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Structural organization of the gene for prostaglandin D synthase in the rat brain

(prostaglandin D_2/gene structure/lipocalin superfamily/molecular evolution)

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ABSTRACT **A 3-kilobase-pair gene for rat brain prostaglandin D synthase [(5*Z*,13*E*)-(15*S*)-9α,11α-epidoxy-15-hydroxyprosta-5,13-dienoate D-isomerase, EC 5.3.99.2], which belongs to the lipocalin family, was isolated from a rat genomic DNA library by plaque hybridization with the cDNA for the enzyme. The gene contains seven exons, and all the splice donor and acceptor sites conform to the GT/AG rule. Transcription initiates at a guanine residue 39 base pairs upstream of the translation initiation codon, as determined by primer-extension analysis of rat brain mRNA. The 5′-flanking region of the gene lacks typical transcriptional regulatory sequences, such as TATA and CAAT boxes, but contains several sets of inverted repeats, direct repeats, and sequences resembling the transcriptional factor Sp1-binding site. The gene structure of prostaglandin D synthase is remarkably analogous to those of other lipocalins, such as β-lactoglobulin, α_2-urinary globulin, placental protein 14, and α_1-microglobulin, in terms of number and sizes of exons and phase of splicing of introns. Furthermore, in a multiple alignment of the deduced amino acid sequences, positions of exon/intron junction of the prostaglandin D synthase gene are highly conserved and located around the positions of those of the genes for other lipocalins despite a weak homology.**

Prostaglandin (PG) D_2 is a major PG produced in rat brain and functions as a neuromodulator of several central actions such as sleep–wake cycles, body temperature, luteinizing hormone release, and odor responses (for reviews, see refs. 1 and 2).

Among several enzymes catalyzing the conversion of PG H_2 to produce PG D_2, glutathione-independent prostaglandin D (PGD) synthase [prostaglandin-H_2 D-isomerase; (5*Z*,13*E*)-(15*S*)-9α,11α-epidioxy-15-hydroxyprosta-5,13-dienoate D-isomerase, EC 5.3.99.2)] (3) is responsible for biosynthesis of PG D_2 in the central nervous system (4), retina (5), and cochlea (6) but not in various other tissues (4). Furthermore, the principal cellular localization of the enzyme changes postnatally from neurons in the brain of 1- to 2-week-old rats to oligodendrocytes in adult animals (7). It is, therefore, likely that the enzyme plays important roles in both maturation and maintenance of the central nervous system and also that the expression of this enzyme is controlled by distinct mechanisms operating at each specific developmental stage of the cells in those tissues.

Previously, we isolated cDNAs encoding this enzyme in the brain of rats (8) and humans (9). By a homology search in data bases of protein primary structure, the enzyme was shown to be a member of the lipocalin superfamily consisting of hydrophobic molecule transporters (9, 10). Here we reveal the gene structure of rat brain PGD synthase‖ and show that the exon/intron splicing sites of this enzyme are also conserved between some, but not all, of the members of this protein family. This information is useful for further studies on the regulation of PGD synthase expression and the evolution of the highly divergent lipocalin family (11).

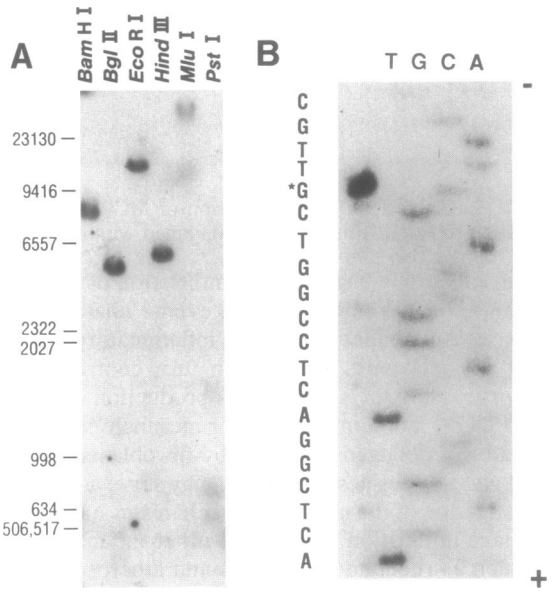

FIG. 1. (*A*) Southern blot analysis of rat genomic DNA. Rat genomic DNA (20 μg) was digested with various endonucleases and hybridized with the cDNA for rat brain PGD synthase. λDNA digested with *Hin*dIII was used as a size marker (in kb). (*B*) Primer-extension analysis. A 5′-end-labeled oligonucleotide (5′-CCACAGCATTGGAAGAGCAGCCAT-3′, depicted by arrow in Fig. 2) (1 pmol) was hybridized to 1 μg of rat brain mRNA and extended at 42°C for 1 hr with 20 units of reverse transcriptase; the reaction products were fractionated on a sequencing gel. The adjacent lanes T, G, C, and A represent complementary sequencing reactions with an *Eco* O109I fragment including the entire exon 1 as a template and the same unlabeled primer. DNA sequence of the coding strand is shown at left. The cap site guanine residue is denoted with a star.

MATERIALS AND METHODS

Screening of Phage Clones Containing the Gene for Rat Brain PGD Synthase. A rat liver genomic DNA (*Eco*RI-partial cut) library constructed with a λ Charon 4A vector was

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Abbreviations: PG, prostaglandin; PGD synthase, prostaglandin D synthase.

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M94134).

obtained from Clontech. An *Eco*RI insert of λDS4 [0.7 kilobase (kb)], which contains full length of the cDNA for rat brain PGD synthase (8), was labeled with [^{32}P]dCTP by multiple priming (12). The radiolabeled cDNA was used for plaque hybridization screening of the genomic library.

Southern Blotting. Rat genomic DNA was extracted from the liver of adult male Wistar rats as described (13). The genomic DNA and DNAs of the cloned phages were digested with various restriction endonucleases, separated by electrophoresis on 0.8% agarose gels, and transferred to a nitrocellulose membrane. The membrane was hybridized to the ^{32}P-labeled oligonucleotide cDNA insert for rat brain PGD synthase.

DNA Sequencing. The nucleotide sequence was determined by the dideoxynucleotide chain-termination procedure (14) after subcloning into M13mp18 and M13mp19 vectors.

Primer-Extension Analysis. A synthetic oligonucleotide primer was ^{32}P-labeled at the 5′ end. Total RNA was extracted from rat brain by the method of Chomczynski and Sacchi (15), and the mRNA was purified by oligo(dT) column chromatography. The mRNA was hybridized to the ^{32}P-labeled oligonucleotide primer, incubated with reverse transcriptase, and electrophoresed on 6% polyacrylamide/8 M urea sequencing gels.

Homology Search, Sequence Alignment, and Construction of Phylogenetic Tree. The deduced amino acid sequence of PGD synthase was subjected to a homology search in data bases of protein primary structure, Protein Research Foundation (release 90.9) (16) and National Biomedical Research Foundation (release 30.0) (17), with a program for weak homology detection (18). A multiple alignment was constructed by a program using a tree-based alignment algorithm (19), in which sequence differences from pairwise comparisons were used as genetic distances for tree construction. An unrooted phylogenetic tree was constructed by the neighbor-joining method (20) from the differences among the aligned sequences after Poisson correction for multiple substitutions at a site (21).

Estimation of Numbers of Nucleotide Substitutions per Site. Numbers of silent nucleotide substitutions per site (K_s^c) and of amino acid-changing nucleotide substitutions per site (K_a^c) were calculated between humans and rodents for PGD synthase (8, 9), α_1-acid glycoprotein (22, 23), retinol-binding protein (24, 25), apolipoprotein D (26, 27) after correction considering multiple substitutions at a site (28).

RESULTS AND DISCUSSION

In Southern blots after endonuclease digestion of the rat genomic DNA with *Bam*HI, *Bgl* II, *Eco*RI, and *Hin*dIII (Fig. 1*A*), the cDNA for rat brain PGD synthase hybridized to single DNA fragments from 5 to 12 kb in length. Because the cDNA contains a unique *Mlu* I restriction site and *Pst* I site, the cDNA probe hybridized to two *Mlu* I-digested and several *Pst* I-digested genomic fragments. This simple restriction map suggests that the rat haploid genome contains a single copy of the gene for rat brain PGD synthase.

After screening ≈2 × 10^6 plaques of the rat genomic library, three positive clones were isolated independently. These clones contained inserts of 15, 17, and 18 kb in length. Southern blot analyses of these cloned DNAs with the cDNA probe showed restriction digest profiles identical to those of the genomic DNA described above, indicating that these phages contained the entire region for the authentic copy of

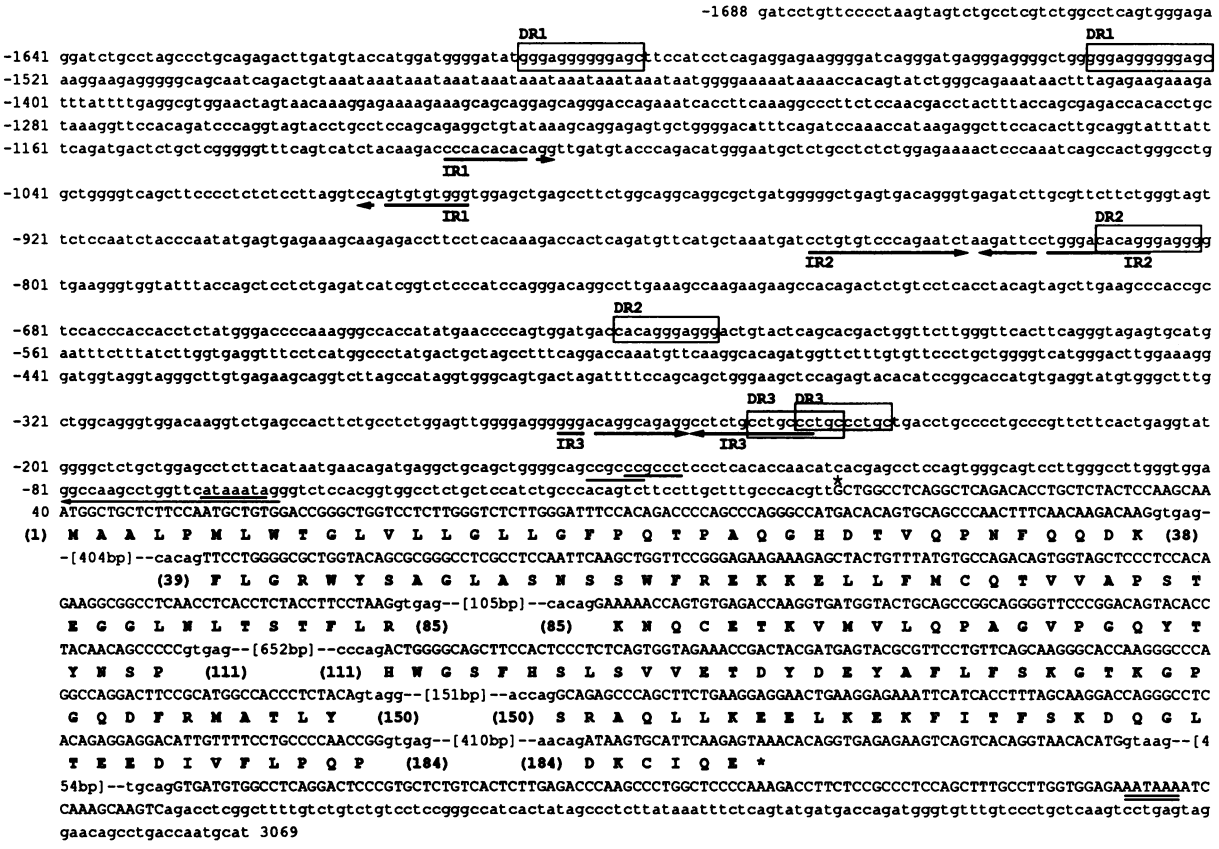

FIG. 2. Nucleotide sequence of the gene for rat brain PGD synthase. The coding sequence of exons is translated and numbered in parentheses from the ATG initiation codon. The overlapped GC boxes, CCGCCC, and the TATA-like sequence are underlined. The cap site is marked with a star. The nucleotide sequence is numbered from the cap site at the left. Direct repeats (DR) are boxed, and inverted repeats (IR) are marked with arrows. A thin arrow indicates a primer used for determining the cap site. Exons are in uppercase letters, and introns and flanking sequences are in lowercase letters. The putative poly(A) signal is double-underlined.

the PGD synthase gene. A *Bam*HI fragment (9 kb) from the cloned phages was subcloned into a pUC18 vector, used for detailed mapping of the gene with various restriction endonucleases and for further subcloning to sequence the DNA. The nucleotide sequence of the gene is shown in Fig. 2.

The cap site as mapped by primer-extension analysis is a guanine residue 39 base pairs (bp) upstream of the translation start codon (ATG) (Fig. 1*B*). The region up to 1.7 kb upstream of the transcription initiation site contains several features. Neither TATA nor CAAT boxes are found. A TATA-like sequence (ATAAATA) is observed at position −66 to −60 bp. However, this position is far from the initiation site as compared with the usual TATA boxes (−20 to −30 bp). Two possible Sp1-binding sites are present at overlapping positions −142 to −137 and −146 to −141 (CCGCCC antisense). Several sets of direct repeat sequences (≥10 bp, 100% matching) and inverted repeat sequences (≥12 bp, >90% matching) are also observed (Fig. 2), but they show no significant similarities to known enhancers or other functional sequences. Because such inverted and palindromic repeats can form a secondary structure, they may be involved in the control and/or modulation of PGD synthase gene expression.

The gene spans ≈3 kb and contains seven exons ranging in size from 56 bp (exon 6) to 153 bp (exon 1) that are split by six introns ranging from 105 bp (intron 2) to 652 bp (intron 3). All splice junction sequences flanking the introns conform to the consensus splice junction sequences and the GT/AG splice rule. The entire 5′-untranslated region is included in exon 1. The coding region is divided into exons 1–6, and the 3′-untranslated region is in exons 6 and 7. The DNA sequence of all exons agrees perfectly with that of the cDNA obtained previously (8), in which we found five mistakes (addition of three guanine residues and two guanine/cytosine substitutions in the corrected sequence**). Because of the corrections, rat brain PGD synthase now has 189 residues with a M_r of 21,219.

As reported (9, 10), PGD synthase is a member of the lipocalin superfamily, which consists of various small (160–190 amino acid residues) secretory proteins sharing a common feature for binding and transport of small hydrophobic molecules (11). Because of the high evolutionary divergence of this superfamily, the homology of the amino acid sequences of the members is very weak; yet the tertiary structure is well conserved to form a remarkably similar β-barrel structure as revealed by x-ray crystallography of β-lactoglobulin (29), retinol-binding protein (30), and bilin-binding protein (31).

Compared with the genes for seven lipocalins already characterized, the structure of the PGD synthase gene is remarkably similar to those of the genes for β-lactoglobulin (32), α_2-urinary globulin (33), placental protein 14 (34), and α_1-microglobulin (35); all corresponding exons are similar in size, all corresponding introns are identically spliced in phase, and all the genes except the α_1-microglobulin gene possess an entirely noncoding exon 7 (Fig. 3). α_1-Microglobulin links to an HI-30 peptide at the amino terminus

**The corrected sequence has been deposited in the GenBank data base (accession no. M61900).

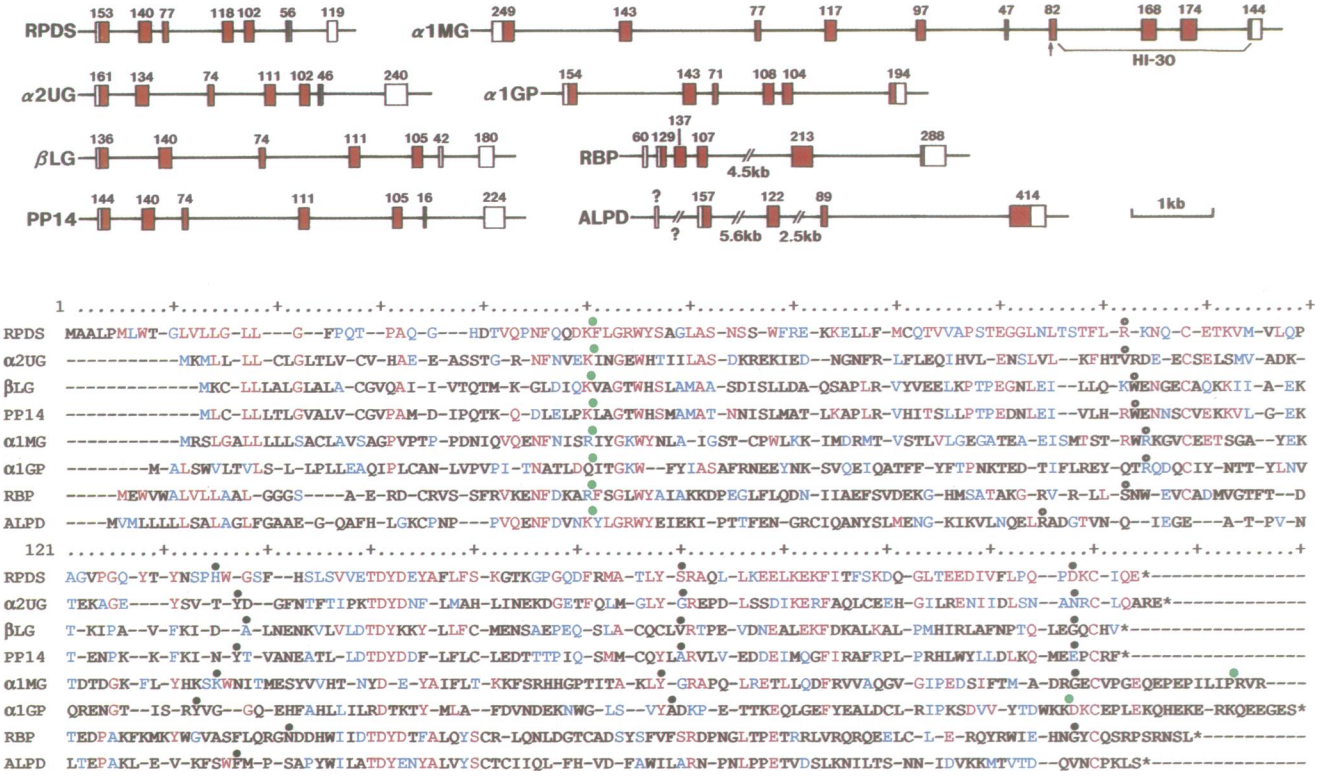

FIG. 3. Alignment of the deduced amino acid sequences of rat brain PGD synthase and seven lipocalins with known gene structures. Structures of the genes for rat brain PGD synthase and the seven lipocalins are shown at top. Exons are shown as open boxes, and their coding region are shown in red; sizes of exons are indicated above the boxes. Introns and flanking regions are depicted as a thin line. Scale bar represents 1-kb length. The boundary of α_1-microglobulin and HI-30 peptide regions is indicated by an arrow (36). In a multiple alignment, the amino acid residues with exact matches and conservative substitutions against those of PGD synthase are shown in red and blue, respectively. Stars indicate stop codons. Positions of the exon/intron junction are indicated by solid green circles between residues (codon–codon splicing) and open circles (splicing between the first and second base of a codon) or solid black circles (splicing between the second and third base of a codon) above the residue. RPDS, rat brain PGD synthase; α2UG, mouse α_2-urinary globulin (murine urinary protein); βLG, ovine β-lactoglobulin; PP14, human placental protein 14; α1MG, human α_1-microglobulin; α1GP, human α_1-acid glycoprotein; RBP, rat retinol-binding protein; ALPD, human apolipoprotein D.

in the seventh exon, constructing a protein named inter-α-trypsin inhibitor (36). The corresponding exons of α_1-acid glycoprotein gene (37) are similar in size to those of PGD synthase, although one intron (intron 6) is out-of-phase and the seventh noncoding exon is lost in the α_1-acid glycoprotein gene. The structures of the genes for retinol-binding protein (38) and apolipoprotein D (27) differ from the other gene structures. These two genes have an entirely noncoding exon 1 and a fused downstream exon that corresponds to exons 4 and 5 or exons 4–6 of the PGD synthase gene, respectively. The positions of exon/intron junctions are well conserved among members of the lipocalin superfamily (32). In a multiple alignment of the deduced amino acid sequences (Fig. 3), the exon/intron junctions of PGD synthase were also present at the corresponding positions to those of other lipocalins despite a weak homology (13.1–24.7% identity and 28.8–44.2% similarity).

The phylogenetic tree of 26 members of the superfamily reflects the structural similarity in terms of both gene organization and disulfide bonding patterns (Fig. 4). Retinol-binding protein and apolipoprotein D, encoded by genes that show the fusion of the downstream exons, form a distinct cluster apart from PGD synthase and other lipocalins. In this cluster, apolipoprotein D, bilin-binding proteins, and crustcyanins form a subcluster, all of which possess the distinctive disulfide bonding pattern (aligned positions of 73 and 220 in Fig. 3) different from that of other lipocalins (aligned positions of 109 and 220). Pyrazine-binding protein, which is only one lipocalin free from disulfide bond (43), does not exist in the subcluster but is homologous to aphrodisin and odorant-binding protein. Among the members of the superfamily, PGD synthase shows the greatest homology (32.5% identity and 52.2% similarity) with 24p3 protein, an oncogene product recently identified as a lipocalin (10, 44), suggesting a close evolutionary relationship between this protein and PGD synthase. PGD synthase, together with 24p3 protein and human complement component C8 γ chain, is part of a cluster distinct from other clusters in the phylogenetic tree. Most lipocalins other than PGD synthase have been characterized as soluble secretory proteins, except that an isoform of probasin, which is translated from the second AUG, reportedly translocates to the nucleus (45). Two distinctive characteristics of PGD synthase, as an enzyme and as a membrane-associated protein, were probably acquired after the divergence from these two lipocalins during evolution of the superfamily.

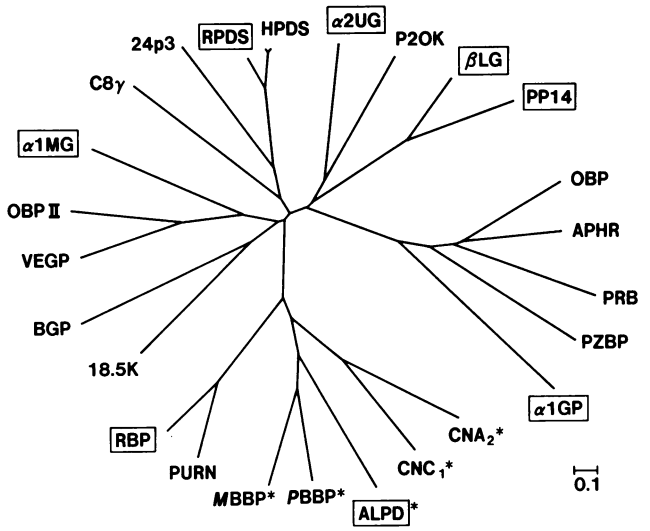

FIG. 4. Phylogenetic tree of 26 members of the lipocalin family. Scale bar represents branch length corresponding to 0.1 amino acid substitution per site. Lipocalins of known gene structures that possess the distinctive disulfide bonding pattern are boxed and starred. Abbreviations, sources, and accession numbers in the National Biomedical Research Foundation data base or references are as follows: RPDS, rat brain PGD synthase; HPDS, human brain PGD synthase (10); 24p3, mouse 24p3 protein (S07397); C8γ, human complement component C8 γ chain (C8HUG); α2UG, rat α_2-urinary globulin (39); p20K, chicken quiescence-specific polypeptide 20K (A30230); βLG, ovine β-lactoglobulin (A25136); PP14, human placental protein 14 (A35570); OBP, rat odorant-binding protein (A28713); APHR, hamster aphrodisin (A31243); PRB, rat probasin (A32602); PZBP, bovine pyrazine-binding protein (S06843); α1GP, human α_1-acid glycoprotein (OMHU1); α1MG, human α_1-microglobulin (HCHU); OBPII, rat odorant-binding protein II (40); VEGP, rat von Ebner's gland protein (S08161); BGP, frog (*Rana pipiens*), Bowman's gland protein (OVFGP); 18.5K, rat androgen-dependent epididymal 18.5K protein (SQRTAD); RBP, rat retinol-binding protein (VART); PURN, chicken retinol-binding protein (purpurin) (A26969); *M*BBP, tobacco hornworm (*Manduca sexta*) bilin-binding protein (insecticyanin) (CUWOI); *P*BBP, butterfly (*Pieris brassica*) bilin-binding protein (S00819); ALPD, human apolipoprotein D (A26958); CNA_2, lobster (*Homarus gammarus*), crustcyanin A_2 (41); CNC_1, lobster (*Homarus gammarus*) crustcyanin C_1 (42).

When the numbers of nucleotide substitutions were calculated between rats and humans at the silent and amino acid-changing nucleotide sites (K_s^c and K_a^c, respectively), the K_s^c values were found to be 0.76 for PGD synthase, 0.69 for retinol-binding protein, 0.89 for α_1-acid glycoprotein, and 0.82 for apolipoprotein D. The values are in a range of K_s^c values (0.43–0.95) of any pairs of orthologous nuclear autosomal genes between rodents and humans (46), suggesting that the genes for these lipocalins are also orthologous between the two species. On the other hand, the K_a^c values were 0.20 for PGD synthase, 0.10 for retinol-binding protein, 0.18 for apolipoprotein D, and 0.44 for α_1-acid glycoprotein, indicating that the evolutionary rate of PGD synthase is in the middle of the range shown by other lipocalins.

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1. Hayaishi, O. (1991) *FASEB J.* **5**, 2575–2581.
2. Ito, S., Narumiya, S. & Hayaishi, O. (1989) *Prostaglandins Leukotrienes Essent. Fatty Acids* **37**, 219–234.
3. Urade, Y., Fujimoto, N. & Hayaishi, O. (1985) *J. Biol. Chem.* **260**, 12410–12415.
4. Ujihara, M., Urade, Y., Eguchi, N., Hayashi, H., Ikai, K. & Hayaishi, O. (1988) *Arch. Biochem. Biophys.* **260**, 521–531.
5. Goh, H., Urade, Y., Fujimoto, N. & Hayaishi, O. (1987) *Biochim. Biophys. Acta* **921**, 302–311.
6. Tachibana, M., Fex, J., Urade, Y. & Hayaishi, O. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7677–7680.
7. Urade, Y., Fujimoto, N., Kaneko, T., Konishi, A., Mizuno, N. & Hayaishi, O. (1987) *J. Biol. Chem.* **262**, 15132–15136.
8. Urade, Y., Nagata, A., Suzuki, Y., Fujii, Y. & Hayaishi, O. (1989) *J. Biol. Chem.* **264**, 1041–1045.
9. Nagata, A., Suzuku, Y., Igarashi, M., Eguchi, N., Toh, H., Urade, Y. & Hayaishi, O. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4020–4024.
10. Peitsch, M. C. & Bougski, M. S. (1991) *Trends Biochem. Sci.* **16**, 363.
11. Pervaiz, S. & Brew, K. (1987) *FASEB J.* **1**, 209–214.
12. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
13. Gross-Bellard, M., Oudet, P. & Chambon, P. (1973) *Eur. J. Biochem.* **36**, 32–38.
14. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
15. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
16. Seto, Y., Ihara, S., Kohtsuki, S., Ooi, T. & Sakakibara, S. (1988) in *Computational Molecular Biology*, ed. Lesk, A. M. (Oxford Univ. Press, New York), pp. 27–37.

17. Dayhoff, M. O., ed. (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington), Vol. 5, Suppl. 3.
18. Nishikawa, K., Nakashima, H., Kanehisa, M. & Ooi, T. (1987) *Protein Sequences Data Anal.* **1**, 107–116.
19. Feng, D. F. & Doolittle, R. F. (1987) *J. Mol. Evol.* **25**, 351–360.
20. Saitou, N. & Nei, M. (1987) *Mol. Biol. Evol.* **4**, 406–425.
21. Nei, M. (1987) *Molecular Evolutionary Genetics* (Columbia Univ. Press, New York).
22. Merritt, C. M. & Board, P. G. (1988) *Gene* **66**, 97–106.
23. Cooper, R., Eckley, D. M. & Papaconstantinou, J. (1987) *Biochemistry* **26**, 5244–5250.
24. Colantuoni, V., Romano, V., Bensi, G., Santoro, C., Costanzo, F., Raugei, G. & Cortese, R. (1983) *Nucleic Acids Res.* **11**, 7769–7776.
25. Laurent, B. C., Nilsson, M. H. L., Bavik, B. O., Jones, T. A., Sundelin, J. & Peterson, P. A. (1985) *J. Biol. Chem.* **260**, 11476–11480.
26. Drayna, D., Fielding, C., McLean, J., Baer, B., Castro, G., Chen, E., Comstock, L., Henzel, W., Kohr, W., Rhee, L., Wion, K. & Lawn, R. (1987) *DNA* **6**, 199–204.
27. Spreyer, P., Schaal, H., Kuhn, G., Rothe, T., Unterbeck, A., Olek, K. & Muller, H. W. (1990) *EMBO J.* **9**, 2479–2484.
28. Jukes, T. H. & Cantor, C. R. (1969) in *Mammalian Protein Metabolism*, ed. Munro, H. N. (Academic, New York), Vol. 3, pp. 21–132.
29. North, A. C. T. (1989) *Int. J. Biol. Macromol.* **11**, 56–58.
30. Cowan, S. W., Newcomer, M. E. & Jones, T. A. (1990) *Proteins* **8**, 44–61.
31. Huber, R., Schneider, M., Epp, O., Mayr, I., Messerschmidt, A., Pflugrath, J. & Kayser, H. (1987) *J. Mol. Biol.* **195**, 423–434.
32. Ali, S. & Clark, A. J. (1988) *J. Mol. Biol.* **199**, 415–426.
33. Clark, A. J., Ghazal, P., Bingham, R. W., Barrett, D. & Bishop, J. O. (1985) *EMBO J.* **4**, 3159–3165.
34. Vaisse, C., Atger, M., Potier, B. & Milgrom, E. (1990) *DNA Cell. Biol.* **9**, 401–413.
35. Vetr, H. & Gebhard, W. (1990) *Biol. Chem. Hoppe-Seyler* **371**, 1185–1196.
36. Kaumeyer, J. F., Polazzi, J. O. & Kotick, M. P. (1986) *Nucleic Acids Res.* **14**, 7839–7850.
37. Dente, L., Pizza, M. G., Metspalu, A. & Cortese, R. (1987) *EMBO J.* **6**, 2289–2296.
38. Laurent, B. C., Nilsson, M. H. L., Bavik, B. O., Jones, T. A., Sundelin, J. & Peterson, P. A. (1985) *J. Biol. Chem.* **260**, 11476–11480.
39. Ichiyoshi, Y., Endo, H. & Yamamoto, M. (1987) *Biochim. Biophys. Acta* **910**, 45–51.
40. Dear, T. N., Campbell, K. & Rabbitts, T. H. (1991) *Biochemistry* **30**, 10376–10382.
41. Keen, J. N., Caceres, I., Eliopoulos, E. E., Zagalsky, P. F. & Findlay, J. B. C. (1991) *Eur. J. Biochem.* **197**, 407–417.
42. Zagalsky, P. F., Eliopoulos, E. E. & Findlay, J. B. C. (1990) *Comp. Biochem. Physiol. B* **97**, 1–18.
43. Tirindell, R., Keen, J. N., Cavaggioni, A., Eliopoulos, E. E. & Findlay, J. B. C. (1989) *Eur. J. Biochem.* **185**, 569–592.
44. Flower, D. R., North, A. C. T. & Attwood, T. K. (1991) *Biochem. Biophys. Res. Commun.* **180**, 69–74.
45. Spence, A. M., Sheppard, P. C., Davie, J. R., Matuo, Y., Nishi, N., McKeehan, W. L., Dodd, J. G. & Matusik, R. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7843–7847.
46. Miyata, T., Hayashida, H., Kuma, K. & Yasunaga, T. (1987) *Proc. Jpn. Acad.* **63**, 327–331.