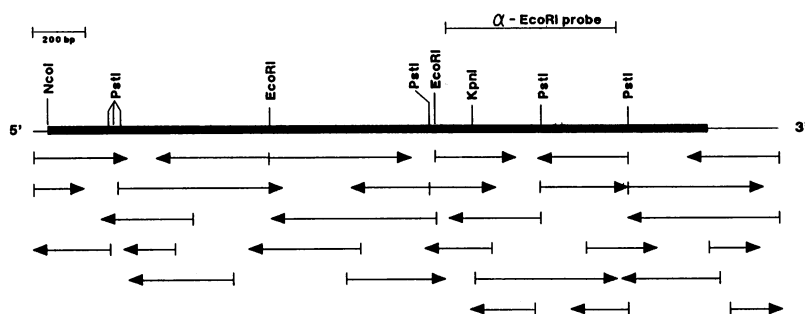


FIG. 1. Probe and sequencing strategy for a bovine cone PDE cDNA clone (p21-1). The α -EcoRI probe, derived from a rod PDE α subunit cDNA, was used to select the cone clone. The restriction sites marked were used to prepare subclones or directionally deleted clones for sequencing.

probe (Fig. 1). Hybridization of the probe under conditions of $6\times$ SSC at 58°C and washing with $2\times$ SSC at 58°C identified 36 clones. These clones were further fractionated by differentially washing at given temperatures and concentrations of SSC towards increasingly higher stringency. A subgroup of 20 clones was selected that lost the ability to cross hybridize upon washing at $0.2\times$ SSC above 67°C . The largest clone in this group (p21) contained an insert of ≈ 1.1 kb; it was sequenced and used for further screening. To obtain full-length clones, additional bovine retinal cDNA libraries were prepared, as described by Kimmel and Berger (16) by using a cDNA synthesis kit from BRL and the λ Zap vector (Stratagene). The insert size averages ≈ 1.5 kb. Eight nearly full-length cone PDE clones were selected at high stringency with p21; these clones were excised as Bluescript SK(-) phagemids and mapped. One clone, p21-1, was fully sequenced, and others were partially sequenced.

Restriction mapping and cross hybridization of another set of clones showed that they were a distinct group from those encoding rod PDE α subunit or cone PDE α' subunit. These clones were confirmed to encode PDE β subunit by hybridization to a 50-nucleotide oligomer synthesized according to a partial nucleotide sequence for PDE β subunit cDNA provided by V. Lipkin (personal communication) and by sequence analysis. The insert of clone p21-22 is 2.0 kb and recognizes the same 3.4-kb transcript in RNA blots as the 50-mer synthetic probe.

RESULTS

Isolation of Cone PDE Clones. To identify clones that might encode additional members of the PDE family, a retinal cDNA library was probed at low stringency with a DNA fragment encoding the α subunit of rod PDE. The fragment chosen (Fig. 1) encompasses most of the catalytic core that has been identified as common to many PDEs (5, 17). Homologous cDNA clones were selected allowing for $\approx 35\%$ or less base mismatch and further sorted by washing at increased stringencies. Three small clones were sequenced and shown to derive from the same gene. The deduced amino acid sequence had $\approx 70\%$ amino acid identity with rod PDE α subunit. Full-length clones were selected from an unamplified bovine retinal λ Zap library, using one of the three clones (p21). Eight nearly full-length clones were analyzed; three clones started before the putative translation initiation site and contained full-length 3' untranslated regions with polyadenylation. The probe used to select these clones and the sequencing strategy for a 2900-bp cDNA (p21-1) is given in Fig. 1.

Sequence and Identification of a Cone PDE cDNA. The sequence of the p21-1 cDNA is shown in Fig. 2. Analysis of PDE mRNA by 5' primer extension indicates that the 5' end extends ≈ 107 bases beyond the first methionine codon in the sequence (data not shown). Addition of a ≈ 250 -base poly(A) tail at the 3' untranslated end and ≈ 53 additional bases indicated for the 5' untranslated end to the 2900-base cloned fragment would predict a transcript size of ≈ 3.4 kb, close to the size measured by RNA blotting of retinal mRNA (see Fig.

4). Although no stop codons precede the first methionine codon indicated, we have tentatively assigned this ATG as the point of translational initiation because it is colinear in position with the N-terminal methionine of the PDE α subunit, and the surrounding bases are compatible with the Kozak consensus initiation site for eukaryotic mRNAs (18). An open reading frame of 2565 bases encodes an 855-amino acid polypeptide the calculated M_r of which is 98,748. In the 3'-untranslated region, two acceptable sequences for polyadenylation (19) are found; a less frequently used sequence is 21 bases upstream of the terminal polyadenines and a second, 80 bases upstream (Fig. 2). The predicted sequence is colinear with the 859-amino acid rod PDE α subunit polypeptide throughout the coding area. The two PDEs are 66% identical at the nucleotide level and 63% identical in their deduced amino acid sequences.

The deduced amino acid sequence from the p21-1 clone differs in 26 out of the 68 amino acid residues that have been published for the rod PDE β -subunit polypeptide (5). In contrast, the deduced sequence corresponds completely with 19 peptides corresponding to 43% of the bovine cone PDE protein that Charbonneau *et al.* (20) have isolated and sequenced. We conclude that the cDNA clone (p21-1) encodes the large subunit (α') of the bovine cone cGMP PDE that has been characterized by Gillespie and Beavo (4).

Distinct Genes and Transcripts Encode Rod and Cone PDE Subunits. To further distinguish the identities of the three known α , β , and α' (cone) subunits of PDE, probes for each of the cloned cDNAs were used for Southern analysis of the bovine genome and Northern analysis of the transcripts. Each probe detects multiple genomic restriction fragments, indicative of large genes or multiple gene copies (Fig. 3). However, the patterns observed for each probe are distinct; no common bands are detected for any given restriction enzyme. These results and the lack of regions of sequence identity indicate that each of the rod and cone subunits is encoded by a distinct gene.

Northern analysis with the same probes indicates that transcript sizes and abundance vary as well (Fig. 4). The cone PDE probe identifies a ≈ 3.4 -kb retinal mRNA but fails to detect any transcripts from brain tissue. The relative abundance was measured by densitometry of films exposed to the blots. The probes used were equal in concentration and specific activity, and a correction for length of the β -PDE and opsin probes was made. The cone transcript is ≈ 10 -fold less abundant than rod α and β transcripts, which are roughly equal in abundance. In a separate Northern blotting experiment (data not shown), the 3.4-kb β transcript was shown to be ≈ 80 -fold less abundant than opsin transcripts. The major PDE α -subunit transcript is ≈ 4.6 kb; a less abundant species, which may be incompletely processed, or a minor alternative transcript is apparent at ≈ 5.8 kb. We interpret the faint hybridization seen with the α probe at 3.4 kb to be cross hybridization with β transcripts; however, it might reflect a true minor PDE α -subunit species.

-GGTGTCTGAGGGTCTCTCCCTTTAGACAGCAGCAGCAGCAGCCAGCCATGGGTGAGATGACGAAAGAGCGGTGGAGAAATACCTGGAGGCCAACCTCAGTTCGCAAGGATTTTCAACGAAAGTTCAGGTGGAGGTG 150
 M G E I S Q E T V E K Y L E A N P Q F A K E Y F N R K L Q V E V 32
 CGAGCGGGCGGGGACAGCCAGCCGCGCCCTCTCCCGGGCGGACGCTGGCGGAGGAGCGCCCTGTACCTGGAGTCTGTGGAGGCTTGTGGAGGAGCGGGCAGCGTGGCGCACACAGGGCCCTGCAGAGGCTG 300
 P S G G A Q A P A S A S F P G R T L A E E A A L Y L E L L E V L L E E A G S V E L A A H R A L Q R L 82
 GCGCAGCTGCTGCGGACAGCCGCTGCAGCATGTTCTGTGTGCGGCGCCGCAACGCCAGCCGCGGAGGTGGCTTCAAGCTGCTGATGTCACCCCACTTCCAAGTTTGAGGACAACCTGGTGGTCCCGACAGAGAAGCTGTGTTCCG 450
 A Q L L Q A D R C S M F L C R A R N G T P E V A S K L L D V T P T S K F E D N L V V P D R E A V F P 132
 TTGGAGTGGGATAGTGGGTGGTCTCACACGAAGAAACCTTTAATGTTCCAGATGTGAAAAGAAGACGCCATTTTCTGACTTACGGCAACAAACCGGGTATGTCACCGAAGCTGCTGGCAACCCCATCGTGGTGGG 600
 L D V G I V G W V A H T K K T F N V P D V K K N S H F S D F M D K Q T G Y V T R N L L A T P I V M G 182
 AAGGAGTCTGCTGTGTTTATGGCAGTAAACAAGTAGACGCATCCGAATTTTCAACAGGATGAAGAGGCTCTTCCAAATACCTCAGCTTGTGTTCTATCATCTTAAAGCTTCCACACCACTACTCTGACAATTAATGATCC 750
 K E V L A V F M A V N K V D A S E F S K Q D E E V F S K Y L S F V S I I L K L H H T N Y L Y N I E S 232
 CGAAGAAGTCAGATCTTATGTGGTCAGCCAACAAGTATTGAAGAGCTCACAGATGTTGAGCGACAGTTTCAAAAGCACCTACACTGTCAGGACATACCTGAACTGCGAAGCATACTCCATTGGGCTGCTGGACATGACCAAGGAG 900
 R R S Q I L M W S A N K V F E E L T D V E R Q F H K A L Y T V R T Y L N C E R Y S I G L L D M T K E 282
 AAGGAATCTATGATGAGTGGCAGTCAAGCTGGGAAGTCGAGCCTTACAAGGTCGAAGACACAGATGCGAGAGGATCATCTTTATAAAATCATCGATTACATTTACATGGAAGAAGAGATCAAGTTCATCCGACACCT 1050
 K E F Y D E W P V K L G E V E P Y K G P K T P D G R E V I F Y K I I D Y I L H G K E I K W I P T P 332
 CCCATGGACCCTGGACTCTCATTAGTGGGTGCCAACATATGTTGCTGAAATGGATTATCTGCAACATGCTGAACCGCCCGGGGATGAATCTCAGCTTTCAGAAAGGACCTGTAGATGAACTGGCTGGGTCATTAATAATGTC 1200
 P M D H W T L I S G L P T Y V A E N G F I C N M L N A P A D E Y F T F Q K G P V D E T G W V I K N V 382
 TTGTCCTTGCCTATTGTCAACAAAAGGAAGACATCGTGGCGTAGCTACATTTTACAACAGGAAGGATGGAAAGCCTTTTGGATGATGAGCAGACATCGCTGAGACTCTCACAGGTTTCTGGATGCTCTCTAAATACGAC 1350
 L S L P I V N K K E D I V G V A T F Y N R K D G K P F D E Y D E H I A E T L T Q F L G W S L L N T D 432
 ACCTATGAGAAAATGAATAGCTGGAGAACAGAAAGACATAGCCAGGAAATGCTCATGAACACACCAAGGCTACACCTGATGAGATCAAGTCTATTTGAAATTTAAAGAGAAATTAATATAGATGTAATGAAGACTGTGAAGAA 1500
 T Y E K M N K L E N R K D I A Q E M L M N H T K A T P D E I K S I L K F K E K L N I D V I E D C E E 482
 AAACAGCTGTGACAAATTTGAAGGAGGACCTGCCAGACCACGGACTGCAGACCTGTATGAATTCGCTTCAGGCACTGCCATCACAGAGCAGCAACTGATTAAAGTGGACTTCGGCTGTTTTTGAATAAAGCTGGTGGAGAAA 1650
 K Q L V T I L K E D L P D P R T A D L Y E F R F R H L P I T E H E L I K C G L R L F F E I N V V E K 532
 TTCAAAGTACCTGTAGAGGTTCTCACCAGATGGATGTACACGGTGAAGGAGGGTACCGGGCGTCCACATCCACAACTGGCGACACGGGTTAAAGTGGGAGACCATGTTCACTTTGCTGATGACAGGAAGACTGAAGAAATAC 1800
 F K V P P Y E V L T R W M Y T V R K G Y R A V T Y H N R H G F N V G Q T M F T L L M T G R L K K Y Y 582
 ACAGACCTGGAAGCTTTGCCATGCTGCTGCTGCTGCTGCGCAGCATTGACACAGAGGACCAATAATTTGTATCAGATGAAGTCCACATCTCCGCTAGCAAGGCTTCACGGCTCTTCACTCTGGAGAGGACACCTGGAGTAC 1950
 T D L E A F A M L A A A F C H D I D H R G T N N L Y Q M K S T S P L A R L H G S S I L E R H H L E Y 632
 AGTAAGACGCTCTGACAGGATGAGGTTTAAACATATTTCCAGAACTAAATAAAGCCAGATGAAGACGTTTATCATTTTGAAGTTCGATAATAGCAGCTGACCTGGCTTTATTTCAAGAGAGAACCATGTTTCAAAAAAT 2100
 S K T L L Q D E S L N I F Q N L N K R Q Y E T V I H L F E V A I I A T D L A L Y F K K R T M F Q K I 682
 GTTATGCTGTGAAAAATGAAACTGAAGAGAGGCCATCAATATGTAACCATGATCAACAAAAAAGAGATATCATGGCAATGATGATGACTGGCTGTGATCTGCTGAGTATAACCAAGCCCTGGGAGGTGCAAGTCAAGT 2250
 V D A C E K M E T E E E A I K Y V T I D P T K K E I I M A M M M T A C D L S A I T K W E V Q S Q V 732
 GCACCTCTGGTGCAAATGAATTTGGGAACAGGAGATCTGGAGAGAACAGTCTGCAGCAACAACCCATCTCATGATGGACAGAAAACAAAGATGAACCTAAACTCAAGTGGATTATTGACTTTGTCTGACTTTTGT 2400
 A L L V A N E F W E Q G D L E R T V L Q Q Q P I P M M D R N K K D E L P K L Q V G F I D F V C T F V 782
 TATAAGGAGTTTTCACGGTTTCAACAAAGAAATCACACCTATGCTGAATGGTCTTCAAAACACAGAGTGGAAATCCCTAGCTGATGAGTATGATGAAAAGATGAAGGTAATGGAAGAGATGAAAAGCAGGAAGAAAGGAAATACG 2550
 Y K E F S R F H K E I T P M L N G L Q N N R V E W K S L A D E Y D E K M K V I E E M K K Q E E G N T 832
 ACAGAAAAGCTGAGAAGATTCAGGAGGTGGTGGTGTGACAAGAAATCCAAACATGCTCAATGTTGTAAGAAATCAAAATGTTTCTACATTTGAAGAAAACATAAAAAAGAACATCAGCAAAAATTC 2700
 T E K A V E D S G G D D K S K T C L M L * 855
 ATTATCAAAACCTCAAGTACAGAGACTCTTCAGGAAGGCTACCTGTGACTAGCATAGAATAATAGTCTAGCTGAGCTGAAAGCCATGAGGATAAATAAGTTCACAAAAGTGCAAAATAGACAAAATAAGTGCAATTTTGGTGC 2850
 CAATTTGTTTAAATAATAATAATCTGAAAAAATAAAAAA 2900

Fig. 2. Nucleotide sequence and derived amino acid sequence of the bovine cone cGMP PDE. The underlined residues correspond to bovine cone peptides sequenced by Charbonneau *et al.* (20). The deduced sequence is identical with 362 peptide amino acids. Residue Asn-453 (N) is found as aspartic acid (D) in the peptide. Boxed residues indicate consensus sequences for poly(A) addition (19).

DISCUSSION

Light triggers visual responses in vertebrate rod and cone photoreceptors by activating components of cGMP cascades (1). The general physiological properties of signaling are shared in both cell types, but several functional aspects, such

as response times, sensitivities, and adaptation, suggest that underlying molecular mechanisms of these cells must differ (21). By identifying the particular molecular components of transduction in each photoreceptor type, functional distinction may be further corroborated. Molecular cloning of

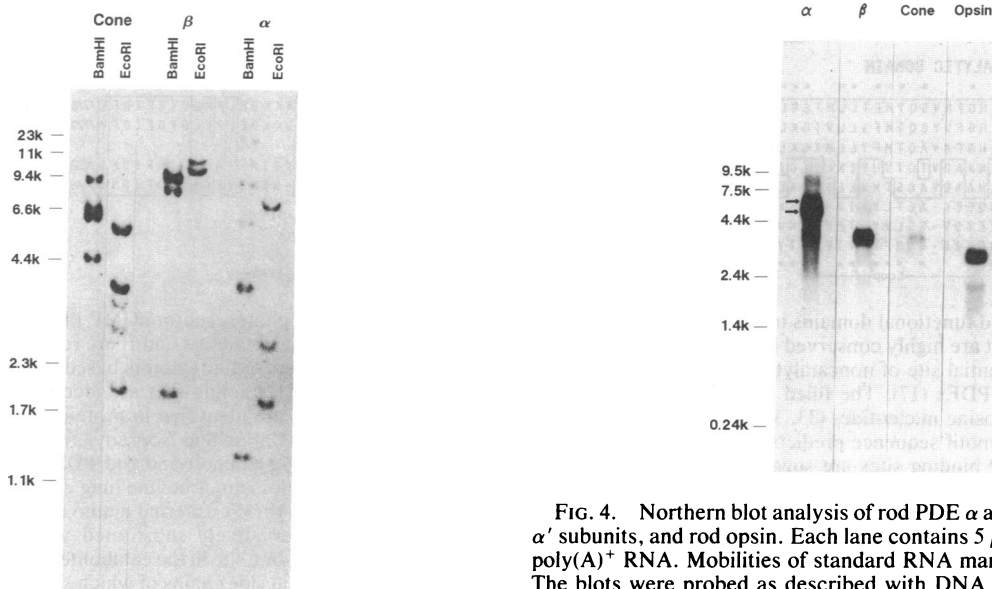


FIG. 3. Genomic Southern blot analysis of the bovine cone PDE α' subunit, rod PDE α -subunit and β -subunit genes. Each lane contains 5 μ g of DNA. The *Bam*HI and *Eco*RI digests were probed at high stringency with fragments of cone PDE α' subunit, rod PDE α subunit, and rod PDE β subunit as described.

FIG. 4. Northern blot analysis of rod PDE α and β and cone PDE α' subunits, and rod opsin. Each lane contains 5 μ g of bovine retinal poly(A)⁺ RNA. Mobilities of standard RNA markers are indicated. The blots were probed as described with DNA fragments of equal specific activity and concentration. Each was hybridized at the same stringency and exposed for 16 hr. Opsin was probed with a 140-base N-terminal probe to achieve comparable signal intensity; correcting for probe length indicates the intensity would be \approx 20-fold greater. The arrows in lane α indicate discrete bands observed for shorter exposure.

photoreceptor proteins has shown that the rod rhodopsin and the red, green, and blue cone opsins are each encoded by distinct genes (15, 22). The rod and cone transducins are also encoded by distinct cDNAs (23). Through biochemical isolation, Beavo and colleagues (4, 24) have established that rod and cone cGMP PDEs are composed of distinct polypeptides that have different properties of activation and cGMP binding. This work establishes that subunits of the rod and cone cGMP PDEs are encoded by distinct genes (Fig. 3). As for a cDNA clone encoding cone transducin (23), it is unclear whether the cone PDE here characterized is expressed commonly in red, green, and blue cones, or whether additional genes for individual cell types exist.

The relative abundance of mRNAs encoding components of the cGMP cascade parallels the molar ratio of proteins and the ratio of rods to cones. In the rod outer segment, rhodopsin is ≈ 100 -fold more abundant than cGMP PDE (2). The transcript encoding opsin is likewise ≈ 80 -fold greater than those of PDE α or β subunit (Fig. 4). The rod PDE mRNAs are ≈ 10 -fold greater than the cone PDE mRNA levels, which correlates with the ratio (10:1) of rods to cones seen in bovine retina (25). Given the ratio of rod opsin transcripts to cone PDE transcripts (1:800) and the abundance of opsin mRNA in the retina (0.5%) (15), the cone PDE transcript may be calculated to be $\approx 0.0006\%$ of retinal mRNA.

The 855-amino acid residue cone PDE α' subunit (Fig. 2) is slightly smaller than the 859-residue rod PDE α subunit. Neither of the calculated molecular masses, 98,748 Da (cone)

or 99,248 Da (α -PDE), is consistent with the molecular masses assigned from the mobilities determined by SDS gel electrophoresis—90,000 Da (cone) (4) and 88,000 Da (PDE α subunit). The inconsistency is not from presence of carbohydrate, for the rod PDE is not glycosylated (2). However, both the rod α and cone α' subunits may be modified at their C-termini. Each contains a terminal (Cys-aliphatic-aliphatic-Xaa) motif (26) (Figs. 1 and 4), suggesting that the last 3 amino acids may be removed (27), and the remaining cysteine residue may be modified as an isoprenyl thioester (28) and carboxymethylated (29). Evidence for carboxymethylation of the α subunit of rod PDE has been seen (30) to support such a modification. Such alterations tend to decrease the apparent molecular mass measured by SDS gel electrophoresis (27, 28). These modifications may serve to tether the protein to the membrane, either for interaction with other functional components or for proper translocation from the site of synthesis (26).

Comparing the deduced amino acid sequences of the cone PDE, the rod PDE α subunit and a fragment of the rod PDE β subunit (5) with cAMP PDEs (17, 31, 32) strengthens the observation that the photoreceptor enzymes belong to the family of PDEs (17, 20). Inspection enables one to predict sequence elements that might be unique for cGMP binding (Fig. 5). The photoreceptor PDE proteins are highly conserved (80%) among themselves in the C-terminal regions, covering residues 515–827 in cone (Figs. 2 and 5). Based on similarities with other PDEs, this region is assigned as the

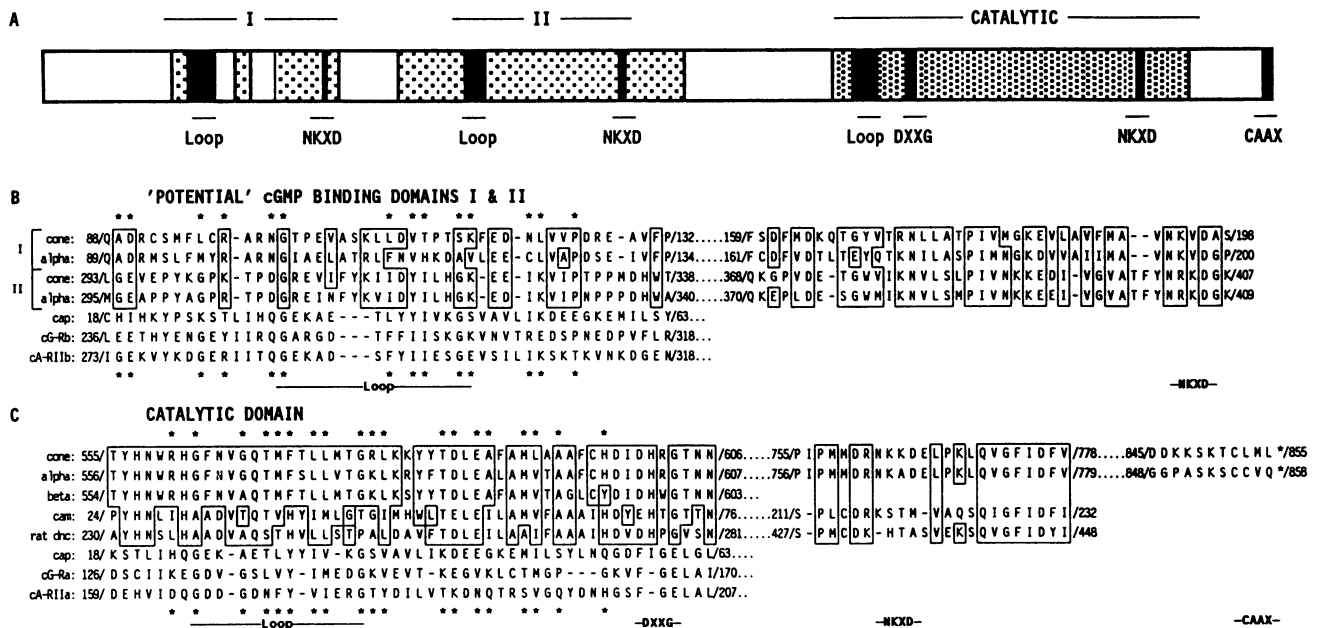


FIG. 5. Proposed functional domains in the primary amino acid structures of rod and cone photoreceptor cGMP PDEs. (A) Dot-filled areas indicate regions that are highly conserved between rod PDE α subunit and cone PDE α' subunit. Regions I and II are related as a tandem repeat (20). Each is a potential site of noncatalytic, high-affinity cGMP binding. The assignment of catalytic region is based on its similarity to core domains of cAMP PDEs (17). The filled boxes denote residues potentially involved in cGMP binding that are identical or similar to those described for guanosine nucleotide- (33, 34) and cyclic nucleotide- (41, 42) binding sites that are identified in protein crystal structures. The C-terminal CAAX motif sequence predicts posttranslational modification of proteolysis, methylation and isoprenylation (26–30). (B) Potential noncatalytic cGMP binding sites are suggested by comparing regions I and II of cone PDE α' subunit and rod PDE α subunit (5) with the *Escherichia coli* cAMP-binding catabolite activator protein CAP (37, 38), the β -chain binding domain of bovine lung cGMP protein kinase (36) and the β -chain binding domain of bovine heart cAMP protein kinase regulatory subunit type II (35). Internal amino acid similarities between the PDE regions I and II are boxed. Asterisks mark residues that are identical or conservatively substituted with those of the cyclic nucleotide-binding proteins listed. The glycine-rich "loop" is identified as part of the cAMP-binding site in the catabolite activator protein crystal structure (39–42). NKXD (Asn-Lys-Xaa-Asp) is a conserved consensus sequence the amino acid side chains of which specify guanosine binding (33, 34). (C) Potential catalytic cGMP-binding sites are suggested by comparing C-terminal sequences for photoreceptor cone PDE α' subunit, rod PDE α subunit, and rod PDE β subunit (5) with bovine brain calmodulin-dependent cAMP PDE (cam) (17), the rat brain dunce cAMP PDE *dnc* (31, 32), the cAMP-binding catabolite activator protein (cap) (37, 38), the A-chain binding domain of bovine lung cGMP protein kinase (cG-Ra) (37), and the A-chain binding domain of bovine heart cAMP protein kinase regulatory subunit type II (cA-RIIa) (35). Boxed residues denote amino acid identities or conservative substitutions among three or more members of the PDE family. Asterisks denote residues that are identical or similar to those of the known cyclic nucleotide-binding sites.

catalytic core (17, 20). Proteins that bind guanine nucleotides are generally found to have three conserved elements that specify a binding site: a glycine-rich loop Gly-Xaa_n-Gly, a Mg²⁺ binding element Asp-Xaa₂-Gly, and the guanine ring-binding G-box Asn-Lys-Xaa-Asp (33, 34). The catalytic cores of photoreceptor PDEs contain a conserved Gly-Xaa₃-(Gly/Ala)-Xaa₉-Gly-(Lys/Arg) sequence reminiscent of the glycine-rich, antiparallel β -strand loops found in the cyclic nucleotide-binding proteins (35–38, 40, 41). As modeled from the crystal structure of the cAMP-binding catabolite activator protein, CAP (40, 41), such loops are part of a β -roll structure encompassing a cyclic nucleotide-binding domain. In GTP- or ATP-binding proteins, a shorter Gly-Xaa-Gly-Xaa₂-Gly loop connects a β -strand and α -helix for a phosphate-binding site (33, 34). The catalytic cores of the PDEs contain the Asp-Xaa₂-Gly elements, which provide aspartic residues for Mg²⁺ ternary complexation with substrate and protein in GTP-binding proteins (33). The presence of this element in PDEs may be consistent with the Mg²⁺ requirement for substrate binding and catalytic activity in these enzymes (43). Finally, the catalytic core of the photoreceptor cGMP enzymes contains the G-box elements Asn-Lys-Xaa-Asp that may specify guanine binding; the element is distinctly absent in cAMP enzymes (Fig. 5).

Rod and cone PDEs have noncatalytic sites that bind cGMP with high affinity (24, 44–46). Single sites are reported for the bovine rod (24) and cone (44) enzymes, whereas two classes of high-affinity, noncatalytic cGMP-specific sites are observed for the frog enzyme (45, 46). Although somewhat speculative, elements for cGMP binding are apparent in the N-terminal portion of the photoreceptor enzymes (Fig. 5A regions I and II). The N-terminal regions of the rod PDE α subunit and cone PDE α' subunit (no sequence is available for PDE β subunit in this region) are more divergent than the catalytic core regions. However, a tandem repeat of sequences, noted as regions I and II in Fig. 5, is conserved. The repeat is more thoroughly analyzed by Charbonneau *et al.* (20) for the photoreceptor enzymes and the cGMP-stimulated PDE of bovine heart. A similar tandem repeat is the motif used for the two cyclic nucleotide-binding sites found in the regulatory domains of the cAMP and cGMP protein kinases (35–42). In the cone PDE, elements that may specify cGMP binding are readily recognizable in region II (residues 250–450) and less so in region I (residues 90–230) (Fig. 5B). Each site contains residues corresponding to a glycine-rich antiparallel β -loop, but the amino acid side chains that have been suggested to specify guanine (42) and adenine (41) binding in the regulatory domains of protein kinases are not obvious in the rod and cone PDEs. However, well-conserved G-boxes that may specify guanine ring binding are present in these regions, and it will be interesting to learn whether these elements are important for cGMP binding.

These speculations about domains will require experimental verification. Moreover, domains that stabilize the rod $\alpha\beta$ heterodimer, the cone α' homodimer, binding sites for the small γ subunits, and for transducin G_t α (47) remain to be identified. The presence of noncatalytic cGMP-binding sites suggests that the activation by transducin and/or catalytic activity of photoreceptor PDEs may be regulated by the prevailing cytoplasmic concentrations of cGMP. Defining the role for these sites will be important for understanding how the PDE activity is modulated during triggering and upon light adaptation of rod and cone photoreceptors.

We thank Dr. V. Lipkin for providing partial DNA sequences for β -PDE cDNA before publication. Professor Joe Beavo and his colleagues generously provided peptide sequences of the bovine cone PDE that confirmed our clone's identity. We appreciate their interactions and discussions. We thank Fran Jurnak for stimulating us to examine the structural elements for cGMP binding. The work

was supported, in part, by National Institutes of Health Grant EY04801 and a grant from the George Gund/National Retinitis Pigmentosa Foundation. M.L.A. is the recipient of a Jules and Doris Stein Research to Prevent Blindness Professorship.

1. Stryer, L. (1986) *Annu. Rev. Neurosci.* **9**, 87–119.
2. Baehr, W., Devlin, M. J. & Applebury, M. L. (1979) *J. Biol. Chem.* **254**, 11669–11677.
3. Deterre, P., Bigay, J., Robert, M. & Chabre, M. (1986) *Proc. Natl. Acad. Sci. USA* **85**, 2424–2428.
4. Gillespie, P. G. & Beavo, J. A. (1988) *J. Biol. Chem.* **263**, 8133–8141.
5. Ovchinnikov, Y. A., Gubanov, V. V., Khromtsov, N. V., Ischenko, K. A., Zagranichny, V. E., Muradov, K. G., Shuraeva, T. M. & Lipkin, V. M. (1987) *FEBS Lett.* **223**, 169–173.
6. Maniatis, T., Fritsch, E. G. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
7. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K., eds. (1989) *Current Protocols in Molecular Biology* (Wiley, New York).
8. McKnight, S. L. & Kingsbury, R. (1982) *Science* **217**, 316–324.
9. Jones, K. A., Yamamoto, K. R. & Tjian, R. (1985) *Cell* **42**, 559–572.
10. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
11. Chirgwin, J. M., Przybyla, A. A., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
12. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
13. Frischauf, A.-M., Lehrach, H., Poustka, A. & Murray, N. (1983) *J. Mol. Biol.* **170**, 827–842.
14. Martin, R. L., Wood, C., Baehr, W. & Applebury, M. L. (1986) *Science* **232**, 1266–1269.
15. Nathans, J. & Hogness, D. S. (1983) *Cell* **34**, 807–814.
16. Kimmel, A. R. & Berger, S. L. (1987) *Methods Enzymol.* **152**, 307–389.
17. Charbonneau, H., Beier, N., Walsh, K. A. & Beavo, J. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9308–9312.
18. Kozak, M. (1986) *Cell* **44**, 283–292.
19. Birnstiel, M. L., Busslinger, M. & Strub, K. (1985) *Cell* **41**, 349–359.
20. Charbonneau, H., Prusti, R. K., LeTrong, H., Sonnenburg, W. K., Mullaney, P. J., Walsh, K. A. & Beavo, J. A. (1990) *Proc. Natl. Acad. Sci. USA*, pp. 288–292.
21. Pugh, E. N. & Cobbs, W. H. (1986) *Vision Res.* **26**, 1613–1643.
22. Nathans, J., Thomas, D. & Hogness, D. S. (1986) *Science* **232**, 193–202.
23. Lerea, C. L., Somers, D. E., Hurley, J. B., Klock, I. B. & Bunt-Milam, A. H. (1986) *Science* **234**, 77–80.
24. Hurwitz, R. L., Bunt-Milam, A. H., Chang, M. L. & Beavo, J. A. (1984) *J. Biol. Chem.* **260**, 568–573.
25. Krebs, W. & Friedrich, I. (1982) in *The Structure of the Eye*, ed. Hollyfield, J. G. (Elsevier, NY), pp. 175–182.
26. Magee, T. & Hanley, M. (1988) *Nature (London)* **335**, 114–115.
27. Tamanoi, F., Hsueh, E. C., Goodman, L. E., Cobitz, A. R., Detrick, R. J., Brown, W. R. & Fujiyama, A. (1988) *J. Cell. Biochem.* **36**, 261–273.
28. Hancock, J. F., Magee, T., Childs, J. E. & Marshall, C. J. (1989) *Cell* **57**, 1167–1177.
29. Clarke, S., Vogel, J. P., Deschenes, R. J. & Stock, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4643–4647.
30. Swanson, R. J. & Applebury, M. L. (1983) *J. Biol. Chem.* **258**, 10599–10605.
31. Colicelli, J., Birchmeier, C., Michaeli, T., O'Neill, K., Riggs, M. & Wigler, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3599–3603.
32. Davis, R. L., Takayasu, H., Eberwine, M. & Myres, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3604–3608.
33. Jurnak, F. (1988) *Trends Biochem. Sci.* **13**, 196–198.
34. Holbrook, S. R. & Kim, S. H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1751–1755.
35. Takio, K., Smith, S. B., Krebs, E. G., Walsh, K. A. & Titani, K. (1984) *Biochemistry* **23**, 4200–4206.
36. Takio, K., Wade, R. D., Smith, S. B., Krebs, E. G., Walsh, K. A. & Titani, K. (1984) *Biochemistry* **23**, 4193–4199.
37. Aiba, H., Fujimoto, S. & Ozaki, N. (1982) *Nucleic Acids Res.* **10**, 1345–1361.
38. Cossart, P. & Gicquel-Sanzey, B. (1982) *Nucleic Acids Res.* **10**, 1363–1378.
39. McKay, D. B. & Steitz, T. A. (1982) *Nature (London)* **290**, 744–749.
40. McKay, D. B., Weber, I. T. & Steitz, T. A. (1982) *J. Biol. Chem.* **257**, 9518–9524.
41. Weber, I. T., Steitz, T. A., Bubis, J. & Taylor, S. S. (1987) *Biochemistry* **26**, 343–351.
42. Weber, I. T., Shabb, J. B. & Corbin, J. D. (1989) *Biochemistry* **28**, 6122–6127.
43. Cheung, W. Y. (1967) *Biochemistry* **6**, 1079–1087.
44. Gillespie, P. G. & Beavo, J. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4311–4315.
45. Yamazaki, A., Sen, I., Bitensky, M. W., Casnellie, J. E. & Greengard, P. (1980) *J. Biol. Chem.* **255**, 11619–11624.
46. Yamazaki, A., Batucca, F., Ting, A. & Bitensky, M. W. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3702–3706.
47. Kroll, S., Phillips, W. J. & Cerione, R. A. (1989) *J. Biol. Chem.* **264**, 4490–4497.