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Cloning and Characterization of the cDNA Encoding the α-Subunit of cGMP-Phosphodiesterase in Canine Retinal Rod Photoreceptor Cells

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Rod photoreceptor cyclic GMP-phosphodiesterase (cGMP-PDE, EC 3.1.4.17) is a key enzyme in the phototransduction cascade of the vertebrate retina. The enzyme is composed of α- and beta-catalytic subunits, and two identical inhibitory gamma-subunits. Once the phototransduction cascade is initiated by the absorption of light by rhodopsin, activated cGMP-PDE rapidly hydrolyses cGMP, depletion of which shuts cGMP-gated cation channels in the plasma membrane. The consequent hyperpolarization of the photoreceptor outer segment represents a large signal amplification and generates the visual neural impulse. Aberrant function of cGMP-PDE is causally associated with retinal degenerative diseases in man and animals. Mutations in the genes for the α- and beta-subunits of cGMP-PDE (*PDEA* and *PDE6B*, respectively) cause retinitis pigmentosa (OMIM entry) in some human families [6,8,9]. Defects in *PDE6B* also cause retinal degeneration in the *rd* mouse [3,11] and the *rcd1*dog [5,13,14]. Rod-cone dysplasia 2 (*rcd2*), which affects the collie dog, also represents a defect of retinal cyclic GMP metabolism since retinal cGMP levels are significantly elevated and cGMP-PDE activity is deficient [16]; however, *rcd2* is not caused by a defect in *PDE6B*[1]. Thus it is likely that the *rcd2* locus codes for either another cGMP-PDE structural subunit [*PDEA*, or *PDEG* (the gene for the gamma-subunit of cGMP-PDE)], or for one of the other proteins in the phototransduction cascade that activates cGMP-PDE. Since the canine chromosomal locations of the *rcd2* locus and the genes involved in phototransduction are not known, none of these candidate genes can be ruled out based on their map location relative to the *rcd2* locus.

In order to identify the mutation responsible for the *rcd2*disorder, we have begun to examine the different candidate genes that code for the phototransduction cascade proteins in the dog. The cDNAs for the α-subunit of cGMP-PDE (PDEα) have been cloned and characterized from man [12], mouse [2], and cow [10,12]. However, no information is available in the literature on the canine PDEα cDNA.

In this study we characterize the canine PDEα cDNA from normal dog, present evidence for usage of alternate polyadenylation sites to generate the two different transcripts described in multiple species [2,7,12], and compare the deduced amino acid sequences for conservation through evolution.

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RESULTS

Initially two different segments of canine PDEα cDNA were obtained by reverse transcription (RT) and polymerase chain reaction (PCR) using total retinal RNA and consensus primer pairs (PDEA-1/PDEA-2 and PDEA-3/PDEA-4; Table 1) based on the coding regions of known PDEα cDNA sequences from other species. These RT-PCR amplified fragments were cloned (1-PDEA and 2-PDEA) in pCRII vector (Invitrogen; San Diego, CA) and sequenced to confirm authenticity of the retina-specific PDE cDNA sequence. From the confirmed canine

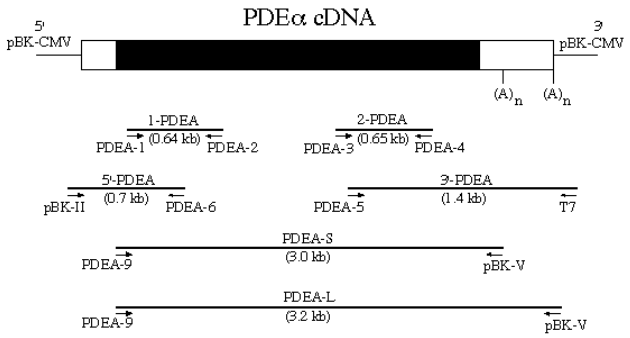

Figure 1. Strategy for cloning the canine PDEα cDNA. The cDNA containing the coding sequence (shaded box), the untranslated regions at the 5'- and 3'-end (open boxes) and two sites where poly (A) are added, is shown as an insert in the vector pBK-CMV (Stratagene, La Jolla, CA) used for the construction of the library. Clones containing different overlapping regions of the cDNA (1-PDEA, 2-PDEA, 5'-PDEA, 3'-PDEA, PDEA-S and PDEA-L), the sizes of the cDNA fragments in the clones, and the primers (see Table 1 for sequence) used for generating those fragments are identified. The clones 1-PDEA and 2-PDEA were obtained by reverse transcription and polymerase chain reaction (RT-PCR) from canine retinal total RNA using the RT-PCR kit (Perkin-Elmer; Foster City, CA) as recommended by the manufacturer. Amplification of cDNA to obtain 1-PDEA and 2-PDEA was done for 30 cycles at 94 °C (1 min), 54 °C (2 min), 72 °C (2 min). Other clones (5'-PDEA, 3'-PDEA, PDEA-S and PDEA-L) were obtained by screening a canine retinal cDNA library using a PCR based method. The PCR for screening the canine retinal cDNA library was done in 50 µl containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 10% DMSO, and 1.25 units Taq polymerase (Life Technologies; Grand Island, NY). The conditions for PCR amplification of each fragment were as follows: 5'-PDEA and 3'-PDEA were obtained by 30 cycles at 94 °C (1 min), 60 °C (1.5 min), 72 °C (2 min); PDEA-S and PDEA-L were obtained by 30 cycles at 94 °C (1 min), 58 °C (2 min), 72 °C (3 min). All reactions were concluded with a single step extension reaction at 72 °C for 10 min.

PDEα cDNA sequence, new canine-specific primers were designed to amplify both the 5'- and 3'-ends of canine PDEα cDNA from a canine retinal cDNA library by PCR (Figure 1).

To amplify the 5'-end of the cDNA, forward vector-specific (pBK-II) and *PDEA*-specific reverse (PDEA-6) primers were used. From the PCR products, the largest fragment showing evidence for *PDEA*specificity, based on PCR using internal primers, was cloned (5'-PDEA). To amplify the 3'-end of the cDNA, gene specific forward (PDEA-5) and vector specific reverse (T7) primers were used, and the amplified DNA fragment was cloned (3'-PDEA). The clone 3'-PDEA, however, lacked the poly (A) tail. We therefore designed a canine-specific primer (PDEA-9) from the 5'-end of the cDNA and used it in combination with the vector-specific reverse primer (pBK-V) for amplification of the entire open reading frame (ORF) and 3'-untranslated region (UTR) of PDEα cDNA from the cDNA library. The PCR resulted in amplification of two DNA fragments (3.0 and 3.2 kb), both of which hybridized to canine PDEα cDNA in Southern blots (data not shown). These two putative PDEα cDNA fragments (PDEA-L and PDEA-S) were cloned as described above.

Sequence of the PDEα cDNA was obtained from clones 1-PDEA, 2-PDEA, 5'-PDEA, 3'-PDEA, and PDEA-S, which contain overlapping fragments, from both directions. The identity of the larger clone (PDEA-L) as PDEα cDNA was confirmed by (a) partial sequencing (800 bp) of the 3'-end and two other upstream regions of the insert; (b) amplification of multiple overlapping fragments identical in size to those obtained from PDEA-S by PCR using the same set of primers; and (c) identical and predicted restriction enzyme digestion pattern of PCR amplified DNA fragments from both the clones (Figure 2). Sequences of all the primers used and their location in the canine PDEα cDNA or vector DNA are listed in Table 1.

TABLE 1. SEQUENCE AND LOCATION OF PRIMERS USED FOR PCR

Primer sequence(5'to3')	Source of Primer	Name of primer	Location
gCTTTGCCAAACAGTACTACAACC	hPDEα cDNA	PDEA1	192-215
AgTTGTGcAGGTAACTCAGGTG	hPDEα cDNA	PDEA2	815-836
GGCCCCTGGTGCGgTTC	hPDEα cDNA	PDEA3	1759-1774
ATGGGATTCTGtTGCAGCAC	hPDEα cDNA	PDEA4	2393-2412
TTCAACGTGGGGCAGACCAT	cPDEα cDNA	PDEA5	1832-1851
CACTACGTCCTTCCCATTCATTATGG	cPDEα cDNA	PDEA6	681-706
GTCATaAAGAAGCTGTGCTTCCTCC	cPDEα cDNA	PDEA7	380-404
GTCATGGGTGAGGTGACAGCAGAG	cPDEα cDNA	PDEA9	137-160
cCAACGTTTTGCCGAACTCCAAG	cPDEα cDNA	PDEA11	2032-2054
TCCACCCTATTCTGGTCCCA	cPDEα cDNA	PDEA16	1036-1055
ATGGTCTGCCCCACGTTGAAGCC	cPDEα cDNA	PDEA17	1829-1851
TTGCTTGGCTGTCTGCTGCTT	cPDEα cDNA	PDEA18	2624-2644
GCAGGTCGACACTAGTGGATCC	pBKCMV	pBKII	1092-1113
CCGCTCTAGAAGTA**CTCTCGAGTT**	pBKCMV	pBKV	1052-1067
CGACTCACTATAGGGCGAATT	pBKCMV	T7	980-1000

Primers (PDEA-1 to PDEA-4) used for RT-PCR correspond to the human (h) PDEα cDNA sequence, selected from the consensus region in different species. All other PDEA primers correspond to canine (c) PDEα cDNA sequence. Location of all the PDEA primers are shown with respect to cPDEα cDNA sequence. Nucleotides (shown in lower case) in some primers have mismatches with the canine sequence because those primers were selected either from the human sequence, or from preliminary canine sequence. Phagemid vector (pBK-CMV) specific primers were selected either from the multiple cloning site or from the flanking region. The bold-italicized region of primer pBK-V represents the linker used to make the canine cDNA library.

The two full length canine rod PDEα cDNAs are 2988 and 3233 nucleotides long, including the poly (A) tail (Figure 3). We have previously reported the presence of two distinct PDEα mRNAs, 3.3 and 3.0 kb, expressed in equivalent amounts in canine retina by northern blot [7]. Thus the sizes of the canine PDEα cDNAs described here (GenBank accession number U52868) are in complete agreement with our observation from northern analysis [7]. We have not formally determined the transcription start site; however, the clone containing the most upstream 5'-noncoding region contains 139 nucleotides in the 5'-UTR followed by the ATG codon for initiation of translation. The stop codon (TAA) corresponds to positions 2723 through 2725 of the cDNA sequence. The 3'-UTRs of the 3.0 kb and 3.3 kb transcripts, represented by clones PDEA-S and PDEA-L, are 263 and 508 nucleotides respectively. A non-consensus putative polyadenylation signal (ATTAAA) is present in the corresponding locations of the smaller (PDEA-S) and larger (PDEA-L) clones. In PDEA-S this first polyadenylation signal (Figure 3, nucleotides 2948 through 2953) is 15 nucleotides upstream of the poly (A) tail.

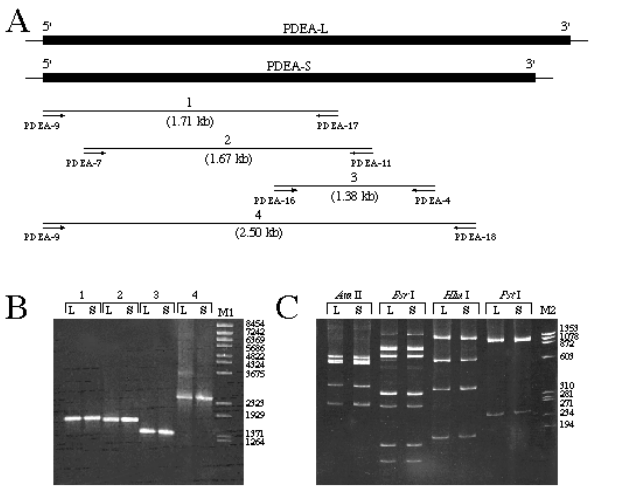

Figure 2. Identity of PDEA-L clone as PDEa cDNA sequence. (A) Relative size of the inserts in the PDEA-L and PDEA-S clones, the DNA fragments amplified by PCR, primers (see Table 1 for sequence) used, and the predicted sizes of the amplified fragments are shown. PCR condition for amplified fragments are as follows: fragments 1 and 2 were obtained by 30 cycles at 94 °C (1 min), 64 °C (1 min), 72 °C (2 min); fragment 3 was obtained by 30 cycles at 94 °C (1 min), 54 °C (1 min), 72 °C (1.5 min); and fragment 4 was obtained by 35 cycles at 94 °C (1 min), 58 °C (2 min), 72 °C (3.5 min). All reactions were concluded with a single step extension reaction at 72 °C for 5 min.
(B) Agarose gel (0.8%) electrophoresis of PCR products from PDEA-S (S) and PDEA-L (L) clones. Numbers 1 through 4 identify DNA fragments amplified from regions of the clones represented in panel A. Lane M1 represents BstE II-digested lambda DNA markers
(C) Polyacrylamide gel (6%) electrophoresis of restriction enzyme digestion of PCR product (4 in panel A) from PDEA-S (S) and PDEA-L (L) clones. Lane M2 represents Hae III-digested phi X 174 markers. The length of the DNA fragments (bp) in both markers (M1 and M2) are shown next to the marker lanes. The PCR and the restriction enzyme digestion results indicate that both the clones are identical in the regions examined.

In the larger clone (PDEA-L) there is a second polyadenylation signal (Figure 3, nucleotides 3187 through 3192) 23 nucleotides upstream of the poly (A) tail. The ORF of the canine sequence predicts a protein of 99.7 kDa containing 861 amino acids.

DISCUSSION

Comparison of the ORF nucleotides of the canine PDEα cDNA with that of other species shows that it shares similar nucleotide identity with the bovine (91.0%) and human (90.4%) sequences, and slightly lower identity with the mouse (86.5%) sequence. At the amino acid level, however, the similarity between the canine sequence and that of other species is higher (94%) than at the nucleotide level. Two transcripts have been reported to be present for human (4.9 and 5.3 kb), bovine (4.0 and 4.6 kb) and canine (3.0 and 3.3 kb) PDEα [2,7,12]. The reported human and bovine clones, however, contain only 2.9-3.2 kb of the PDEα cDNA sequence [10,12]. The size of the transcript in mouse has not been reported. Since only a single ORF with high homology between different species has been identified for PDEα cDNAs, it is reasonable that the inter- and intra-species difference in the sizes of the transcripts could be due to either different transcription start sites or use of alternative poly (A) addition sites. The sequence presented here clearly demonstrates that two differently sized canine PDEα transcripts are generated by use of different polyadenylation

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CTTGCAGGTCGACACTAGTGGATCCAAAGAATTCGGCACGAGAAAACACCTCCTGACTCT 60
GTCTTGCCCCCAGCTATAGACCTTCCCTGGGCAGGCCAGCTTTAGGCTCTCCTGGGAAGT 120
AGCCAGCGGGATTCCAGTC ATG GGT GAG GTG ACA GCA GAG CAG GTG GAG  169
                    Met Gly Glu Val Thr Ala Glu Gln Val Glu  10
AAG TTC CTG GAC TCG AAT ATT ATC TTT GCC AAA CAG TAC TAC AAC  214
Lys Phe Leu Asp Ser Asn Ile Ile Phe Ala Lys Gln Tyr Tyr Asn  25
CTC CGC TAC CGG GCC AAG GTC ATC TCA GAC ATG CTG GGG GCC AAG  259
Leu Arg Tyr Arg Ala Lys Val Ile Ser Asp Met Leu Gly Ala Lys  40
GAG GCA GCG GTG GAC TTC AGC AAC TAC CAC TCG CTG AGC AGT GTG  304
Glu Ala Ala Val Asp Phe Ser Asn Tyr His Ser Leu Ser Ser Val  55
GAG GAG AGT GAA ATC ATC TTT GAC CTC CTG CGA GAC TTC CAG GAG  349
Glu Glu Ser Glu Ile Ile Phe Asp Leu Leu Arg Asp Phe Gln Glu  70
AAT TTG CAG GCC GAG AGA TGC ATC TTC AAT GTC ATG AAG AAG CTG  394
Asn Leu Gln Ala Glu Arg Cys Ile Phe Asn Val Met Lys Lys Leu  85
TGC TTC CTC CTT CAG GCA GAT CGC ATG AGC CTG TTC ATG TAC AGG  439
Cys Phe Leu Leu Gln Ala Asp Arg Met Ser Leu Phe Met Tyr Arg 100
GTC CGA AAT GGC ATC GCA GAG CTA GCC ACC CGG CTC TTC AAT GTC  484
Val Arg Asn Gly Ile Ala Glu Leu Ala Thr Arg Leu Phe Asn Val 115
CAC AAG GAT GCT GTC CTT GAG GAA TGC CTG GTG GCG CCC GAC TCA  529
His Lys Asp Ala Val Leu Glu Glu Cys Leu Val Ala Pro Asp Ser 130
GAG ATT GTG TTC CCC CTG GAC ATG GGT GTG GTG GGT CAC GTT GCC  574
Glu Ile Val Phe Pro Leu Asp Met Gly Val Val Gly His Val Ala 145
CAC TCT AAA AAG ATC GCC AAC GTC GTC AAC ACA GAA GAG GAT GAG  619
His Ser Lys Lys Ile Ala Asn Val Val Asn Thr Glu Glu Asp Glu 160
CAT TTC TGT GAC TTT GTG GAC ACC CTC ACT GAG TAC CAG ACC AAG  664
His Phe Cys Asp Phe Val Asp Thr Leu Thr Glu Tyr Gln Thr Lys 175
AAC ATC CTG GCT TCC CCC ATA ATG AAT GGG AAG GAC GTA GTG GCA  709
Asn Ile Leu Ala Ser Pro Ile Met Asn Gly Lys Asp Val Val Ala 190
GTA ATC ATG GCT GTG AAT AAA GTG GAC GAG CCC CAC TTC ACC AAG  754
Val Ile Met Ala Val Asn Lys Val Asp Glu Pro His Phe Thr Lys 205
AGA GAT GAA GAG ATT CTT CTC AAG TAC CTC AAT TTT GCA AAC CTA  799
Arg Asp Glu Glu Ile Leu Leu Lys Tyr Leu Asn Phe Ala Asn Leu 220
ATC ATG AAG GTA TAC CAC CTG AGT TAC CTA CAC AAT TGC GAG ACT  844
Ile Met Lys Val Tyr His Leu Ser Tyr Leu His Asn Cys Glu Thr 235
CGG CGT GGC CAG ATA CTG CTG TGG TCT GGG AGC AAA GTC TTT GAA  889
Arg Arg Gly Gln Ile Leu Leu Try Ser Gly Ser Lys Val Phe Glu 250
GAG CTT ACG GAC ATC GAG AGG CAG TTC CAC AAG GCC CTG TAC ACA  934
Glu Leu Thr Asp Ile Glu Arg Gln Phe His Lys Ala Leu Tyr Thr 265
GTC CGG GCC TTC CTC AAC TGT GAC AGA TAT TCT GTG GGA CTC TTA  979
Val Arg Ala Phe Leu Asn Cys Asp Arg Tyr Ser Val Gly Leu Leu 280
GAC ATG ACC AAG CAG AAG GAA TTT TTT GAT GTG TGG CCA GTC CTG 1024
Asp Met Thr Lys Gln Lys Glu Phe Phe Asp Val Try Pro Val Leu 295
ATG GGG GAG GCT CCA CCC TAT TCT GGT CCC AGG ACT CCG GAT GGA 1069
Met Gly Glu Ala Pro Pro Tyr Ser Gly Pro Arg Thr Pro Asp Gly 310
AGG GAA ATC AAC TTT TAC AAG GTC ATT GAC TAT ATC CTA CAC GGC 1114
Arg Glu Ile Asn Phe Tyr Lys Val Ile Asp Tyr Ile Leu His Gly 325
AAA GAA GAC ATC AAA GTA ATC CCG AAT CCA CCT CCT GAT CAT TGG 1159
Lys Glu Asp Ile Lys Val Ile Pro Asn Pro Pro Pro Asp His Try 340
GCT TTA GTA AGT GGT CTG CCC ACT TAT GTT GCC CAG AAT GGC CTG 1204
Ala Leu Val Ser Gly Leu Pro Thr Tyr Val Ala Gln Asn Gly Leu 355
ATT TGC AAC ATC ATG AAT GCA CCT GCA GAG GAC TTT TTT GCA TTC 1249
Ile Cys Asn Ile Met Asn Ala Pro Ala Glu Asp Phe Phe Ala Phe 370
CAG AAA GAG CCT CTG GAT GAG TCT GGA TGG ATG ATT AAG AAT GTC 1294
Gln Lys Glu Pro Leu Asp Glu Ser Gly Try Met Ile Lys Asn Val 385
CTT TCT TTG CCA ATT GTG AAC AAG AAG GAG GAA ATT GTT GGG GTG 1339
Leu Ser Leu Pro Ile Val Asn Lys Lys Glu Glu Ile Val Gly Val 400
GCC ACG TTT TAC AAT CGC AAA GAT GGA AAA CCC TTT GAT GAA ATG 1384
Ala Thr Phe Tyr Asn Arg Lys Asp Gly Lys Pro Phe Asp Glu Met 415
GAT GAG ACC CTC ATG GAG TCT TTG GCT CAA TTC CTG GGC TGG TCC 1429
Asp Glu Thr Leu Met Glu Ser Leu Ala Gln Phe Leu Gly Try Ser 430
GTC TTA AAT CCT GAT ACT TAC GAG TCA ATG AAC AGA CTT GAA AAC 1474
Val Leu Asn Pro Asp Thr Tyr Glu Ser Met Asn Arg Leu Glu Asn 445
AGG AAG GAT ATT TTC CAG GAC ATG GTA AAA TAC CAC GTG AAG TGT 1519
Arg Lys Asp Ile Phe Gln Asp Met Val Lys Tyr His Val Lys Cys 460

GAC AAT GAA GAG ATC CAG AAA ATC CTG AAA ACC AGA GAG GTG TAT  1564
Asp Asn Glu Glu Ile Gln Lys Ile Leu Lys Thr Arg Glu Val Tyr  475
GGG AAG GAG CCG TGG GAG TGC GAG GAA GAG GAA CTC GCT GAG ATC  1609
Gly Lys Glu Pro Try Glu Cys Glu Glu Glu Glu Leu Ala Glu Ile  490
CTG CAA GGA GAG CTG CCA GAT GCA GAG AAA TAT GAA ATC AAT AAA  1654
Leu Gln Gly Glu Leu Pro Asp Ala Glu Lys Tyr Glu Ile Asn Lys  505
TTC CAC TTC AGC GAC TTG CCC CTG ACC GAA CTG GAG CTG GTG AAA  1699
Phe His Phe Ser Asp Leu Pro Leu Thr Glu Leu Glu Leu Val Lys  520
TGT GGG ATA CAG ATG TAC TAT GAG CTC AAA GTG GTG GAT AAA TTT  1744
Cys Gly Ile Gln Met Tyr Tyr Glu Leu Lys Val Val Asp Lys Phe  535
CAC ATT CCT CAG GAG GCC CTG GTG CGC TTC ATG TAC TCG CTG AGC  1789
His Ile Pro Gln Glu Ala Leu Val Arg Phe Met Tyr Ser Leu Ser  550
AAG GGC TAC CGC AGG ATC ACC TAC CAC AAC TGG CGG CAC GGC TTC  1834
Lys Gly Tyr Pro Arg Ile Thr Tyr His Asn Try Arg His Gly Phe  565
AAC GTG GGG CAG ACC ATG TTC TCC TTG CTG GTG ACC GGA AAG CTG  1879
Asn Val Gly Gln Thr Met Phe Ser Leu Leu Val Thr Gly Lys Leu  580
AAG CGA TAC TTC ACA GAC CTA GAG GCC TTG GCC ATG GTC ACC GCT  1924
Lys Arg Tyr Phe Thr Asp Leu Glu Ala Leu Ala Met Val Thr Ala  595
GCC TTC TGC CAT GAC ATT GAC CAC AGA GGC ACC AAC AAT CTC TAC  1969
Ala Phe Cys His Asp Ile Asp His Arg Gly Thr Asn Asn Leu Tyr  610
CAG ATG AAG TCC CAG AAC CCA CTG GCC AAG CTC CAT GGG TCC TCC  2014
Gln Met Lys Ser Gln Asn Pro Leu Ala Lys Leu His Gly Ser Ser  625
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Ile Leu Glu Arg His His Leu Glu Phe Gly Lys Thr Leu Leu Arg  640
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Asp Glu Ser Leu Asn Ile Phe Gln Asn Leu Asn Arg Arg Gln His  655
GAG CAC GCC ATC CAC ATG ATG GAC ATA GCA ATC ATT GCC ACA GAC  2149
Glu His Ala Ile His Met Met Asp Ile Ala Ile Ile Ala Thr Asp  670
CTC GCC CTG TAT TTC AAG AAG AGG ACA ATG TTC CAA AAG ATC GTG  2194
Leu Ala Leu Tyr Phe Lys Lys Arg Thr Met Phe Gln Lys Ile Val  685
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Asp Gln Ser Lys Thr Tyr Glu Thr Gln Gln Glu Try Thr Gln Tyr  700
ATG ATG CTG GAG CAG ACA CGG AAG GAA ATT GTT ATG GCC ATG ATG  2284
Met Met Leu Glu Gln Thr Arg Lys Glu Ile Val Met Ala Met Met  715
ATG ACC GCC TGT GAT CTC TCA GCC ATC ACC AAG CCC TGG GAG GTG  2329
Met Thr Ala Cys Asp Leu Ser Ala Ile Thr Lys Pro Try Glu Val  730
CAG AGT AAG GTA GCT CTA CTG GTT GCT GCC GAA TTC TGG GAA CAA  2374
Gln Ser Lys Val Ala Leu Leu Val Ala Ala Glu Phe Try Glu Gln  745
GGT GAC CTG GAG CGC ACA GTG CTG CAG CAG AAT CCC ATT CCC ATG  2419
Gly Asp Leu Glu Arg Thr Val Leu Gln Gln Asn Pro Ile Pro Met  760
ATG GAC AGG AAC AAG GCA GAT GAA CTC CCC AAG CTT CAA GTC GGC  2464
Met Asp Arg Asn Lys Ala Asp Glu Leu Pro Lys Leu Gln Val Gly  775
TTC ATT GAC TTT GTT TGC ACC TTT GTC TAC AAG GAA TTC TCC CGT  2509
Phe Ile Asp Phe Val Cys Thr Phe Val Tyr Lys Glu Phe Ser Arg  790
TTC CAC GAG GAG ATC ACT CCC ATG CTG GAT GGG ATC ACC AAC AAC  2554
Phe His Glu Glu Ile Thr Pro Met Leu Asp Gly Ile Thr Asn Asn  805
CGC AAG GAG TGG AAG GCG CTC GCC GAT GAG TAC GAC ACC AAG ATG  2599
Arg Lys Glu Try Lys Ala Leu Ala Asp Glu Tyr Asp Thr Lys Met  820
AAG GCC CTG GAG GAG GAG AAG CAG CAG ACA GCC AAG CAA  2644
Lys Ala Leu Glu Glu Glu Lys Gln Lys Gln Gln Thr Ala Lys Gln  835
GGG GCG GCA GGA GAT CAG CCG GGG GGC AAC CCC AGC CCG GCC GGG  2689
Gly Ala Ala Gly Asp Gln Pro Gly Gly Asn Pro Ser Pro Ala Gly  850
GGC GCA CCT GCA TCC AAG TCC TGC TGC ATC CAG TAA CGCTGCCTGGCA 2737
Gly Ala Pro Ala Ser Lys Ser Cys Cys Ile Gln ***              861
TCAGCTGCACCGAATGGTACCACCCTTCCTGGAAAGAGACCACCCAAGCCAGCAGAAAAC 2797
CAAAACCCTGCTTGTGAAGTAAAATAGTAATCGGATTTGAAAGCTGGGAGAGAATTTAGC 2857
TTACTTTCATCTAGTGGTTTTTGAACATTTTTTCAGTTTTGAATACTTTTTACTGAGCTA 2917
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TGCTTGCTTAGAATCATTTTCACTCTTATACTTCCATTTTATGATTTCTTATAACAATTG 3037
TCTAGCATCCTCAAAAGTAGAGAATTGTTCAGTGAATCCCTCCTCCTCATCACCCAGATT 3097
CCAATATCAGGGTTTTGCCCCATTTGCTTCATCCATTCTTTTACATTTCTTTCGCATTTT 3157
TTTCTTAGCTGAAGTGAAGTATTTTCTTT ATTAAA TTGATGGTCTTTTTTCTTTTTTAA 3217
AAAAAAAAAAAAAAA                                              3233
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Figure 3. The nucleotide sequence of PDEα cDNA from canine retina (GenBank accession number U52868), and the deduced amino acid sequence. The position of the nucleotides and the deduced amino acids are indicated on the right. The two extra amino acids that are inserted in comparison to human and mouse sequences are shown in ***bold italics*** [***Ala*** (849), ***Gly*** (850)]. The stop codon (TAA) is marked with asterisks (***), and the sequence motif (ATTAAA) present at the two polyadenylation sites is boldfaced. The 3'-untranslated region (UTR) of the larger transcript is shown in red to where a poly (A) tail containing 20 ‘A’s is added (not shown) in the smaller transcript.

sites. The same mechanism could well account for the observed different transcript sizes within other species. The canine sequence does not contain the canonical polyadenylation signal (AATAAA); instead a similar sequence motif (ATTAAA) was identified in both appropriate locations (Figure 3). This alternative motif has been demonstrated to serve as a surrogate polyadenylation signal with ~80% efficiency [15]. A non-consensus polyadenylation signal (AATACA) has also been reported to be present in human PDEα cDNA [12].

It is noteworthy that while PDEα cDNA clones from 3 other species contain an ORF capable of coding for a polypeptide of 859 amino acids, the canine PDEα polypeptide is predicted to contain 861 amino acids. With respect to the human and mouse sequences, the two extra amino acids are located as the 849th (Ala) and 850th (Gly) residues (Figure 3). Similar to the comparison made between human and bovine PDEα sequences [12], we noted that the differences in amino acid sequence among the four species (human, mouse, bovine, and canine) are clustered in the 225 N-terminal and 45 C-terminal residues (data not shown). The conserved region includes the domain present in several eukaryotic cyclic nucleotide phosphodiesterases [4]. Alignment of amino acid sequences of canine PDEα and PDEbeta shows a 72% overall identity, and the domains that are most dissimilar are at the N-terminus (first 50 residues) and C-terminus (last 30 residues).

The data presented here have been used to identify the possible mechanism for the presence of two transcripts for PDEα in different species, and to compare nucleotide/amino acid identity among rod-specific PDEα sequences of these species. Characterization of the wild type canine PDEα cDNA sequence will allow us to detect PDEA mutations in dogs affected with *rcd2* or other inherited retinal degenerations. Because collie dogs affected with *rcd2* have elevated retinal cGMP levels secondary to low PDE activity, we are currently investigating the possibility of a PDEα mutation in this disease.

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REFERENCES

1. Acland GM, Fletcher RT, Gentleman S, Chader GJ, Aguirre GD. Non-allelism of three genes (rcd1, rcd2 and erd) for early-onset hereditary retinal degeneration. Exp Eye Res 1989; 49:983-98.
2. Baehr W, Champagne MS, Lee AK, Pittler SJ. Complete cDNA sequences of mouse rod photoreceptor cGMP phosphodiesterase α- and beta-subunits, and identification of beta'-, a putative beta-subunit isozyme produced by alternative splicing of the beta-subunit gene. FEBS Lett 1991; 278:107-14.
3. Bowes C, Li T, Danciger M, Baxter LC, Applebury ML, Farber DB. Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. Nature 1990; 347:677-80.
4. Charbonneau H, Beier N, Walsh KA, Beavo JA. Identification of a conserved domain among cyclic nucleotide phosphodiesterases from diverse species. Proc Natl Acad Sci U S A 1986; 83:9308-12.
5. Clements PJ, Gregory CY, Peterson-Jones SM, Sargan DR, Bhattacharya SS. Confirmation of the rod cGMP phosphodiesterase beta-subunit (PDE beta) nonsense mutation in affected rcd-1 Irish setters in the UK and development of a diagnostic test. Curr Eye Res 1993; 12:861-6.
6. Danciger M, Blaney J, Gao YQ, Zhao DY, Heckenlively JR, Jacobson SG, Farber DB. Mutations in the PDE6B gene in autosomal recessive retinitis pigmentosa. Genomics 1995; 30:1-7.
7. Farber DB, Danciger JS, Aguirre G. The beta subunit of cyclic GMP phosphodiesterase mRNA is deficient in canine rod-cone dysplasia 1. Neuron 1992; 9:349-56.
8. Huang SH, Pittler SJ, Huang X, Oliveira L, Berson EL, Dryja TP. Autosomal recessive retinitis pigmentosa caused by mutations in the α subunit of rod cGMP phosphodiesterase. Nat Genet 1995; 11:468-71.
9. McLaughlin ME, Ehrhart TL, Berson EL, Dryja TP. Mutation spectrum of the gene encoding the beta subunit of rod phosphodiesterase among patients with autosomal recessive retinitis pigmentosa. Proc Natl Acad Sci U S A 1995; 92:3249-53.
10. Ovchinnikov YuA, Gubanov VV, Khramtsov NV, Ischenko KA, Zagranichny VE, Muradov KG, Shuvaeva TM, Lipkin VM. Cyclic GMP phosphodiesterase from bovine retina. Amino acid sequence of the α-subunit and nucleotide sequence of the corresponding cDNA. FEBS Lett 1987; 223:169-73.
11. Pittler SJ, Baehr W. Identification of a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase beta-subunit gene of the rd mouse. Proc Natl Acad Sci U S A 1991; 88:8322-6.
12. Pittler SJ, Baehr W, Wasmuth JJ, McConnell DG, Champagne MS, vanTuinen P, Ledbetter D, Davis RL. Molecular characterization of human and bovine rod photoreceptor cGMP phosphodiesterase α-subunit and chromosomal localization of the human gene. Genomics 1990; 6:272-83.
13. Ray K, Baldwin VJ, Acland GM, Blanton SH, Aguirre GD. Cosegregation of codon 807 mutation of the canine rod cGMP phosphodiesterase beta gene and rcd1. Invest Ophthalmol Vis Sci 1994; 35:4291-9.
14. Suber ML, Pittler SJ, Qin N, Wright GC, Holcombe V, Lee RH, Craft CM, Lolley RN, Baehr W, Hurwitz RL. Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase beta-subunit gene. Proc Natl Acad Sci U S A 1993; 90:3968-72.
15. Wickens M. How the messenger got its tail: addition of poly(A) in the nucleus. Trends Biochem Sci 1990; 15:277-81.
16. Woodford BJ, Liu Y, Fletcher RT, Chader GJ, Farber DB, Santos-Anderson R, Tso MO. Cyclic nucleotide metabolism in inherited retinopathy in collies: a biochemical and histochemical study. Exp Eye Res 1982; 34:703-14.

The typographical corrections below were made to the article on the date noted. These changes have been incorporated in the article and the details are documented here. 29 March 1999: The text "retinitis pigmentosa (RP)" was changed to "retinitis pigmentosa (OMIM entry 268000)" in the first paragraph of the introduction. The abbreviations "Fig." and "no." were expanded to "Figure" and "number", respectively, throughout the article. The characters enclosing citations to references were changed from parentheses () to brackets []. Several instances of incorrect spacing between a word and puntuation have been corrected.