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(54) CYCLIC GMP-BINDING, CYCLIC GMP-SPECIFIC PHOSPHODIESTERASE MATERIALS AND METHODS

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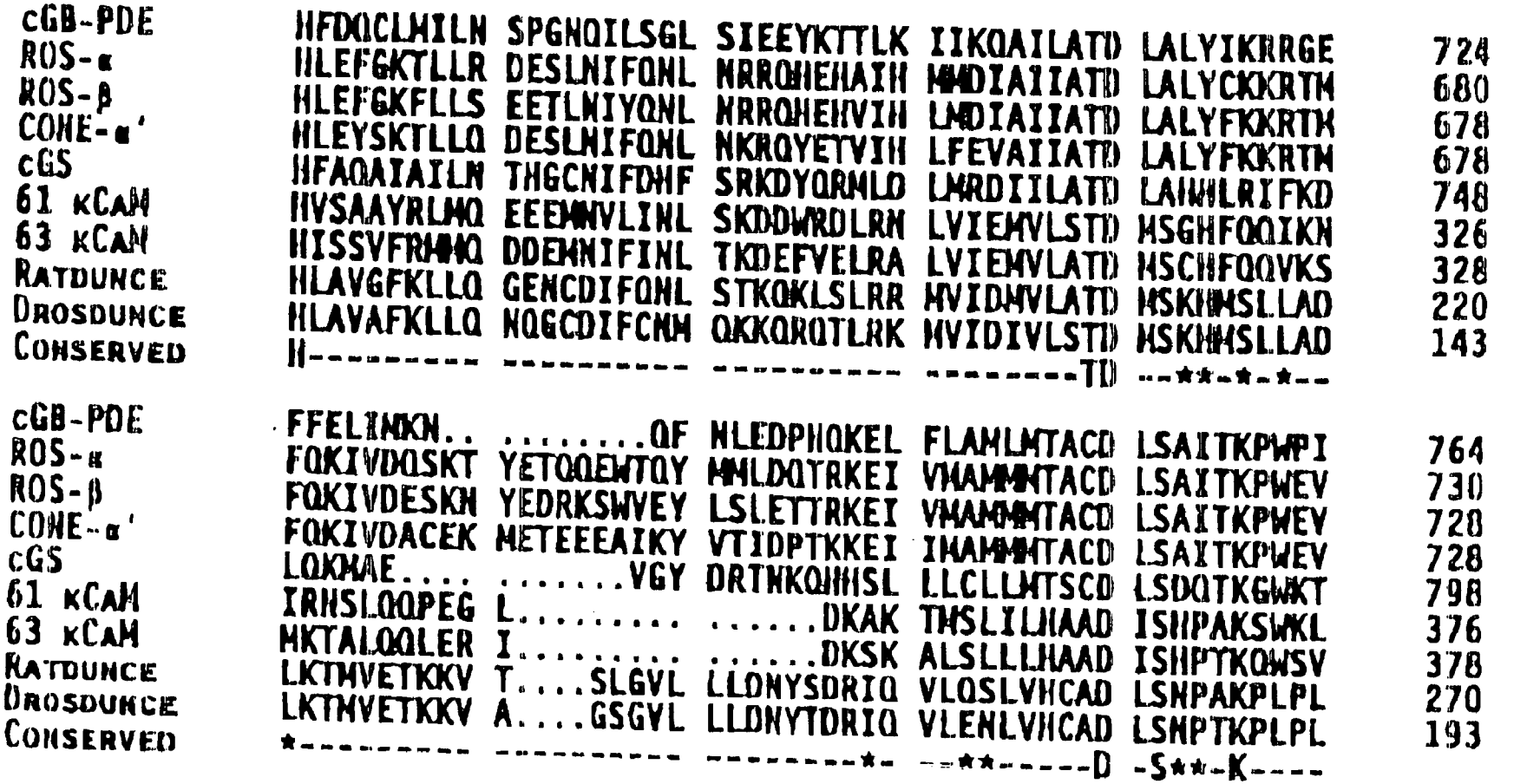
(57) **ABSTRACT**

The present invention provides novel purified and isolated nucleotide sequences encoding the cGMP-binding. cGMP-specific phosphodiesterase designated cGB-PDE. Also provided by the invention are methods and materials for the recombinant production of cGB-PDE polypeptide products and methods for identifying compounds which modulate the enzymatic activity of cGB-PDE polypeptides.

```
cGB-PDE       FQMKHEVLCK WILSVKKNYR K.NVAYHNWR HAFNTAQCMF AALKAGKIQK   626
ROS-α         FHIPQEALVR FNYSLSKGYR R..ITYHNWR HGFNVGQTMF SLLVTGKLKR   582
ROS-β         FQIPQEVLVR FLFSVSKGYR R..ITYHNWR HGFNVAQTMF TLLMTGKLKS   580
CONE-α'       FKVPVEVLTR WMTYVRKGYR A..VTYHNWR HGFNVGQTMF TLLMTGRLKK   580
cGS           YKIDCPTLAR FCLMVKKGYR D.P.PYHNWM HAFSVSHFCY LLYKNLELTN   639
61 kCaM       FKIPVSCLIA FAEALEVGYS KYKNPYHNLI HAADVTQTVH YIMLHTGIMH   242
63 kCaM       FKIPTVFLMT FLDALETGYG KYKNPYHNQI HAADVTQTVH CFLLRTGMVH   244
RatDunce      FQIPADTLLR YLLTLEGHYH S.NVAYHNSI HAADVVQSAH VLLGTPALEA   125
DrosDunce     .MIPPKTFLN FMSTLEDHYV K.DNPFHNSL HAADVTQSTN VLLNTPALEG    48
Conserved     -----*----- --------Y- ------HN-* H-------** -----*----

cGB-PDE       RLTDLEILAL LIAALSHDLD HRGVNNSYIQ RSEHPLAQLY CH..SIMEHH   674
ROS-α         YFTDLEALAM VTAAFCHDID HRGTNNLYQM KSQNPLAKLH GS..SILERH   630
ROS-β         YYTDLEAFAM VTAGLCHDID HRGTNNLYQM KSQNPLAKLH GS..SILERH   628
CONE-α'       YYTDLEAFAM LAAAFCHDID HRGTNNLYQM KSTSPLARLH GS..SILERH   628
cGS           YLEDMEIFAL FISCMCHDLD HRGTNNSFQV ASKSVLAALY SSEGSVMERH   709
61 kCaM       WLTELEILAM VFAAAIHDYE HIGTTNNFHI QTRSDVAILY .NDRSVLENH   291
63 kCaM       CLSEIEVLAI IFAAAIHDYE HIGTTNSFHI QTKSEQAILY .NDRSVLENH   293
RatDunce      VFTDLEVLAA IFACAIHDVD HPGVSNQFLI NTNSELALMY .NDSSVLENH   174
DrosDunce     VFTPLEVGGA LFAACIHDVD HPGLTNQFLV NSSSELALMY .NDESVLENH    97
Conserved     -----E---- ------HD-- H-G--N-*-- ----*-A--- ----S--E-H
```

FIGURE 1A


cGB-PDE HFDQCLMILN SPGNQILSGL SIEEYKTTLK IIKQAILATD LALYIKRRGE 724
ROS-α HLEFGKTLLR DESLNIFQNL NRRQHEHAIH MADIAIIATD LALYCKKRTM 680
ROS-β HLEFGKFLLS EETLNIYQNL NRRQHEHVIH LMDIAIIATD LALYFKKRTK 678
CONE-α' HLEYSKTLLQ DESLNIFQNL NKRQYETVIH LFEVAIIATD LALYFKKRTM 678
cGS HFAQQAIAILN THGCNIFDHF SRKDYQRMLD LMRDIILATD LAIMLRIFKD 748
61 kCaM HVSAAYRLMQ EEEMNVLINL SKDDWRDLRM LVIEMVLSTD MSGHFQQIKN 326
63 kCaM HISSVFRMMQ DDEMNIFINL TKDEFVELRA LVIEMVLATD MSCHFQQVKS 328
RatDunce HLAVGFKLLQ GENCDIFQNL STKQKLSLRR MVIDMVLATD MSKHMSLLAD 220
DrosDunce HLAVAFKLLQ NQGCDIFCNM QKKQRQTLRK MVIDIVLSTD MSKHMSLLAD 143
Conserved H--------- ---------- ---------- --------TD --**-*-*-*--

cGB-PDE FFELINKN.. ....QF NLEDPHQKEL FLAMLMTACD LSAITKPWPI 764
ROS-α FQKIVDQSKT YETQQEWTQY MMLDQTRKEI VMAMMMTACD LSAITKPWEV 730
ROS-β FQKIVDESKN YEDRKSWVEY LSLETTRKEI VMAMMMTACD LSAITKPWEV 720
CONE-α' FQKIVDACEK METEEEAIKY VTIDPTKKEI IMAMMMTACD LSAITKPWEV 728
cGS LQKMAE.... ....VGY DRTMKQHMSL LLCLLMTSCD LSDQTKGWKT 798
61 kCaM IRHSLQQPEG L......... ......DKAK TMSLILHAAD ISHPAKSWKL 376
63 kCaM MKTALQQLER I......... ......DKSK ALSLLLHAAD ISHPTKQWSV 378
RatDunce LKTMVETKKV T....SLGVL LLDNYSDRIQ VLQSLVHCAD LSNPAKPLPL 270
DrosDunce LKTMVETKKV A....GSGVL LLDNYTDRIQ VLENLVHCAD LSNPTKPLPL 193
Conserved *--------- ---------- ---------*- --**------D -S**-K----

FIGURE 1B

```
CGB-POE   QQRIAELVAT EFFQQGDRER KELNIEPADL MNREKKNKIP SMQVGFID..  812
ROS-α     QSKVALLVAA EFWEQGDLER TVLQQNPIPM MDRNKADELP KLQVGFID..  778
ROS-β     QSKVALLVAA EFWEQGDLER TVLDQQPIPM MDRNKAAELP KLQVGFID..  776
CONE-α'   QSQVALLVAN EFWEQGDLER TVLQQQPIPM MDRNKKDELP KLQVGFID..  776
CGS       TRKIAELIYK EFFSQGDLEK A.MGNRPMEM MDREKAY.IP ELQISFME..  844
61 KCAM   HHRWTMALME EFFLQGDKEA EL..GLPFSP LCDRKSTMVA QSQIGFID..  422
63 KCAM   HSRWTKALME EFFRQGDKEA EL..GLPFSP LCDRTSTLVA QSQIGFID..  424
RATDUNCE  YRQWTERIMA EFFQQGDRER ES..GLDISP MCDKHTASVE KSQVGFID..  316
DROSDUNCE YKRWVALLME EFFLQGDKER ES..GMDISP MCDRHNATIE KSQVGFID..  239
CONSERVED *------**- EF--QGD-E- ---------- --Q--F----
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FIGURE 1C

```
cGB-PDE      LLELVKDISS HLDVTALCHK IFLHIHGLIS ADRYSLFLVC EDSSNDKFLI   188
cGS          ILQLCGELYD .LDASSLQLK VLQYLQQETQ ASRCCLLLVS EDN..LQ.LS   245
CONE-α'      LLEVL..LEE AGSVELAAHR ALQRLAQLLQ ADRCSMFLCR ARNGTPE.VA   106
ROS-β        LFELVQDMQE NVNMERVVFK ILRRLCSILH ADRCSLFMYR QRNGVAE.LA   107
ROS-α        ...LLRDFQD NLQAEKCVFN VMKKLCFLLQ ADRMSLFMYR ARNGIAE.LA   109
CONSERVED    ---------- ---------- ---------- A-R------- ----------

cGB-PDE      SRLFDVAEGS TLEE...ASN NCIRLEWNKG IVGHVAAFGE PLNIKDAYED   237
cGS          CKVIG...DK VLEE..... .EISFPLTTG RLGQVVEDKK SIQLKDLTSE   292
CONE-α'      SKLLDVTPTS KFEDNLVVPD REAVFPLDVG IVGWVAHTKK TFNVPDVKKN   154
ROS-β        TRLFSVQPDS VLEDCLVPPD SEIVFPLDIG VVGHVAQTKK MVNVQDVMEC   155
ROS-α        TRLFNVHKDA VLEECLVAPD SEIVFPLDMG VVGHVALSKK IVNVPNTEED   157
CONSERVED    ---------- --E------- ---------G --G-V----- ----------

cGB-PDE      PRFNAEVDQI TGYKTQSILC MPIKNHR.EE VVGVAQAINK KSGNGGTFTE   287
cGS          DM..QQLQSM LGCEVQAMLC VPVISRATDQ VVALACAFNK ..LGGDLFTD   342
CONE-α'      SHFSDFMDKQ TGYVTRNLLA TPIV..MGKE VLAVFMAVNK ..VDASEFSK   204
ROS-β        PHFSSFADEL TDYVTRNILA TPIM..NGKD VVAVIMAVNK ..LDGPCFTS   205
ROS-α        EHFCDFVDTL TEYQTKNILA SPIM..NGKD VVAIIMAVNK ..VDGPHFTE   207
CONSERVED    ---------- --------L- -P-------- V-----A-NK -------F--
```

FIGURE 2A

```
cGB-PDE      KDEKDFAAYL AFCGIVLHNA QLYETSLLEN KRNQVLLDLA SLIFEEQQSL    337
cGS          QDEHVIQHCF HYTSTVLTST LAFQKEQKLK CECQALLQVA KNLFTHLDDV    390
CONE-α'      QDEEVFSKYL SFVSIILKLH HTNYLYNIES RRSQILMWSA NKVFEELTDV    252
ROS-β        EDEDVFLKYL NFGTLNLKIY HYSYLHNCET RRGQVLLWSA NKVFEELTDI    253
ROS-α        NDEEILLKYL NFANLIMKVF HLSYLHNCET RRGQILLWSG SKVFEELTDI    255
CONSERVED    -DE------- ---------- ---------- ---Q-L---- ---F------

cGB-PDE      EVILKKIAAT IISFMQVQKC TIFIVD.EDC SDSFSSVFHM ECEELEKSSD    361
cGS          SVLLQEIITE ARNLSNAEIC SVFLID...Q NELVAKVFDG GVLEDESY..    409
CONE-α'      ERQFHKALYT VRTYLNCERY SIGLLDMTKE KEFY.DEWPV KPGEVEPYKG    301
ROS-β        ERQFHKAFYT VRAYLNCDRY SVGLLDMTKE KEFF.DVWPV LMGEAQAYSG    302
ROS-α        ERQFHKALYT VRAFLNCDRY SVGLLDMTKQ KEFF.DVWPV LMGEAPPYAG    304
CONSERVED    ---------- ---------- -----D---- ---------- ---E------

cGB-PDE      TLTRE..... .......... .......... ..RDANRINY MYAQYVKNTM    411
cGS          .......... .......... ...EIRI... .PADQ..... GIAGHVATTG    459
CONE-α'      PKTPDGREVI FYKIIDYILH GKEEIKVIPT PPMDHWTLIS GLPTYVAENG    351
ROS-β        PRTPDGREIL FYKVIDYILH GKEDIKVIPS PPADHWALAS GLPTYVAESG    352
ROS-α        PRTPDGREIN FYKVIDYILH GKEDIKVIPN PPPDHWALVS GLPTYVAQNG    354
CONSERVED    ---------- ---------- ---------- ---D------ -----V----
```

FIGURE 2B

```
cGB-PDE       EPLNIPDVSK DKRFPWTNEN MGNINQQCIR SLLCTPIKNG KKNKVIGVCQ    459
cGS           QILNIPDAYA HPLFY..RGV DDSTGRF.TR NILCFPIKN. ENQEVIGVAE    499
CONE-α'       FICNMLNAPA DEYFTFQKGP VDETGWV.IK NVLSLPIVN. KKEDIVGVAT    399
ROS-β         FICNIMNAPA DEMFNFQEGP LDDSGWI.VK NVLSMPIVN. KKEEIVGVAT    400
ROS-α         LICNIMNAPS EDFFAFQKEP LDESGWM.IK NVLSMPIVN. KKEEIVGVAT    402
CONSERVED     ---N------ ---F------ ---------- --L--PI-N- ------GV--

cGB-PDE       LVNKMEETTG KVKAFNRNDE QFLEAFVIFC GLGIQNTQMY EAVERAMAKQ    506
cGS           LVNKING... ..PWFSKFDE DLATAFSIYC GISIAHSLLY KKVNEAQYRS    541
CONE-α'       FYNRKDG... ..KPFDEYDE HIAETLTQFL GWSLLNTDTY EKMNKLENRK    441
ROS-β         FYNRKDG... ..KPFDEQDE VLMESLTQFL GWSVLNTDTY DKMNKLENRK    442
ROS-α         FYNRKDG... ..KPFDEMDE TLMESLAQFL GWSVLNPDTY ELMNKLENRK    444
CONSERVED     --N------- ----F---DE ---------- G--------Y ----------

cGB-PDE       MVTLEVLSYH ASAAEEE                                        526
cGS           HLANEMMMYH MKVSDDE                                        561
CONE-α'       DIAQEMLMNH TKATPDE                                        461
ROS-β         DIAQDMVLYH VRCDREE                                        462
ROS-α         DIFQDMVKYH VKCDNEE                                        464
CONSERVED     ---------H ------E
```

FIGURE 2C

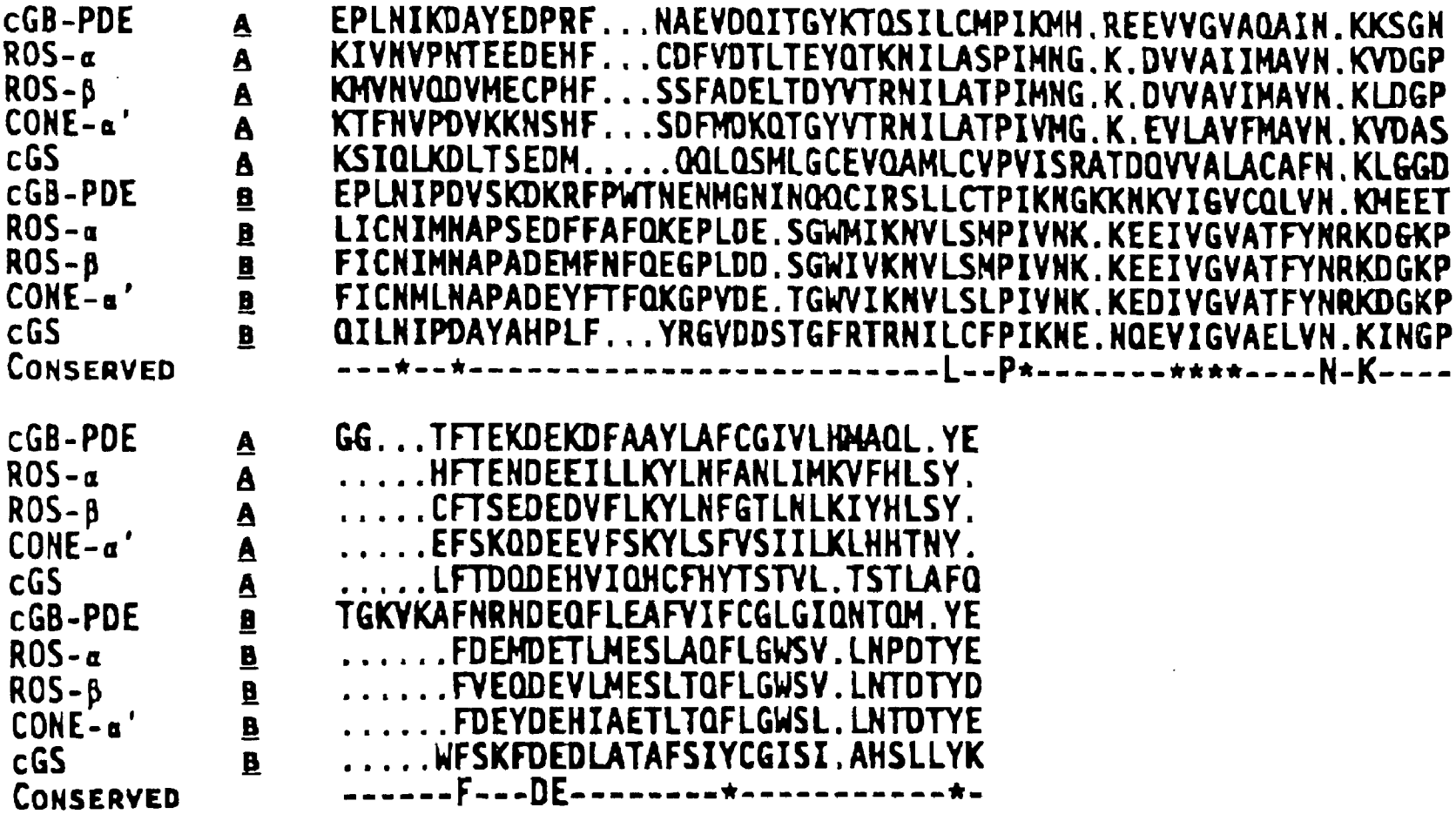
cGB-PDE A EPLNIKDAYEDPRF...NAEVDQITGYKTQSILCMPIKMH.REEVVGVAQAIN.KKSGN
ROS-α A KIVNVPNTEEDEHF...CDFVDTLTEYQTKNILASPIMNG.K.DVVAIIMAVN.KVDGP
ROS-β A KMVNVQDVMECPHF...SSFADELTDYVTRNILATPIMNG.K.DVVAVIMAVN.KLDGP
CONE-α' A KTFNVPDVKKNSHF...SDFMDKQTGYVTRNILATPIVMG.K.EVLAVFMAVN.KVDAS
cGS A KSIQLKDLTSEDM.....QQLQSMLGCEVQAMLCVPVISRATDQVVALACAFN.KLGGD
cGB-PDE B EPLNIPDVSKDKRFPWTNENMGNINQQCIRSLLCTPIKNGKKNKVIGVCQLVN.KMEET
ROS-α B LICNIMNAPSEDFFAFQKEPLDE.SGWMIKNVLSMPIVNK.KEEIVGVATFYNRKDGKP
ROS-β B FICNIMNAPADEMFNFQEGPLDD.SGWIVKNVLSMPIVNK.KEEIVGVATFYNRKDGKP
CONE-α' B FICNMLNAPADEYFTFQKGPVDE.TGWVIKNVLSLPIVNK.KEDIVGVATFYNRKDGKP
cGS B QILNIPDAYAHPLF...YRGVDDSTGFRTRNILCFPIKNE.NQEVIGVAELVN.KINGP
CONSERVED ---*--*----------L--P*--------****----N-K----
cGB-PDE A GG...TFTEKDEKDFAAYLAFCGIVLHMAQL.YE
ROS-α A .....HFTENDEEILLKYLNFANLIMKVFHLSY.
ROS-β A .....CFTSEDEDVFLKYLNFGTLNLKIYHLSY.
CONE-α' A .....EFSKQDEEVFSKYLSFVSIILKLHHTNY.
cGS A .....LFTDQDEHVIQHCFHYTSTVL.TSTLAFQ
cGB-PDE B TGKVKAFNRHDEQFLEAFVIFCGLGIQNTQM.YE
ROS-α B ......FDEMDETLMESLAQFLGWSV.LNPDTYE
ROS-β B ......FVEQDEVLMESLTQFLGWSV.LNTDTYD
CONE-α' B ......FDEYDEHIAETLTQFLGWSL.LNTDTYE
cGS B .....WFSKFDEDLATAFSIYCGISI.AHSLLYK
CONSERVED ------F---DE--------*----------*-

FIGURE 3

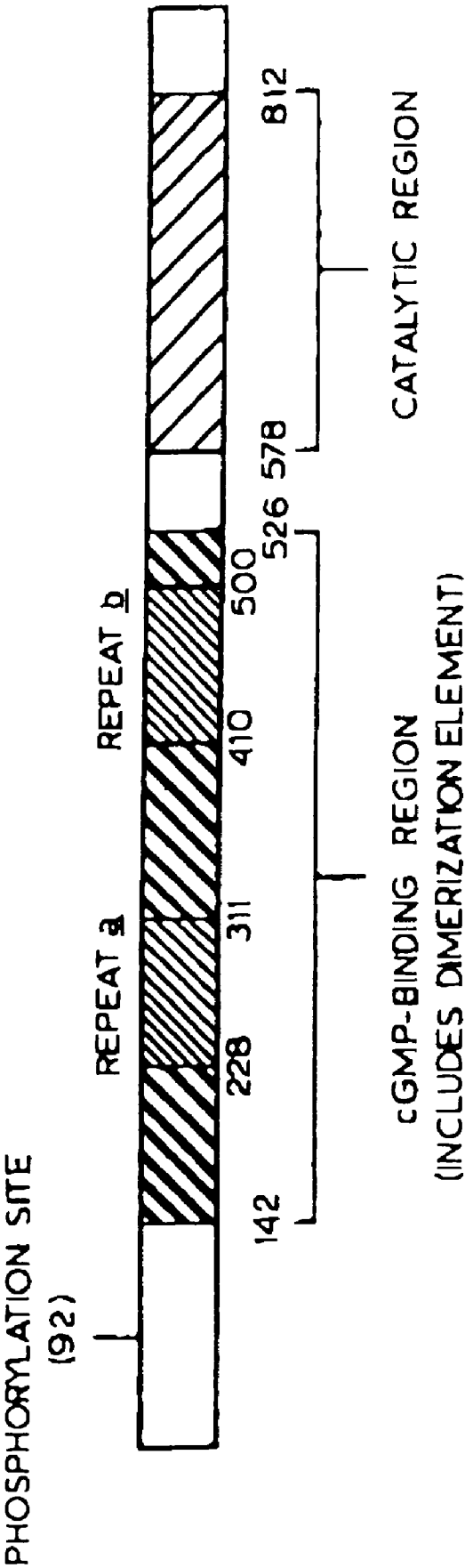
PHOSPHORYLATION SITE
(92)
REPEAT a
REPEAT b
142
228
311
410
500
526
578
812
cGMP-BINDING REGION
(INCLUDES DIMERIZATION ELEMENT)
CATALYTIC REGION

FIGURE 4

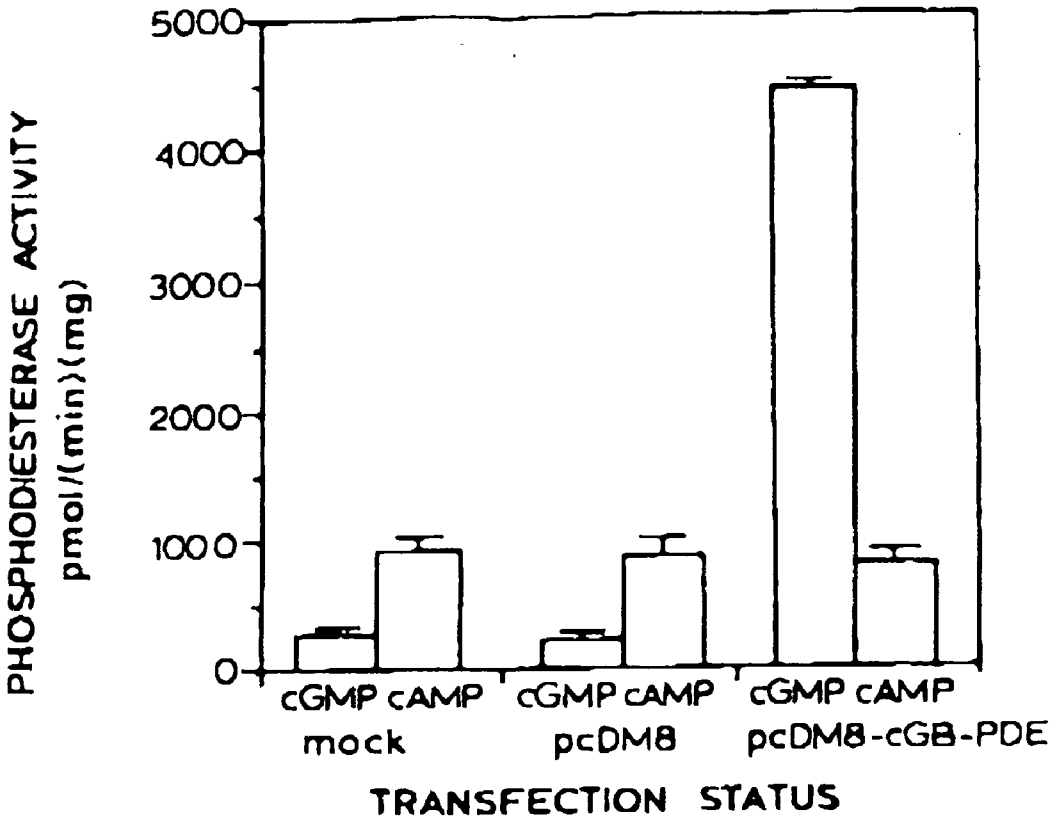

FIGURE 5

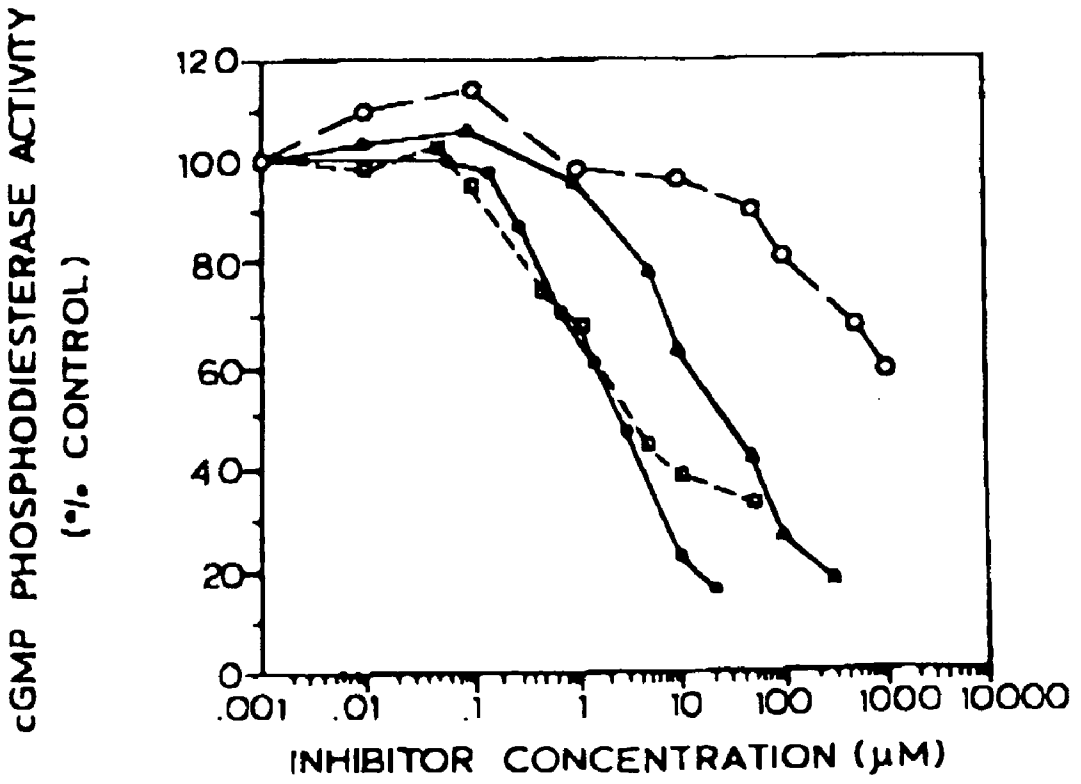

FIGURE 6

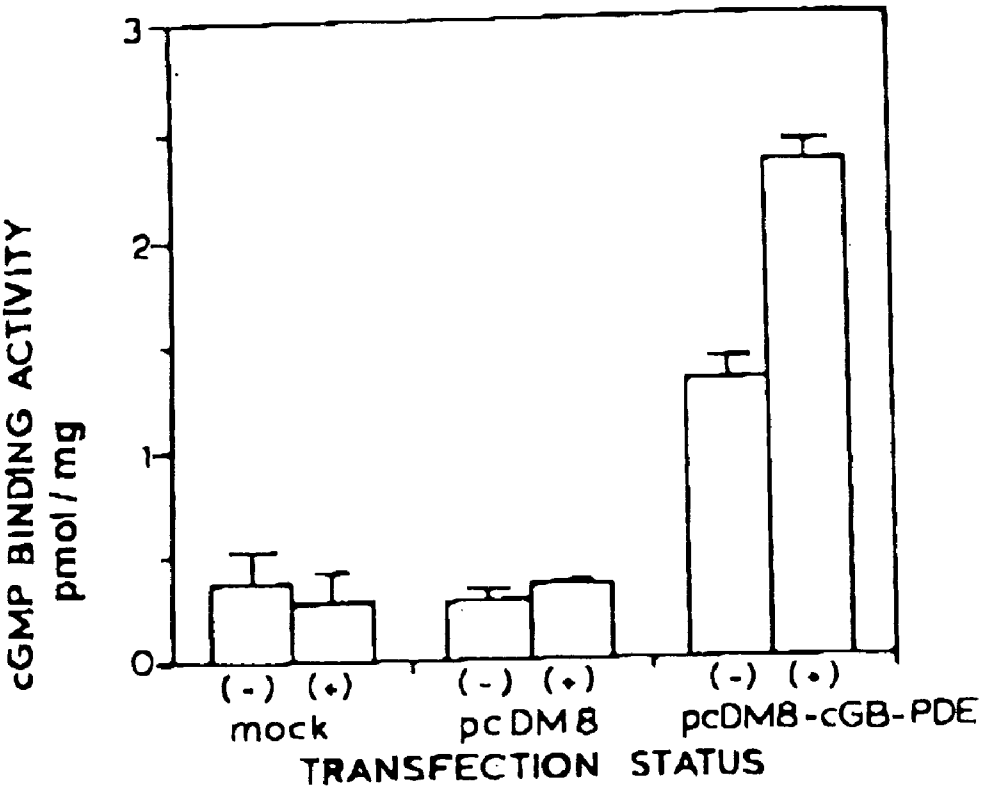

FIGURE 7

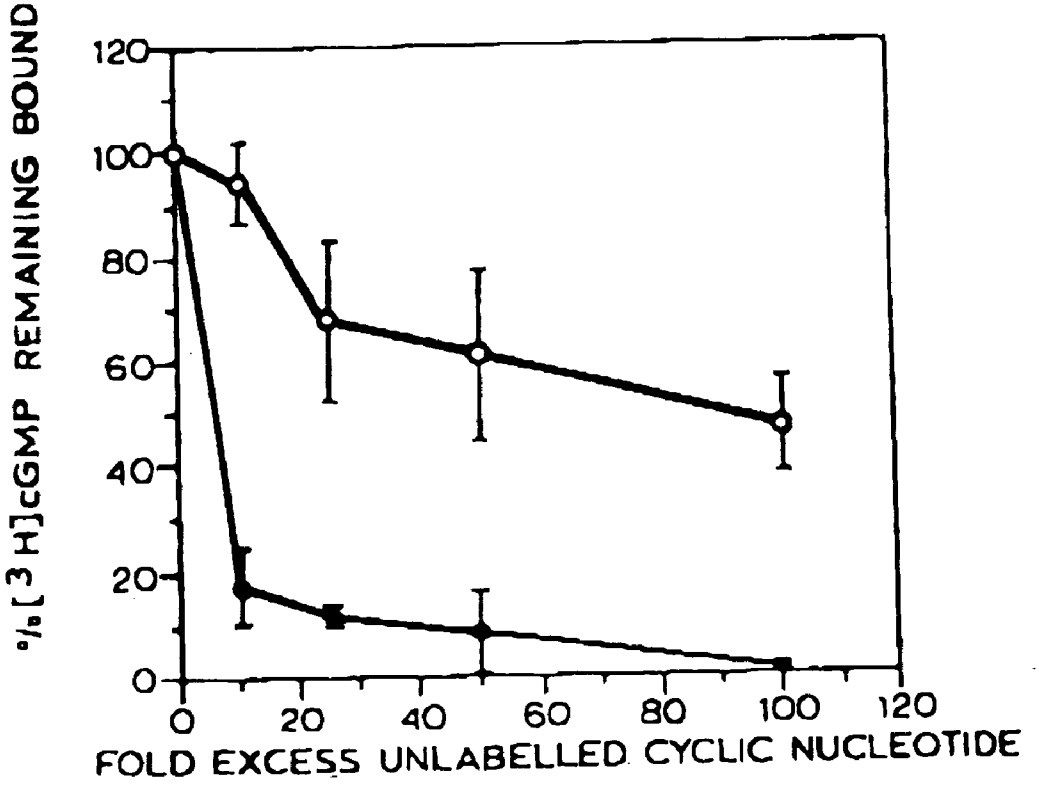

FIGURE 8

CYCLIC GMP-BINDING, CYCLIC GMP-SPECIFIC PHOSPHODIESTERASE MATERIALS AND METHODS

[0001] This application is a continuation-in-part of co-pending U.S. patent application Ser. No. 08/068,051 filed May 27, 1993.

[0002] Experimental work described herein was supported in part by Research Grants GM15731, DK21723, DK40029 and GM41269 and the Medical Scientist Training Program Grant GM07347 awarded by the National Institutes of Health. The United States government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention relates generally to a cyclic guanosine monophosphate-binding, cyclic guanosine monophosphate-specific phosphodiester designated cGB-PDE and more particularly to novel purified and isolated polynucleotides encoding cGB-PDE polypeptides, to methods and materials for recombinant production of cGB-PDE polypeptide, and to methods for identifying modulators of cGB-PDE activity.

BACKGROUND

[0004] Cyclic nucleotide phosphodiesterases (PDEs) that catalyze the hydrolysis of 3'5' cyclic nucleotides such as cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (CAMP) to the corresponding nucleoside 5' monophosphates constitute a complex family of enzymes. By mediating the intracellular concentration of the cyclic nucleotides, the PDE isoenzymes function in signal transduction pathways involving cyclic nucleotide second messenger.

[0005] A variety of PDEs have been isolated from different tissue sources and many of the PDEs characterized to date exhibit differences in biological properties including physicochemical properties, substitute specificity, sensitivity to inhibitor, immunological reactivity and mode of regulation. [See Beavo et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, John Wiley & Sons, Chichester, U.K. (1990)]. Comparison of the known amino acid sequences of various PDEs indicates that most PDEs are chimeric multidomain proteins that have distinct catalytic and regulatory domains. [See Charbonneau, pp. 267 in Beavo et al., supra] All mammalian PDEs characterized to date share a sequence of approximately 250 amino acid residues in length that app to comprise the catalytic site and is located in the carboxyl terminal region of the enzyme. PDE domains that interact with allosteric or regulatory molecules are thought to be located within the amino-terminal regions of the isoenzymes. Based on their biological properties, the PDEs may be classified into six general families: the Ca^{2+}/calmodulin-stimulated PDEs (Type I), the cGMP-stimulated PDEs (Type II), the cGMP-inhibited PDEs (Type III), the cAMP-specific PDEs (Type IV), the cGMP-specific phosphodiesterase COB-PDE (Type V) which is the subject of the present invention and the cGMP-specific photoreceptor PDEs (Type VI).

[0006] The cGMP-binding PDEs (Type II, Type V and Type VI PDEs), in addition to having a homologous catalytic domain near their carboxyl terminus, have a second conserved sequence which is located closer to their amino terminus and which may comprise an allosteric cGMP-binding domain. See Charbonneau et al., *Proc. Nail. Acad. Sci. USA,* 87: 288-292 (1990).

[0007] The Type II cGMP-stimulated PDEs (cGs-PDEs) am widely distributed in different tissue types and are thought to exist as homodimers of 100-105 kDa subunits. The cGs-PDEs respond under physiological conditions to evaluated cGMP concentrations by increasing the rate of cAMP hydrolysis. The amino acid sequence of a bovine heart cGs-PDE and a partial cDNA sequence of a bovine adrenal cortex cGS-PDE am reported in LeTrong et. al., *Biochemistry,* 29: 10280-10288 (1990) and full length bovine adrenal and human fetal brain cGB-PDE cDNA sequences are described in Patent Cooperation Treaty International Publication No. WO 92/18541 published an Oct. 29, 1992. The full length bovine adrenal cDNA sequence is also described in Sonnenburg et al., *J. Biol. Chem.,* 266: 17655-17661 (1991).

[0008] The photoreceptor PDEs and the cGB-PDE have been described as cGMP-specific PDEs because they exhibit a 50-fold or greater selectivity for hydrolyzing cGMP over cAMP.

[0009] The photoreceptor PDEs are the rod outer segment PDE (ROS-PDE) and the cone PDE (COS-PDE). The holoenzyme structure of the ROS-PDE consists of two large subunits α (88 kDa) and β (84 kDa) which are both catalytically active and two smaller γ regulatory subunits (both 11 kDa) A soluble form of the ROS-PDE has also been identified which includes α, β, and γ subunits and a δ subunit (15 kDa) that appears to be identical to the COS-PDE 15 kDa subunit. A full-length cDNA corresponding to the bovine membrane-associated ROS-PDE α subunit is described in Ovchinnikov et al., *FEBS Lett.,* 223: 169-173 (1987) and a full length cDNA corresponding to the bovine rod outer segment PDE β subunit is described in Lipkin et al., *J. Biol. Chem.,* 265: 12955-12959 (1990). Ovchinnikov et. al., *FEBS Lett,* 204: 169-173 (1986) presents a full-length cDNA corresponding to the bovine ROS-PDE γ subunit and the amino acid sequence of the δ subunit. Expression of the ROS-PDE has also been reported in brain in Collins et al., *Genomics,* 13: 698-704 (1992). The COS-PDE is composed of two identical α' (94 kDa) subunits and three smaller subunit of 11 kDa, 13 kDa and 15 kDa. A full-length cDNA corresponding to the bovine COS-PDE α' subunit is reported in Li et al., *Proc. Natl. Acad. Sci. USA,* 87: 293-297 (1990).

[0010] cGB-PDE has been purified to homogeneity from rat [Francis et. al., *Methods Enzymol.,* 159: 722-729 (1988)] and bovine lung tissue [Thomas et. al., *J. Biol. Chem.,* 265: 14964-14970 (1990), hereinafter "Thomas I"]. The presence of this or similar enzymes has been reported in a variety of tissues and species including rat and human platelets [Hamet et al., *Adv. Cyclic Nucleotide Protein Phosphorylation Res.,* 16: 119-136 (1984)], rat spleen [Coquil et al., *Biochem. Biophys. Res. Commun.,* 127: 226-231 (1985)], guinea pig lung [Davis et. al., *J. Biol. Chem.,* 252: 4078-4084 (1977)], vascular smooth muscle [Coquil et al., *Biochim. Biophys. Acia,* 631: 148-165 (1980)], and sea urchin sperm [Francis et al., *J. Biol. Chem,* 255: 620-626 (1979)]. cGB-PDE may be a homodimer comprised of two 93 kDa subunits. [See Thomas I, supra] cGB-PDE has been shown to contain a single site not found in other known cGMP-binding PDEs

which is phosphorylated by cGMP-dependent protein kinase (cGK) and, with a lower affinity, by cAMP-dependent protein kinase (cAK). [See Thomas et al., *J. Biol. Chem.,* 265: 14971-14978 (1990), hereinafter "Thomas II"]The primary amino acid sequence of the phosphorylation site and of the amino-terminal end of a fragment generated by chymotryptic digestion of cGB-PDE are described in Thomas II, supra, and Thomas I; supra, respectively. However, the majority of the amino acid sequence of cGB-PDE has not previously been described.

[0011] Various inhibitors of different types of PDEs have been described in the literature. Two inhibitors that exhibit some specificity for Type V PDEs are zaprinast and dipyridamole. See Francis et al., pp. 117-140 in Beavo et. al., supra.

[0012] Elucidation of the DNA and amino acid sequences encoding the cGB-PDE and production of cGB-PDE polypeptide by recombinant methods would provide information and material to allow the identification of novel agent that selectively modulate the activity of the cGB-PDEs. The recognition that there are distinct types or families of PDE isoenzymes and that different tissues express different complements of PDEs has led to an interest in the development of PDE modulation which may have therapeutic indications for disease states that involve signal transduction pathways utilizing cyclic nucleotides as second messenger. Various selective and non-selective inhibitors of PDE activity are discussed in Murray et al., *Biochem. Soc. Trans.,* 20(2): 460-464 (1992). Development of PDE modulators without the ability to produce a specific PDE by recombinant DNA techniques is difficult because all PDEs catalyze the same basic reaction, have overlapping substrate specificities and occur only in trace amounts. As a result, purification to homogeneity of many PDEs is a tedious and difficult process.

[0013] There thus continues to exist a need in the an for DNA and amino acid sequence information for the cGB-PDE, for methods and mates for the recombinant production of cGB-PDE polypeptides and for methods for identifying specific modulators of cGB-PDE activity.

SUMMARY OF THE INVENTION

[0014] The present invention provides novel purified and isolate polynucleotides (e.g., DNA sequences and RNA transcripts, both sense and antisense strands, including splice variants thereof) encoding the cGMP-binding, cGMP-specific PDE designated cGB-PDE. Preferred DNA sequences of the invention include genomic and cDNA sequences we well as wholly or partially chemically synthesized DNA sequences. DNA sequences encoding cGB-PDE that are set out in SEQ ID NO: 9 or 20 and DNA sequences which hybridize thereto under stringent conditions or DNA sequences which would hybridize thereto but for the redundancy of the genetic code are contemplated by the invention. Also contemplated by the invention are biological replicas (i.e., copies of isolated DNA sequences made in vivo or in vitro) of DNA sequences of the invention. Autonomously replicating recombinant constructions such as plasmid and viral DNA vectors incorporating cGB-PDE sequences and especially vectors wherein DNA encoding cGB-PDE is operatively linked to an endogenous or exogenous expression control DNA sequence and a transcriptional terminator are also provided. Specifically illustrating expression plasmids of the invention is the plasmid hcgb-met156-2 6n in *E. coli* strain JM109 which was deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852, on May 4, 1993 as Accession No. 69296.

[0015] According to another aspect of the invention, host cells including procaryotic and eucaryotic cells, are stably transformed with DNA sequences of the invention in a manner allowing the desired polypeptides to be expressed therein. Host cells expressing cGB-PDE products can serve a variety of useful purposes. Such cells constitute a valuable source of immunogen for the development of antibody substances specifically immunoreactive with cGB-PDE. Host cells of the invention are conspicuously useful in methods for the large scale production of cGB-PDE polypeptides wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells or from the medium in which the cells are grown by, for example, immunoaffinity purification.

[0016] cGB-PDE products may be obtained as isolates from natural cell sources or may be chemically synthesized, but are preferably produced by recombinant procedures involving host cells of the invention. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., glycosylation, truncation, lipidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention. cGB-PDE products of the invention may be full length polypeptides, fragments or variants. Variants may comprise cGB-PDE polypeptide analogs wherein one or more of the specified (i.e., naturally encoded) amino acids is deleted or replaced or wherein one or more nonspecified amino acids a added: (1) without loss of one or more of the biological activities or immunological characteristics specific for cGB-PDE; or (2) with specific disablement of a particular biological activity of cGB-PDE.

[0017] Also comprehended by the present invention are antibody substances (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, CDR-grafted antibodies and the like) and other binding proteins specific for cGB-PDE. Specific binding proteins can be developed using isolated or recombinant cGB-PDE or cGB-PDE variants or cells expressing such products. Binding proteins are useful, in turn, in compositions for immunization as well as for purifying cGB-PDE polypeptides and detection or quantification of cGB-PDE polypeptides in fluid and tissue samples by known immunological procedure. They are also manifestly useful in modulating (i.e., blocking, inhibiting or stimulating) biochemical activities of cGB-PDE, especially those activities involved in signal transduction. Anti-idiotypic antibodies specific for anti-cGB-PDE antibody substances are also contemplated.

[0018] The scientific value of the information contributed through the disclosures of DNA and amino acid sequence of the present invention is manifest. As one series of examples, knowledge of the sequence of a cDNA for cGB-PDE males possible the isolation by DNA/DNA hybridization of genomic DNA sequences encoding cGB-PDE and specifying cGB-PDE expression control regulatory sequences such as promoters, operators and the like. DNA/DNA hybridization procedures carried out with DNA sequences of the

invention under stringent conditions am likewise expected to allow the isolation of DNAs ending allelic variants of cGB-PDE, other structurally related proteins sharing one or more of the biochemical and/or immunological properties specific to cGB-PDE, and non-human species proteins homologous to cGB-PDE. Polynucleotides of the invention when suitably labelled are useful in hybridization assays to detect the capacity of cells to synthesize cGB-PDE. Polynucleotides of the invention may also be the basis for diagnostic methods useful for identifying a genetic alteration(s) in the cGB-PDE locus that underlies a disease state or states. Also made available by the invention ae anti-sense polynucleotides relevant to regulating expression of cGB-PDE by those cells which ordinarily express the same.

[0019] The DNA and amino acid sequence information provided by the present invention also makes possible the systematic analysis of the structure and function of cGB-PDE and definition of those molecules with which it will interact. Agents that modulate cGB-PDE activity may be identified by incubating a putative modulator with lysate from eucaryotic cells expressing recombinant cGB-PDE and determining the effect of the putative modulator on cGB-PDE phosphodiesterase activity. In a preferred embodiment the eucaryotic cell lacks endogenous cyclic nucleotide phosphodiesterase activity. Specifically illustrating such a eucaryotic cell is the yeast strain YKS45 which was deposited with the ATCC on May 19, 1993 as Accession No. 74225. The selectivity of a compound that modulates the activity of the cGB-PDE can be evaluated by comparing ius activity on the cGB-PDE to its activity on other PDE isozymes. The combination of the recombinant cGB-PDE products of the invention with other recombinant PDE products in a series of independent assays provides a system for developing selective modulators of cGB-PDE.

[0020] Selective modulators may include, for example, antibodies and other proteins or peptides which specifically bind to the cGB-PDE or cGB-PDE nucleic acid, oligonucleotides which specifically bind to the cGB-PDE or cGB-PDE nucleic acid and other non-peptide compounds (e.g., isolated or synthetic organic molecules) which specifically react with cGB-PDE or cGB-PDE nucleic acid. Mutant forms of cGB-PDE which affect the enzymatic activity or cellular location of the wild-type cGB-PDE are also contemplated by the invention. Presently preferred targets for the development of selective modulators include, for example: (1) the regions of the cGB-PDE which contact other proteins and/or localize the cGB-PDE within a cell, (2) the regions of the cGB-PDE which bind substrate, (3) the allosteric cGMP-binding site(s) of cGB-PDE, (4) the phosphorylation site(s) of cGB-PDE and (5) the regions of the cGB-PDE which are involved in dimerization of cGB-PDE subunits. Modulators of cGB-PDE activity may be therapeutically useful in treatment of a wide range of diseases and physiological conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] Numerous other aspects and advantages of the print invention will be apparent upon consideration of the following detailed description thereof, reference being made to the drawing wherein:

[0022] **FIG. 1A** to **1**C is an alignment of the conserved catalytic domains of several PDE isoenzymes wherein residues which are identical in all PDEs listed are indicated by their one letter amino acid abbreviation in the "conserved" line, residues which are identical in the cGB-PDE and photoreceptor PDEs only are indicated by a star in the "conserved" line and gaps introduced for optimum alignment are indicated by periods;

[0023] **FIG. 2A** to **2**C is an alignment of the cGMP-binding domains of several PDE isoenzymes wherein residues which are identical in all PDEs listed are indicated by their one letter amino acid abbreviation in the "conserved" line and gaps introduced for optimum alignment are indicated by periods;

[0024] **FIG. 3** is an alignment of internally homologous repeats from several PDE isoenzymes wherein residues identical in each repeat A and B from all cGMP-binding PDEs listed are indicated by their one letter amino acid abbreviation in the "conserved" line and stars in the "conserved" line represent positions in which all residues are chemically conserved;

[0025] **FIG. 4** schematically depicts the domain organization of cGB-PDE,

[0026] **FIG. 5** is a bar graph representing the results of experiments in which extracts of COS cells transfected with bovine cGB-PDE sequences or extracts of untransfected COS cells were assayed for phosphodiesterase activity using either 20 μM cGMP or 20 μM cAMP as the substrate;

[0027] **FIG. 6** is a graph depicting results of assays of extracts from cells transfected with bovine cGB-PDE sequences for cGMP phosphodiesterase activity in the presence of a series of concentrations of phosphodiesterase inhibitors including dipyridamole (closed squares), zaprinast (closed circles), methoxymethylxanthine (closed triangles) and rolipram (open circles);

[0028] **FIG. 7** is a bar graph presenting results of experiments in which cell extracts from COS cells transfected with bovine cGB-PDE sequences or control untransfected COS cells were assayed for [^{3}H]cGMP-binding activity in the absence (−) or presence (+) of 0.2 mM IBMX; and

[0029] **FIG. 8** is a graph of the results of assays in which exacts from cells transfected with bovine cGB-PDE sequences were assayed for [^{3}H]cGMP-binding activity in the presence of excess unlabelled cAMP (open circles) or cGMP (closed circles) at the concentrations indicated.

DETAILED DESCRIPTION

[0030] The following examples illustrate the invention. Example 1 describes the isolation of a bovine cGB-PDE cDNA fragment by PCR and subsequent isolation of a full length cGB-PDE cDNA using the PCR fragment as a probe Example 2 presents an analysis of the relationship of the bovine cGB-PDE amino acid sequence to sequences reported for various other PDEs. Northern blot analysis of cGB-PDE mRNA in various bovine tissues is presented in Example 3. Expression of the bovine cGB-PDE cDNA in COS cells is described in Example 4. Example 5 presents results of assays of the cGB-PDE COS cell expression product for phosphodiesterase activity, cGMP-binding activity and Zn^{2+} hydrolase activity Example 6 describes the isolation of human cDNAs homologous to the bovine cGB-PDE cDNA. The expression of a human cGB-PDE cDNA in yeast cells is presented in Example 7 RNase protection

assays to detect cGB-PDE in human tissues are described in Example 8. Example 9 describes the bacterial expression of human cGB-PDE cDNA and the development of antibodies reactive with the bacterial cGB-PDE expression product. Example 10 describes cGB-PDE analogs and fragments. The generation of monoclonal antibodies that recognize cGB-PDE is described in Example 11. Example 12 relates to utilizing recombinant cGB-PDE products of the invention to develop agents that selectively modulate the biological activities of cGB-PDE.

EXAMPLE 1

[0031] The polymerase chain reaction (PCR) was utilized to isolate a cDNA fragment encoding a portion of cGB-PDE from bovine lung first strand cDNA. Fully degenerate sense and antisense PCR primers were designed based on the partial cGB-PDE amino acid sequence described in Thomas I, supra, and novel partial amino-acid sequence information.

[0032] A. Purification of cGB-PDE Protein

[0033] cGB-PDE was purified as described in Thomas I, supra, or by a modification of that method as described below.

[0034] Fresh bovine lungs (5-10 kg) were obtained from a slaughterhouse and immediately placed on ice. The tissue was ground and combined with cold PEM buffer (20 mM sodium phosphate, pH 6.8, containing 2 mM EDTA and 25 mM β-mercaptoethanol). After homogenization and centrifugation, the resulting supernatant was incubated with 4-7 liters of DEAE-cellulose (Whatman, UK) for 3-4 hours. The DEAE slurry was then filtered under vacuum and rinsed with multiple volumes of cold PEM. The resin was poured into a glass column and washed with three to four volumes of PEM. The protein was eluted with 100 mM NaCl in PEM and twelve 1-liter fractions were collected. Fractions were assayed for IBMX-stimulated cGMP binding and cGMP phosphodiesterase activities by standard procedures decribed in Thomas et al., supra. Appropriate fractions were pooled, diluted 2-fold with cold, deionized water and subjected to Blue Sepharose® CL-6B (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) chromatography. Zinc chelate affinity adsorbent chromatography was then performed using either an agarose or Sepharose-based gel matrix. The resulting protein pool from the zinc chelation step treated as described in the Thomas I, supra, or was subjected to a modified purification procedure.

[0035] As decribed in Thomas I, supra, the protein pool was applied in multiple loads to an HPLC Bio-Sil TSK-545 DEAE column (150×21.5 mm) (BioRad Laboratories, Hercules, Calif.) equilibrated in PEM at 4° C. After in equilibration period, a 120-ml wash of 50 mM NaCl in PEM was followed by a 120-ml linear gradient (50-200 mM NaCl in PEM) elution at a flow rate of 2 ml/minute. Appropriate fractions were pooled and concentrated in dialysis tubing against Sephadex G-200 (Boehringer Mannheim Biochemicals, UK) to a final volume of 1.5 ml. The concentrated cGB-PDE pool was applied to an HPLC gel filtration column (Bio-Sil TSK-250, 500×215 mm) equilibrated in 100 mM sodium phosphate, pH 6.8, 2 mM EDTA, 25 mM β-mercaptoethanol and eluted with a flow rate of 2 ml/minute at 4° C.

[0036] If the modified, less cumbersome procedure was performed, the protein pool was dialyzed against PEM for 2 hours and loaded onto a 10 ml preparative DEAE Sephacel column (Pharmacia) equilibrated in PEM buffer. The protein was eluted batchwise with 0.5M NaCl in PEM, resulting in an approximately 10-15 fold concentration of protein. The concentrated protein sample was loaded onto an 800 ml (2.5 cm×154 cm) Sephacryl S400 gel filtration column (Boehringer) equilibrated in 0.1M NaCl in PEM, and eluted at a flow rate: of 1.7 ml/minute.

[0037] The purity of the protein was assessed by Coomassie staining after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Approximately 0.5-3.0 mg of pure cGB-PDE were obtained per 10 kg bovine lung.

[0038] Rabbit polyclonal antibodies specific for the purified bovine cGB-PDE were generated by standard procedures.

[0039] B. Amino Acid Sequencing of cGB-PDE

[0040] cGB-PDE phosphorylated with [^{32}P]ATP and was then digested with protease yield ^{32}P-labelled phosphopeptides. Approximately 100 μg of purified cGB-PDE was phosphorylated in a reaction mixture containing 9 mM $MgCl_2$, 9 μM [^{32}P]ATP, 10 μM cGMP, and 4.2 μg purified bovine catalytic subunit of cAMP-dependent protein kinase (cAK) in a final volume of 900 μl. Catalytic subunit of cAK was prepared according to the method of Flockhart et. al., pp. 209-215 in Marangos et al., *Brain Receptor Methodologies, Part A*, Academic Press, Orlando, Fla. (1984). The reaction was incubated for 30 minutes at 30° C., and stopped by addition of 60 μl of 200 mM EDTA.

[0041] To obtain a first peptide sequence from cGB-PDE, 3.7 μl of a 1 mg/ml solution of a α-chymotrypsin in KPE buffer (10 mM potassium phosphate, pH 6.8, with 2 mM EDTA) was added to 100 μg purified, phosphorylated cGB-PDE and the mixture was incubated for 30 minutes at 30° C. Proteolysis was stopped by addition of 50 μl of 10% SDS and 25 μl of β-mercaptoethanol. The sample was boiled until the volume was reduced to less than 400 μl, and was loaded onto an 8% preparative SDS-polyacrylamide gel and subjected to electrophoresis at 50 mAmps. The separated digestion products were electroblotted onto Immobilon polyvinylidene difluoride (Millipore, Bedford, Mass.), according to the method of Matsudaira, *J. Biol. Chem,* 262: 10035-10038 (1987). Transferred protein was identified by Coomassie Blue staining, and a 50 kDa band was excised from the membrane for automated gas-phase amino acid sequencing. The sequence of the peptide obtained by the α-chymotryptic digestion procedure is set out below as SEQ ID NO: 1.

REXDANRINYMYAQYVKNTM SEQ ID NO: 1

[0042] A second sequence was obtained from a cGB-PDE peptide fragment generated by V8 proteolysis. Approximately 200 μg of purified cGB-PDE was added to 10 mM $MgCl_2$, 10 μM [^{32}P]ATP, 100 μM cGMP, and 1 μg/ml purified catalytic subunit of cAK in a final volume of 1.4 ml. The reaction was incubated for 30 minutes at 30° C., and was terminated by the addition of 160 μl of 0.2M EDTA. Next, 9 μl of 1 mg/ml *Staphylococcal aureus* V8 protease (International Chemical Nuclear Biomedicals, Costa Mesa, Calif.) diluted in KPE was added, followed by a 15 minute

incubation at 30° C. Proteolysis was stopped by addition of 88 μl of 20% SDS and 45 μl β-mercaptoethanol. The digestion products were separated by electrophoresis on a preparative 10% SDS-polyacrylamide gel run at 25 mAmps for 4.5 hours. Proteins were electroblotted and stained as described above. A 28 kDa protein band was excised from the membrane and subjected to automated gas-phase amino acid sequencing. The sequence obtained is set out below as SEQ ID NO: 2.

QSLAAAVVP SEQ ID NO: 2

[0043] C. PCR Amplification of Bovine cDNA

[0044] The partial amino acid sequences utilized to design primers (SEQ ID NO: 3, below, and amino acids 9-20 of SEQ ID NO: 1) and the sequences of the corresponding PCR primers (in IUPAC nomenclature) are set below wherein SEQ ID NO: 3 is the sequence reported in Thomas I, supra.

```
SEQ ID NO: 3
   F   D   N   D   E   G   E   Q
5' TTY GAY AAY GAY GAR GGN GAR CA 3'                    (SEQ ID NO: 4)

3' AAR CTR TTR CTR CTY CCN CTY GT 5'                    (SEQ ID NO: 5)

SEQ ID NO: 1, Amino acids 9-20
   N   Y   M   Y   A   Q   Y   V   K   N   T   M
5' AAY TAY ATG TAY GCN CAR TAY GT 3'                    (SEQ ID NO: 6)

3' TTR ATR TAC ATR CGN GTY ATR CA 5'                    (SEQ ID NO; 7)

3' TTR ATR TAC ATR CGN GTY ATR CAN TTY TTR TGN TAC 5'   (SEQ ID NO: 8)
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[0045] The sense and antisense primers, synthesized using an Applied Biosystems Model 380A DNA Synthesizer (Foster City, Calif.), were used in all possible combinations to amplify cGB-PDE-specific sequences from bovine lung first strand cDNA as described below.

[0046] After ethanol precipitation, pairs of oligonucleotides were combined (SEQ ID NO: 4 or 5 combined with SEQ ID NOs: 6, 7 or 8) at 400 nM each in a PCR reaction. The reaction was run using 50 ng first strand bovine lung cDNA (generated using AMV reverse transcriptase and random primers on oligo dT selected bovine lung mRNA), 200 μM dNTPs, and 2 units of Taq polymerase. The initial denaturation step was carried out at 94° C. for 5 minutes, followed by 30 cycles of a 1 minute denaturation step at 94° C., a two minute annealing step at 50° C., and a 2 minute extension step at 72° C. PCR was performed using a Hybrid Thermal Reactor (ENK Scientific Products, Saratoga, Calif.) and products were treated by gel electrophoresis on a 1% low melting point agarose gel run in 40 mM Tris-acetate, 2 mM EDTA. A weak band of about 800-840 bp was wen with the primers set out in SEQ ID NOs: 4 and 7 and with primers set out in SEQ ID NOs: 4 and 8. None of the other primer pairs yielded visible bands. The PCR product generated by amplification with the primers set out in SEQ ID NOs: 4 and 7 was isolated using the Gene Clean® (Bio101, La Jolla, Calif.) DNA purification kit according to the manufacturer's protocol. The PCR product (20 ng) was ligated into 200 ng of linearized pBluescript KS(+) (Stratagene, La Jolla, Calif.), and the resulting plasmid construct was used to transform *E. coli* XL1 Blue cells (Stratagene Cloning Systems, La Jolla, Calif.). Putative transformation positives were screened by sequencing. The sequences obtained were not homologous to any known PDE sequence or to the known partial cGB-PDE sequences.

[0047] PCR was performed again on bovine lung first strand cDNA using the primers set out in SEQ ID NOs; 4 and 7. A clone containing a 0.8 Kb insert with a single large open reading frame was identified. The open reading frame method a polypeptide that included the amino acids KNTM (amino acids 17-20 of SEQ ID NO: 1 which were not utilized to design the primer sequence which is set out in SEQ ID NO: 7) and that possessed a high degree of homology to the deduced amino acid sequences of the cGs-, ROS- and COS-PDEs. The clone identified corresponds to nucleotides 489-1312 of SEQ ID NO: 9.

[0048] D. Construction and Hybridization Screening of a Bovine cDNA Library

[0049] In order to obtain a cDNA encoding a full-length cGB-PDE, a bovine lung cDNA library was screened using the ^{32}P-labelled PCR-generated cDNA insert as a probe.

[0050] Polyadenylated RNA was prepared from bovine lung as described Sonnenburg et al., *J. Biol. Chem.,* 266: 17655-17661 (1991). First stand cDNA was synthesized using AMV reverse transcriptase (Life Sciences, St. Petersburg, Fla.) with random hexanucleotides primers as described in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1987). Second strand cDNA was synthesized using *E. coli* DNA polymerase in the presence of *E. coli* DNA ligase and *E. coli* RNAse H. Selection of cDNAs larger than 500 bp was performed by Sepharose® CL-4B (Millipore) chromatography. EcoRI adaptors (Promega, Madison, Wis.) were ligated to the cDNA using T4 DNA ligase. Following heat inactivation of the ligase, the cDNA was phosphorylated using T4 polynucleotide kinase. Unligated adaptors were removed by Sepharose® CL-4B chromatography (Pharmacia, Piscataway, N.J.). The cDNA was ligated into EcoRI-digested, dephosphorylated lambda Zap®II arms (Stratagene) and packaged with Gigapack® Gold (Stratagene) extracts according to the manufacturer's protocol. The titer of the unamplified library was 9.9×10^5 with 18% nonrecombinants. The library was amplified by plating 50,000 plaque forming units (pfu) on to twenty 150 mm plates, resulting in a final titer of 5.95×10^6 pfu/ml with 21% nonrecombinants.

[0051] The library was plated on twenty-four 150 mm plates at 50,000 pfu/plate, and screened with the ^{32}P-labelled cDNA clone. The probe was prepared using the method of Feinberg et al., *Anal Biochem,* 137: 266-267 (1984), and the ^{32}P-labelled DNA was purified using Elutip-D® columns (Schleicher and Schuell Inc., Keene, N.H.) using the manu-

facturer's protocol. Plaque-lifts were performed using 15 cm nitrocellulose filters. Following denaturation and neutralization, DNA was fixed onto the filter by baking at 80° C. for 2 hours. Hybridization was carried out at 42° C. overnight in a solution containing 50% formamide, 5×SSC (0.75M NaCl, 0.75M sodium citrate, pH 7), 25 mM sodium phosphate (pH 7.0), 2× Denhardt's solution, 10% dextran sulfate, 90 μg/ml yeast tRNA, and approximately 10^6 cpm/ml ^{32}P-labelled probe (5×10**[text missing or illegible when filed]**cpm/μg). The filters were washed twice in 0.1×SSC, 0.1% SDS at room temperature for 15 minutes per wash, followed by a single 20 minute sash in 0.1×SSC, 1% SDS at 45° C. The fillers were then exposed to X-ray film at −70° C. for several days.

[**0052**] Plaques that hybridized with the labelled probe were purified by several rounds of replating and rescreening. Insert cDNAs were subcloned into the pBluescript SK(−) vector (Stratagene) by the in vivo excision method described by the manufacturer's protocol. Southern blots were performed in order to verify that the rescued cDNA hybridized to the PCR probe. Putative cGB-PDE cDNAs were sequenced using Sequenase® Version 2.0 (United States Biochemical Corporation, Cleveland, Ohio) or TaqTrack® kits (Promega).

[**0053**] distinct cDNA clones designated cGB-2, cGB-8 and cGB-10 were isolated. The DNA and deduced amino acid sequences of clone cGB-8 are set out in SEQ ID NOs: 9 and 10 The DNA sequence downstream of nucleotide 2686 may represent a cloning artifact. The DNA sequence of cGB-10 is identical to the sequence of cGB-8 with the exception of one nucleotide. The DNA sequence of clone cGB-2 diverges from that of clone cGB-8 5' to nucleotide 219 of clone cgb-8 (see SEQ ID NO: 9) and could encode a protein with a different amino terminus.

[**0054**] The cGB-8 cDNA clone is 4474 bp in length and contains a large open reading frame of 2625 bp. The triplet ATG at position 99-101 in the nucleotide sequence is predicted to be the translation initiation site of the cGB-PDE gene because it is preceded by an in-frame stop codon and the surrounding bases are compatible with the Kozak consensus initiation site for eucaryotic mRNAs. The stop codon TAG is located at positions 2724-2726, and is followed by 1748 bp of 3' untranslated sequence. The sequence of cGB-8 does not contain a transcription termination consensus sequence, therefore the clone may not represent the entire 3' untranslated region of the corresponding rRNA.

[**0055**] The open reading frame of the cGB-8 cDNA encodes an 875 ammo acid polypeptide with a calculated molecular mass of 99.5 kD. This calculated molecular mass is only slightly larger than the reported molecular man of purified cGB-PDE, estimated by SDS-PAGE analysis to be approximately 93 kDa. The deduced amino acid sequence of cGB-8 corresponded exactly to all peptide sequences obtained from purified bovine lung cGB-PDE providing strong evidence that cGB-8 encodes cGB-PDE.

EXAMPLE 2

[**0056**] A search of the SWISS-PROT and GEnEmbl data banks (Release of February, 1992) conducted using the FASTA program supplied with the Genetics Computer Group (GCG) Software Package (Madison, Wis.) revealed that only DNA and amino acid sequences reported for other PDEs displayed significant similarity to the DNA and deduced amino acid of clone cGB-8.

[**0057**] Pairwise comparisons of the cGB-PDE deduced amino acid sequence with the sequences of eight other PDEs were conducted using the ALIGN [Dayhoff et al., *Methods Enzymol.,* 92; 524-545 (1983)] and BESTFIT [Wilbur et al., *Proc. Natl. Acad. Sci. USA,* 80: 726-730 (1983)] programs. Like all mammalian phosphodiesterases sequenced to date, cGB-PDE contains a conserved catalytic domain sequence of approximately 250 amino acids in the carboxyl-terminal half of the protein that is thought to be essential for catalytic activity. This segment comprises amino acids 578-812 of SEQ ID NO: 9 and exhibits sequence conservation with the corresponding regions of other PDEs. Table 1 below sets out the specific identity values obtained in pairwise comparisons of other PDEs with amino acids 578-812 of cGB-PDE, wherein "ratdunce" is the rat cAMP-specific PDE; "61 kCaM" is the bovine 61 kDa calcium/calmodulin-dependent PDE; "63 kCaM" is the bovine 63 kDa calcium/calmodulin-dependent PDE, "drosdunce" is the *drosophila* cAMP-specific dunce PDE; "ROS-α" is the bovine ROS-PDE α-subunit; "ROS-β" is the bovine ROS-PDE β-subunit; "COS-α" is the bovine COS-PDE α subunit; and "cGs" is the bovine cGs-PDE (612-844).

TABLE 1

Phosphodiesterase	Catalytic Domain Residues	% Identity
Ratdunce	77–316	31
61 kCaM	193–422	29
63 kcam	195–424	29
drosdunce	1–239	28
ROS-α	535–778	45
ROS-β	533–776	46
COS-α'	533–776	48
cGs	612–844	40

[**0058**] Multiple sequence alignments were performed using the Progressive Alignment Algorithm [Feng et al., *Methods Enzymol.,* 183: 375-387 (1990)] implemented in the PILEUP program (GCG Software). **FIG. 1A** to **1**C shows a multiple sequence alignment of the proposed catalytic domain of cGB-PDE with the all the corresponding regions of the PDEs of Table 1. Twenty-eight residues (see residues indicated by one lette amino acid abbreviations in the "conserved" line on **FIG. 1A** to **1**C) are invariant among the isoenzymes including several conserved histidine residues predicted to play a functional role in catalysis. See Charbonneau et al., *Proc. Natl. Acad. Sci. USA*, supra. The catalytic domain of cGB-PDE more closely resembles the catalytic domains of the ROS-PDEs and COS-PDEs than the corresponding regions of other PDE isoenzymes. That are several conserved regions among the photoreceptor PDEs and cGB-PDE that are not shared by other PDEs. Amino acid positions in these regions that are invariant in the photoreceptor PDE and cGB-PDE sequences am indicated by stars in the "conserved" line of **FIG. 1A** to **1**C. Regions of homology among cGB-PDE and the ROS- and COS-PDEs may serve important roles in conferring specificity for cGMP hydrolysis relative to cAMP hydrolysis or for sensitivity to specific pharmacological agents.

[**0059**] Sequence similarity between cGB-PDE, cGs-PDE and the photoreceptor PDEs, is not limited to the conserved

catalytic domain but also includes the noncatalytic cGMP binding domain in the amino-terminal half of the protein. Optimization of the alignment between cGB-PDE, cGs-PDE and the photoreceptor PDEs indicates that an amino-terminal conserved segment may exist including amino acids 142-526 of SEQ ID NO: 9. Pairwise analysis of the sequence of the proposed cGMP-binding domain of cGB-PDE with the corresponding regions of the photoreceptor PDEs and cGs-PDE revealed 26-28% sequence identity. Multiple sequence alignment of the proposed cGMP-binding domains with the cGMP-binding PDEs is shown in **FIG. 2A** to **2**C wherein abbreviations am the same as indicated for Table 1. Thirty-eight positions in this non-catalytic domain appear to be invariant among all cGMP-binding PDEs (see positions indicated by one letter amino acid abbreviations in the "conserved" line of **FIG. 2A** to **2**C).

[**0060**] The cGMP-binding domain of the cGMP-binding PDEs contains internally homologous repeats which may form two similar but distinct inter- or intra-subunit cGMP-binding sites. **FIG. 3** shows a multiple sequence alignment of the repeats a (corresponding to amino aids 228-311 of cGB-PDE) and b (corresponding to amino acids 410-500 of cGB-PDE) of the cGMP-binding PDEs. Seven residues are invariant in each A and B regions (see residue indicated by one letter amino aid abbreviations in the "conserved" line of **FIG. 3**). Residue that are chemically conserved in the A and B regions are indicated by stars in the "conserved" line of **FIG. 3**. cGMP analog studies of cGB-PDE support the existence of a hydrogen bond between the cyclic nucleotide binding site on cGB-PDE and the 2' OH of cGMP.

[**0061**] Three regions of cGB-PDE have no significant sequence similarly to other PDE isoenzymes. These regions include the sequence flanking the carboxyl-terminal end of the catalytic domain (amino acids 812-875), the sequence separating the cGMP-binding and catalytic domains (amino acids 527-577) and the amino-terminal sequence spanning amino acids 1-141. The site (the serine at position 92 of SEQ ID NO: 10) of phosphorylation of cGB-PDE by cGK is located in this amino-terminal region of sequence. Binding of cGMP to the allosteric site on cGB-PDE is required for its phosphorylation.

[**0062**] A proposed domain structure of cGB-PDE based on the foregoing comparisons with other PDE isoenzymes is presented in **FIG. 4**. This domain structure is supported by the biochemical studies of cGB-PDE purified from bovine lung.

EXAMPLE 3

[**0063**] The presence of cGB-PDE mRNA in various bovine tissues was examined by Northern blot hybridization.

[**0064**] Polyadenylated RNA was purified from total RNA preparations using the Poly(A) Quick® mRNA purification kit (Stratagene) according to the manufacturer's protocol, RNA sample (5 μg) were loaded onto a 1.2% agarose, 67% formaldehyde gel. Electrophoresis and RNA transfer were performed as previously described in Sonnenburg et al., supra. Prehybridization of the RNA blot was carried out for 4 hours at 45° C. in a solution containing 50% formamide, 5×SSC, 25 mM sodium phosphate, pH 7, 2× Denhardt's solution, 10% dextran sulfate, and 0.1 mg/ml yeast tRNA. A random hexanucleotide-primer-labelled probe (5×10**[text missing or illegible when filed]**, cpm/μg) was prepared as described in Feinberg et al., supra, using the 4.7 kb cGB-8 cDNA clone of Example 2 exercised by digestion with AccI and SacII. The probe was heat denatured and injected into a blotting bag (6×10^5 cpm/ml) following prehybridization. The Northern blot was hybridized overnight at 45° C., followed by one 15 minute wash with 2×SSC, 0.1% SDS at room temperature, and three 20 minute washes with 0.1× SSC, 0.1% SDS at 45° C. The blot was exposed to X-ray film for 24 hours at −70° C. The size of the RNA that hybridized with the cGB-PDE probe was estimated using a 0.24-9.5 kb RNA ladder that was stained with ethidium bromide and visualized with UV light.

[**0065**] The ^{32}P-labeled cGB-PDE cDNA hybridized to a single 6.8 kb bovine lung RNA species. A mRNA band of the identical size was also detected in polyadenylated RNA isolated from bovine trachea, aorta, kidney and spleen.

EXAMPLE 4

[**0066**] The cGB-PDE cDNA in clone cGB-8 of Example 2 was expressed in COS-7 cells (ATCC CRL1651).

[**0067**] A portion of the cGB-8 cDNA was isolated following digestion with the restriction enzyme XbaI. XbaI cut at a position in the pBluescript polylinker sequence located 30 bp upstream of the 5' end of the cGB-8 insert and at position 3359 within the cGB-8 insert. The resulting 3399 bp fragment, which contains the entire coding region of cGB-8, was then ligand into the unique XbaI cloning site of the expression vector pCDM8 (Invitrogen, San Diego, Calif.). The pCDM8 plasmid is a 4.5 kb eucaryotic expression vector containing a cytomegalovirus promoter and enhancer, an SV40-derived origin of replication, a polyadenylation signal, a procaryotic origin of replication (derived from pBR322) and a procaryotic genetic marker (supF). *E. coli* MC1061/P3 cells (Invitrogen) were transformed with the resulting ligation products, and transformation positive colonies were screened for proper orientation of the cGB-8 insert using PCR and restriction enzyme analysis. The resulting expression construct containing the cGB-8 insert in the proper orientation is referred to as pCDM8-cGB-PDE.

[**0068**] The pCDM8-cGB-PDE DNA was purified from large-scale plasmid preparations using Qiagen pack-500 columns (Chatsworth, Calif.) according to the manufacturer's protocol. COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 50 μg/ml penicillin and 50 μg/ml streptomycin at 37° C. in a humidified 5% CO_2 atmosphere. Approximately 24 hours prior to transfection, confluent 100 mm dishes of cells were replated at one-fourth or one-fifth the original density. In a typical transfection experiment, cells were washed with buffer containing 137 mM NaCl, 2.7 mM KCl, 1.1 mM potassium phosphate, and 8.1 mM sodium phosphate, pH 7.2 (PBS). Then 4-5 ml of DMEM containing 10% NuSerum (Collaborative Biomedical Products, Bedford, Mass.) was added to each plate. Transfection with 10 μg pCDM8-cGB-PDE DNA or pCDM8 vector DNA mixed with 400 μg DEAE-dextran (Pharmacia) in 60 μl TBS [Tris-buffered saline: 25 mM Tris-HCl (pH 74), 137 mM NaCl, 5 mM KCl, 0.6 mM Na_2HPO_4, 0.7 mM $CaCl_2$, and 0.5 mM $MgCl_2$] was carried out by dropwise addition of the mixture to each plate. The cells were incubated at 37° C., 5% CO_2 for 4 hours, and then treated with 10% dimethyl sulfoxide in PBS for 1 minute. After 2 minutes, the dimethyl sulfoxide was removed, the cells were washed with PBS and

incubated in complete medium. After 48 hours, cells were suspended in 0.5-1 ml of cold homogenization buffer [40 mM Tris-HCl (pH 7.5), 15 mM benzamidine, 15 mM β-mercaptoethanol, 0.7 μg/ml pepstatin A, 0.5 μg/ml leupeptin, and 5 μM EDTA]per plate of cells, and disrupted using a Dounce homogenizer. The resulting whole extracts were assayed for phosphodiesterase activity, cGMP-binding activity, and total protein concentration as described below in Example 5.

EXAMPLE 5

[0069] Phosphodiesterase activity in extracts of the transfected COS cells of Example 4 or in extracts of mock transfected COS cells was measured using a modification of the assay procedure described for the cGs-PDE in Martins et al., *J. Biol. Chem.* 257: 1973-1979 (1982). Cells were ha std and extracts prepared 48 hours after transfection. Incubation mixtures contained 40 mM MOPS buffer (pH 7), 0.8 mM EDTA, 15 mM magnesium acetate, 2 mg/ml bovine serum albumin, 20 μM [^{3}H]cGMP or [^{3}H]cAMP (100,000-200,000 cpm/assay) and COS-7 cell extract in a total volume of 250 μl. The reaction mixture was incubated for 10 minutes at 30° C., and then stopped by boiling. Next, 10 μl of 10 mg/ml *Crotalus atrax* venom (Sigma) was added followed by a 10 minute incubation at 30° C. Nucleoside product were separated from unreacted nucleotides as described in Martins et al., supra. In all studies, less than 15% of the total [^{3}H]cyclic nucleoside was hydrolyzed during the reaction.

[0070] The results of the assays are presented in **FIG. 5** wherein the results shown are averages of three separate transfections. Transfection of COS-7 cells with pCDM8-PDE DNA resulted in the expression of approximately 15-fold higher levels of cGMP phosphodiesterase activity than in mock-transfected cells or in cells transfected with pCDM8 vector alone. No increase in CAMP phosphodiesterase activity over mock or vector-only transfected cells was detected in extracts from cells transfected with pCDM8-cGB-PDE DNA. These results confirm that the cGB-PDE bovine cDNA encodes a cGMP-specific phosphodiesterase.

[0071] Extracts from the transfected COS cells of Example 4 we also assayed for cGMP PDE activity in the presence of a series of concentrations of the PDE inhibitors zaprinast, dipyridamole (Sigma), isobutyl-1-methyl-8-methoxymethylxanthine (MeOxMeMIX) and rolipram.

[0072] The results of the assays are presented in **FIG. 6** wherein PDE activity in the absence of inhibitor is taken as 100% and each data point represents the average of two separate determinations. The relative potencies of PDE inhibitors for inhibition of cGMP hydrolysis by the expressed cGB-BPDE cDNA protein product were identical to those relative potencies reported for native cGB-PDE purified from bovine lung (Thomas I, supra). IC_{50} values calculated from the curves in **FIG. 6** are as follows: zaprinast (closed circles), 2 μM; dipyridamole (closed squares), 3.5 μM; MeOxMeMIX (closed triangles), 30 μM; and rolipram (open circles), >300 μM. The IC_{50} value of zaprinast, a relatively specific inhibitor of cGMP-specific phosphodiesterases, was at least two orders of magnitude lower than that reported for inhibition of phosphodiesterase activity of the cGs-PDE or of the cGMP-inhibited phosphodiesterase (cGi-PDEs) (Reeves et al., pp. 300-316 in Beavo et al., supra). Dipyrimadole, an effective inhibitor of selected cAMP- and cGMP-specific phosphodiesterases, was also a potent inhibitor of the expressed cGB-PDE. The relatively selective inhibitor of calcium/calmodulin-stimulated phosphodiesterase (CaM-PDEs), MeOxMeMIX, was approximately 10-fold less potent than zaprinast and dipyridamole, in agreement with results using cGB-PDE activity purified from bovine lung. Rolipram, a potent inhibitor of low K_m cAMP phosphodiesterases, was a poor inhibitor of expressed cGB-PDE cDNA protein product. These results show that the cGB-PDE cDNA encodes a phosphodiesterase that posses catalytic activity characteristic of cGB-PDE isolated from bovine tissue, thus verifying the identity of the cGB-8 cDNA clone as a cGB-PDE.

[0073] It is of interest to note that although the relative potencies of the PDE inhibitors for inhibition of cGMP hydrolysis were identical for the recombinant and bovine isolate cGB-PDE, the absolute IC_{50} values for all inhibitors tested were 2-7 fold higher for the recombinant cGB-PDE. This difference could not be attributed to the effects of any factors present in COS-7 cell extracts on cGMP hydrolytic activity, since cGB-PDE isolated form bovine tissue exhibited identical kinetics of inhibition as a pure enzyme, or when added back to extracts of mock-transfected COS-7 cells. This apparent difference in pharmacological sensitivity may be due to a subtle difference in the structure of the recombinant cGB-PDE cDNA protein product and bovine lung cGB-PDE, such as a difference in post-translational modification at or near the catalytic-site. Alternatively, this difference may be due to an alteration of the catalytic activity of bovine lung cGB-PDE over several purification steps.

[0074] Cell extracts were assayed for [^{3}H]cGMP-binding activity in the absence or presence of 0.2 mM 3-isobutyl-1-methylaxanthine (IBMX) (Sigma), a competitive inhibitor of cGMP hydrolysis. The cGMP binding assay, modified from the assay described in Thomas I, supra, was conducted in a tow volume of 80 μl. Sixty μl of cell extract was combined with 20 μl of a binding cocktail such that the final concentration of components of the mixture were 1 μM [^{3}H]cGMP, 5 μM cAMP, and 10 μM 8-bromo-cGMP. The cAMP and 8-bromo-cGMP were added to block [^{3}H]cGMP binding to cAK and cGK, respectively. Assays were carried out in the absence and presence of 0.2 mM IBMX. The reaction was initiated by the addition of the cell extract, and was incubated for 60 minutes at 0° C. Filtration of the reaction mixtures was carried out as described in Thomas I, supra. Blanks were determined by parallel incubations with homogenization buffer replacing cell extracts, or with a 100-fold excess of unlabelled cGMP. Similar results were obtained with both methods. Total protein concentration of the cell extracts was determined by the method of Bradford, *Anal. Biochem.,* 72:248-254 (1976) using bovine serum albumin as the standard.

[0075] Results of the assay are set out in **FIG. 7**. When measured at 1 μM [^{3}H]cGMP in the presence of 0.2 ml IBMX, extracts from COS-7 cells transfected with pCDM8-cGB-PDE exhibited 8-fold higher cGMP-binding activity than extracts from mock-transfected cells. No IBMX stimulation of background cGMP binding was observed suggesting that little or no endogenous cGB-PDE was present in the COS-7 cell extracts. In extracts of pCDM8cGB-PDE transfected cells cGMP-specific activity was stimulated approximately 1.8-fold by the addition of 0.2 mm IBMX. The ability of IBMX to stimulate cGMP binding 2-5 fold is a distinctive property of the cGMP-binding phosphodiesterases.

[0076] Cell extracts were assayed as described above for [^{3}H]cGMP-binding activity (wherein concentration of [^{3}H]

cGMP was 2.5 μM) in the presence of excess unlabelled cAMP or cGMP. Results are presented in **FIG. 8** wherein cGMP binding in the absence of unlabelled computer was taken 100% and each data point represents the average of three separate determinations. The binding activity of the problem product encoded by the cGB-PDE cDNA was specific for cGMP relative to cAMP. Less than 10-fold higher concentrations of unlabelled cGMP were required to inhibit [^{3}H]cGMP binding activity by 50% whereas approximately 100-fold higher concentrations of cAMP were required for the same degree of inhibition.

[**0077**] The results presented in this example show that the cGB-PDE cDNA encodes a phosphodiesterase which possesses biochemical activities characteristic of native cGB-PDE.

[**0078**] The catalytic domains of mammalian PDEs and a *Drosophila* PDE contain two tandem conserved sequences ($HX_3HX_{24\text{-}26}E$) that are typical Zn^{2+}-binding motifs in Zn^{2+}hydrolases such as thermolysin [Vallee and Auld, *Biochem.,* 29: 5647-5659 (1990)]. cGB-PDE binds Zn^{2+}in the presence of large excesses of Me^{2+}, Mn^{2+}, Fe^{2+}, Fe^{2+}, Ca^{2+} or Cd^{2+}. In the absence of added, metal, cGB-PDE has a PDE activity that is approximately 20% of the maximum activity that occurs in the presence of 40 mM Mg^{2+}, and this basal activity is inhibited by 1,10-phenanthroline or EDTA. This suggests that a trace metal(s) accounts for the basal PDE activity despite exhaustive treatments to remove metal(s), PDE activity is stimulated by addition of Zn^{2+} (0.02-1 μM) or Co^{2+}(1-20 μM), but not by Fe^{2+}, Fe^{2+}, Ca^{2+}, Cd^{2+}, or Cu^{2+}. Zn^{2+}increases the basal PDE activity up to 70% of the maximum stimulation produced by 40 mM Me^{2+}. The stimulatory effect of Zn^{2+}in these assays may be compromised by an inhibitory effect that is caused by Zn^{2+}concentrations >1 μM. The Zn^{2+}-supported PDE activity and Zn^{2+} binding by cGB-PDE occur at similar concentrations of Zn^{2+}. cGB-PDE thus appears to be a Zn^{2+}hydrolase and Zn^{2+}appears to play a critical role in the activity of the enzyme. See, Colbran et al., *The FASEB J.,* 8: Abstract 2148 (Mar. 15, 1994).

EXAMPLES 6

[**0079**] Several human cDNA clones, homologous to the bovine cDNA clone encoding cGB-PDE, were isolated by hybridization under stringent conditions using a nucleic acid probe corresponding to a portion of the bovine cGB-8 clone (nucleotides 489-1312 of SEQ ID NO: 9).

[**0080**] Isolation of cDNA Fragments Encoding Human cGB-PDE

[**0081**] Three human cDNA libraries (two glioblastoma and one lung) in the vector lambda Zap were probed with the bovine cGB-PDE sequence The PCR-generated clone corresponding to nucleotide 484-1312 of SEQ ID NO: 9 which is described in Example 1 was digested with EcoRI and SalI and the resulting 0.8 kb cDNA insert was isolated and purified by agarose gel electrophoresis. The fragment was labelled with radioactive nucleotides using a random primed DNA labelling kit (Boehringer).

[**0082**] The cDNA libraries were plated on 150 mm petri plates at a density of approximately 50,000 plaques per plate. Duplicate nitrocellulose filter replicas were prepared. The prehybridization buffer was 3×SSC, 0.1% sarkosyl, 10× Denhardt's, 20 mM sodium phosphate (pH 6.8) and 50 μg/ml salmon tests DNA. Prehybridization was carried out at 65° C. for a minimum of 30 minutes. Hybridization was carried out at 65° C. overnight in buffer of the same composition with the addition of 1×10^5 cpm/ml of probe. The filter were washed at 65° C. in 2×SSC, 0.1% SDS. Hybridizing plaques were detected by autoradiography. The number of cDNAs that hybridized to the bovine probe and the number of cDNAs screened are indicated in Table 2 below.

TABLE 2

cDNA Library	Type	Positive Plaques	Plaques Screened
Human SW 1088 glioblastoma	dT-primed	1	1.5×10^4
Human lung	dT-primed	2	1.5×10^6
Human SW 1088 glioblastoma	dT-primed	4	1.5×10^6

[**0083**] Plasmids designated cgbS2.1, cgbS3.1, cgbL23.1, cgbL27.1 and cgbS27.1 were excised in vivo from the lambda Zap clones ard sequenced.

[**0084**] Clone cgbS3.1 contains 2060 bp of a PDE open reading frame followed by a putative intron. Analysis of clone cgbS2.1 reveals that it corresponds to clone cgbS3.1 positions 664 to 2060 and extends the PDE open reading frame an additional 585 bp before reading into a putative intron. The sequence of the putative 5' untranslated region and the protein encoding portions of the cgbS2.1 and cgbS3.1 clones are set out in SEQ ID NOs: 11 and 12, respectively. Combining the two cDNAs yields a sequence containing approximately 2.7 kb of an open reading encoding a PDE. The three other cDNAs did not extend any further 5' or 3' than cDNA cgbS3.1 or cDNA cgbS2.1.

[**0085**] To isolate additional cDNAs, probes specific for the 5' end of clone cgbS3.1 and the 3' end of clone cgbS2.1 were prepared and used to screen a SW1088 glioblastoma cDNA library and a human aorta cDNA library. A 5' probe was derived from clone cgbS3.1 by PCR using the primers cgbS3.1S311 and cgbL23.1A1286 whose sequences am set out in SEQ ID NOs: 8 and 9, respectively, and below,

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Primer cgb53.15311              (SEQ ID NO: 13)
5' GCCACCAGAGAAATGGTC 3'

Primer cgbL23.IA1286            (SEQ ID) NO: 14)
5' ACAATGGGTCTAAGAGGC 3'
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[**0086**] The PCR reaction was carried out in a 50 ul reaction volume containing 50 pg cgbS3.1 cDNA, 0.2 mM dNTP, 10 ug/ml each primer, 50 mM KCl, 10 mM Tris-HCl pH 8.2, 1.5 mM $MgCl_2$ and Taq polymerase. After an initial four minute denaturation at 94° C., 30 cycles of one minute at 94° C., two minutes at 50° C. and four minutes at 72° C. were carried out. An approximately 0.2 kb fragment was generated by the PCR reaction which corresponded to nucleotides 300-496 of clone cgbS3.1.

[**0087**] A 3' probe was derived from cDNA cgbS2.1 by PCR using the oligos cgbL3.1 S1190 and cgbS2.1A231 whose sequences are set out below.

Primer cgbL23.151190 (SEQ 113 NO: 15)
5' TCAGTGCATGTTTGCTGC 3'

Primer cgbS2.1A231 (SEQ ID NO: 16)
5' TACAAACATGTTCATCAG 3'

[0088] The PCR reaction as carried out similarly to that described above for generating the 5' probe, and yielded a fragment of approximately 0.8 kb corresponding to nucleotides 1358-2139 of cDNA cgbS2.1. The 3' 157 nucleotides of the PCR fragment (not shown in SEQ ID NO: 12) are within the presumptive intron.

[0089] The two PCR fragments were purified and isolated by agarose get electrophoresis, and were labelled with radioactive nucleotides by random priming. A random-printed SW1088 glioblastoma cDNA library (1.5×10^6 plaques) was screened with the labelled fragments as described above, and 19 hybridizing plaques were isolated. An additional 50 hybridizing plaques were isolated from a human aorta cDNA library (dT and random primed, Clontech, Palo Alto, Calif.).

[0090] Plasmids were excised in vivo from some of the positive lambda Zap clones and sequenced. A clone designated cgbS53.2, the sequence of which is set out in SEQ ID NO: 17, contains an approximately 1.1 kb insert whose sequence overlaps the last 61 bp of cgbS3.1 and extends the open reading frame an additional 135 bp beyond that found in cgbS2.1. The clone contains a termination codon and approximately 0.3 kB of putative 3' untranslated sequence.

[0091] Generation of a Composite cDNA Encoding Human cGB-PDE

[0092] Clones cgbS3.1, cgbS2.1 and cgbS53.2 were used as described in the following paragraphs to build a composite cDNA that contained a complete human cGB-PDE opening reading frame. The composite cDNA is designated cgbmet156-2 and was inserted in the yeast ADH1 expression vector pBNY6N.

[0093] First, a plasmid designated cgb stop-2 was generated that contained the 3' end of the cGB-PDE open reading fame. A portion of the insert of the plasmid was generated by PCR using clone cgbS53.2 as a template. The PCR primers utilized were cgbS2.1S1700 and cgbstop-2.

Primer cgbS2.IS1700
(SEQ ID NO: 18)
5' TTTGGAAGATCCTCATCA 3'

Primer cgbstop-2
(SEQ ID NO: 19)
5' ATGTCTCGAGTCAGTTCCGCTTGGCCCTG 3'

[0094] The PCR reaction was carried out in 50 ul containing 50 pg template DNA, 0.2 mM dNTPs, 20 mM Tris-HCl pH 8.2, 10 mM KCl, 6 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.1% Triton-X-100, 500 ng each primer and 0.5 units of Pfu polymerase (Stratagene). The reaction was heated to 94° C. for 4 minutes and then 30 cycles of 1 minute at 94° C., 2 minutes at 50° C. and four minutes at 72° C. were performed. The polymerase was added during the first cycle at 50° C. The resulting PCR product was phenol/chloroform extracted, chloroform extracted, ethanol precipitated and cut with the restriction enzymes BclI and XhoI. The restriction fragment was purified on in agarose gel and eluted.

[0095] This fragment was ligated to the cDNA cgbS2.1 that had been grown in darn *E. coli*, cut with the restriction enzymes BclI and XhoI, and gel-purified using the Promega magic PCR kit. The resulting plasmid was sequenced to verify that cgbstop-2 contains the 3' portion of the cGB-PDE open reading frame.

[0096] Second, a plasmid carrying the 5' end of the human cGB-PDE open reading frame was generated. Its insert was generated by PCR using clone cgbS3.1 as a template. PCR was performed as described above using primers cgbmet156 and cgbS2.1A2150.

Primer cgbmet156
(SEQ ID NO: 20)
5' TACAGAATICTGACCATGGAGCGGGCCGGC 3'

Primer cgbS2.1A2150
(SEQ ID NO: 21)
5' CATTCTAAGCGGATACAG 3'

[0097] The resulting PCR fragment was phenol/chloroform extracted, chloroform extracted, ethanol precipitated and purified on a Sepharose CL-6B column. The fragment was cut with the restriction enzymes EcoRV and EoRI, run on an agarose gel and purified by spinning through glass wool. Following phenol/chloroform extraction, chloroform extraction and ethanol precipitation, the fragment was ligated into EcoRI/EcoRV digested BluescriptII SK(+) to generate plasmid cgbmet156. The DNA sequence of the insert and junctions was determined. The insert contains a new EcoRI site and an additional 5 nucleotides that together replace the original 155 nucleotides 5' of the initiation codon. The insert extends to an EcoRV site beginning 531 nucleotides from the initiation codon.

[0098] The 5' and 3' portions of the cGB-PDE open reading frame were then assembled in vector pBNY6a. The vector pBNY6a was cut with EcoRI and XhoI, isolated from a gel and combined with the agarose gel purified EcoRI/EcoRV fragment from cgbmct156 and the agarose gel purified EcoRV/XhoI fragment from cgbstop-2. The junctions of the insert were sequenced and the construct was named hcbgmet15-2 6a.

[0099] The cGB-PDE insert from hcbgmet115-2 6a was then moved into the expression vector pBNY6n. Expression of DNA inserted in this vector is directed from the yeast ADH1 promoter and terminator. The vector contains the yeast 2 micron origin of replication, the pUC19 origin of replication and an ampicillin resistance gene. Vector pBNY6n was cut with EcoRI and XhoI and gel-purified. The EcoRI/XhoI insert from hcgbmet156-2 6a was gel purified using Promega magic PCR construct, and ligated into the cut pBNY6n. All new junctions in the resulting construct, hcgbmet156-2 6n, were sequenced. The DNA and deduced amino acid sequences of the insert of hcgbmet156-2 6n which encodes a composite human cGB-PDE is set out in SEQ ID NOs: 22 and 23. The insert extends from the first methionine in clone cgbS3.1 (nucleotide 156) to the stop codon (nucleotide 2781) in the composite cDNA. Because the methionine is the most 5' methionine in clone cgbS3.1

and because there are no stop codons in frame with the methionine and upstream of it, the insert in pBNY6n may represent a truncated form of the open reading frame.

[0100] Variant cDNAs

[0101] Four human cGB-PDE cDNAs that are different from the hcgbmet156-2 6n composite cDNA have been isolated. One cDNA, cgbL23.1, is missing an internal region of hcgbmet156-2 6n (nucleotides 997-1000 to 1444-1447). The exact end points of the deletion cannot be determined from the cDNA sequence at the positions. Three of the four variant cDNAs have 5' end sequences that diverge from the hcgbmet156-2 6n sequence upstream of nucleotide 151 (cDNAs cgbA7f, cgbA5C, cgbI2). These cDNAs presumably represent alteratively spliced or unspliced mRNAs.

EXAMPLE 7

[0102] The composite human cGB-PDE cDNA construct, hcgbmet56-2 6n, was transformed into the yeast strain YKS45 (ATCC 74225) (MATα his3 trp1 ura3 leu3 pde::HIS3 pde2::TRP1) in which two endogenous PDE genes are deleted. Transformants complementing the leu deficiency of the YKS45 strain were selected and assayed for cGB-PDE activity. Extracts from cells bearing the plasmid hcgbmet156-2 6n were determined to display cyclic GMP-specific phosphodiesterase activity by the assay described below.

[0103] One liter of YKS45 cells transformed with the plasmid cgbmet156-2 6n and grown in SC-leu medium to a density of $1\text{-}2\times10^7$ cells/ml was harvested by centrifugation, washed once with deionized water, frozen in dry ice/ethanol and stored at −70° C. Cell pellets (1-1.5 ml) were thawed on ice in the presence of an equal volume of 25 mM Tris-Cl (pH 8.0)/5 mM EDTA/5 mM EGTA/1 mM o-phenanthroline/0.5 mM AEBSF (Calbiochem)/0.1% β-mercaptoethanol and 10 ug/ml each of aprotinin, leupeptin, and pepstatin A. The thawed cells were added to 2 ml of acid-washed glass beads (425-600 μM. Sigma) in 15 ml Corex tube. Cells were broken with 4 cycle consisting of a 30 second vortexing on setting 1 followed by a 60 second incubation on ice. The cell lysate was centrifuged at 12,000×g for 10 minutes and the supernatant was passed through a 0.8μ filter. The supernatant was assayed for cGMP PDE activity as follows. Samples we incubated for 20 minutes at 30° C. in the presence of 45 mM Tris-Cl (pH 8.0), 2 mM EGTA, 1 mM EDTA, 0.2 mg/ml BSA, 5 mM $MgCl_2$, 0.2 mM o-phenanthroline, 2 ug/ml each of pepstatin A, leupeptin, and aprotinin, 0.1 mM AEBSF, 0.02% β-mercaptoethanol and 0.1 mM [^{3}H]cGMP as substrate. [^{14}C]-AMP (0.5 nCi/assay) was added as a recovery standard. The reaction was terminated with stop buffer (0.1M ethanolamine pH 9.0, 0.5M ammonium sulfate, 10 nM EDTA, 0.05% SDS final concentration). The product was separated from the cyclic nucleotide substrate by chromatography on BioRad Affi-Gel 601. The sample was applied to a column containing approximately 0.25 ml of Affi-Gel 601 equilibrated in column buffer (0.1M ethanolamine pH 9.0 containing 0.5M ammonium sulfate). The column was washed five times with 0.5 ml of column buffer. The product was eluted with four 0.5 ml aliquots of 0.25 acetic acid and mixed with 5 ml Ecolume (ICN Biochemicals). The radioactive product was measured by scintillation counting.

EXAMPLE 8

[0104] Analysis of expression of cGB-PDE mRNA in human tissues was carried out by RNase protection assay.

[0105] A probe corresponding to a portion of the putative cGMP binding domain of cGB-PDE (402 bp corresponding to nucleotides 1450 through 1851 of SEQ ID NO: 13) was generated by PCR. The PCR fragment was inserted into the EcoRI site of the plasmid pBSII SK(−) to generate the plasmid RP3. RP3 plasmid DNA was linearized with XbaI and antisense probes were generated by a modification of the Stratagene T7 RNA polymerase kit. Twenty-five ng of linearized plasmid was combined with 20 microcuries of alpha ^{32}rUTP (800 Ci/mmol, 10 mCi/ml), 1× transcription buffer (40 mM Tris Cl, pH 8, 8 mM $MgCl_2$, 2 mM spermidine, 50 mM NaCl), 0.25 mM each rATP, rGTP and rCTP, 0.1 units of RNase Block II, 5 mM DTT, 8 μM rUTP and 5 units of T7 RNA Polymerase in a total volume of 5 μl. The reaction was allowed to proceed 1 hour at room temperature and then the DNA template was removed by digestion with RNase free DNase. The reaction was diluted into 100 μl of 40 mM TrisCl, pH 8, 6 mM $MgCl_2$ and 10 mM NaCl. Five units of RNase-free DNase were added and the reaction was allowed to continue another 15 minutes at 37° C. The reaction was stopped by a phenol extraction followed by a phenol chloroform extraction. One half volume of 7.5M NH_4OAc was added and the probe was ethanol precipitated.

[0106] The RNase protection assays were carried out using the Ambion RNase Protection kit (Austin, Tex.) and 10 μg RNA isolated from human tissues by an acid guanidinium extraction method. Expression of cGB-PDE mRNA was easily detected in RNA extracted from skeletal muscle, uterus, bronchus, skin, right saphenous vein, aorta and SW1018 glioblastoma cells. Barely detectable expression was found in RNA extracted from right atrium, right ventricle, kidney cortex, and kidney medulla. Only complete protection of the RP3 probe was sees. The lack of particle protection argues against the cDNA cgbL23.1 (a variant cDNA described in Example 7) representing a major transcript, at least in these RNA samples.

EXAMPLE 9

[0107] Polyclonal antisera was raised to *E. coli*-produced fragments of the human cGB-PDE.

[0108] A portion of the human cGB-PDE cDNA (nucleotides 1668-2612 of SEQ ID NO: 22, amino acids 515-819 of SEQ ID NO: 23) was amplified by PCR and inserted into the *E. coli* expression vector pGEX2T (Pharmacia) as a BamHI/EcoRI fragment. The pGEX2T plasmid carries an ampicillin resistance gene, an *E. coli* laq I^q gene and a portion of the *Schisrosoma japonicum* glutathione-S-transferase (GST) gene. DNA inserted in the plasmid can be expressed as a fusion protein with GST and can then be cleaved from the GST portion of the protein with thrombin. The resulting plasmid, designated cgbPE3, was transformed into *E. coli* stain LE392 (Stratagene). Transformed cells were grown at 37° C. to an OD600 of 0.6. IPTG (isopropylthioalactopyranoside) was added to 0.1 mM and the cells were grown at 37° C. for an additional 2 hours. The cells were collected by centrifugation and lysed by sonication. Cell debris was removed by centrifugation and the supernatant was fractionated by SDS-PAGE. The gel was stained with cold 0.4M KCl and the GST-cgb fusion protein band

was excised and electrocuted. The PDE portion of the protein was separated from the GST portion by digestion with thrombin. The digest was fractionated by SDS-PAGE, the PDE protein was electroeluted and injected subcutaneously into a rabbit. The resultant antisera recognizes both the bovine cGB-PDE fragment that was utilized as antigen and the full length human cGB-PDE protein expressed in yeast (see Example 8).

EXAMPLE 10

[0109] Polynucleotides encoding various cGB-PDE analogs and cGB-PDE fragments were generated by standard methods.

[0110] A. cGB-PDE Analogs

[0111] All known cGMP-binding PDEs contain two internally homologous tandem repeats within their putative cGMP-binding domain. In the bovine cGB-PDE of the invention, the repeats span at least residues 228-311 (repeat A) and 410-500 (repeat B) of SEQ ID NO; 10. Site-directed mutagenesis of an aspartic acid that is conserved in repeats A and B of all known cGMP-binding PDEs was used to create analogs of cGB-PDE having either Asp-289 replaced with Ala (D289A) or Asp-478 replaced with Ala (D478A). Recombinant wild type (WT) bovine and mutant bovine cGB-PDEs were expressed in COS-7 cells cGB-PDE purified from bovine lung (native cGB-PDE) and WT cGB-PDE displayed identical cGMP-binding kinetics with a K_d of approximately 2 μM and a curvilinear dissociation profile (t=1.3 hours at 4° C.). This curvilinearity may have been due to the presence of distinct high affinity (slow) and low affinity (fast) sites of cGMP binding. The D289A mutant had significantly decreased affinity for cGMP (K_d>20 μM) and a single rate of cGMP-association (t**[text missing or illegible when filed]**=0.5 hours), that was similar to that calculated for the fast site of WT and native cGB-PDE. This suggested the lost of a slow cGMP-binding site in repeat A of this mutant. Conversely, the D478A mutant showed higher affinity for cGMP (K_d of approximately 0.5 μM) and a single cGMP-dissociation rate (t**[text missing or illegible when filed]**=2.8 hours) that was similar to the calculated rate of the slow site of WT and native cGB-PDE. This suggested the loss of a fast site when repeat was modified. Them results indicate that dimeric cGB-PDE possesses two homologous but kinetically distinct cGMP-binding sites, with the conserved aspartic acid being critical for interaction with cGMP at each site. See, Colbran et al., *FASEB J.,* 8: Abstract 2149 (May 15, 1994).

[0112] B. Amino-Terminal Truncated cGB-PDE Polypeptides

[0113] A truncated human cGB-PDE polypeptide including amino acids 516-875 of SEQ ID NO: 23 was expressed in yeast. A cDNA insert extending from the NcoI site at nucleotide 1555 of SEQ ID NO: 22 through the XhoI site at the 3' end of SEQ ID NO: 22 was inserted into the ADH2 yeast expression vector YEpC-PADH2d [Price et al., *Meth. Enzymol.,* 118: 308-318 (1990)] that had been digested with NcoI and SalI to generate plasmid YEpC-PADH2d HcGB. The plasmid was transformed into spheroplasts of the yeast stain yBJ2-54 (prc1-407 prb1-1122 pep4-3 leu2 trp1 ura3-52 Δpde1::URA3, HIS3 Δpdc::TRP1 cir). The endogenous PDE genes are deleted in this strain. Cells were grown in SC-leu media with 2% glucose to 10^7 cells/ml, collected by filtration and grown 24 hours in YEP media containing 3% glycerol. Cells were pelleted by centrifugation and stored frozen. Cells were disrupted with glass beads and the cell homogenate was assayed for phosphodiesterase activity essentially as described in Prpic et al., *Anal. Biochem.,* 208: 155-160 (1993). The truncated human cGB-PDE polypeptide exhibited phosphodiesterase activity.

[0114] C. Carboxy-Terminal Truncated cGB-PDE Polypeptides

[0115] Two different plasmids encoding carboxy-terminal truncated human cGB-PDE polypeptides were constructed.

[0116] Plasmid pBJ6-84Hin contains a cDNA encoding amino acids 1-494 of SEQ ID NO: 23 inserted into the NcoI and SalI sites of vector YEpC-PADH2d. The cDNA insert extends from the NcoI site at nucleotide position 10 of SEQ ID NO: 22 through the HindIII site at nucleotide position 1494 of SEQ ID NO: 22 followed by a linker and the SalI site of YEpC-PADH2d.

[0117] Plasmid pBJ6-84Ban contains a cDNA encoding amino acids 1-549 of SEQ ID NO: 23 inserted into the NcoI and SalI sites of vector YEpC-PADH2d. The cDNA insert extends from the NcoI site at nucleotide position 10 of SEQ ID NO: 22 through the BanI site at nucleotide position 1657 of SEQ ID NO: 22 followed by a linker and the SalI site of YEpC-PADH2d.

[0118] The truncated cGB-PDE polypeptides are useful for screening for modulators of cGB-PDE activity.

EXAMPLE 11

[0119] Monoclonal antibodies reactive with human cGB-PDE were generated.

[0120] Yeast yB12-54 containing the plasmid YEpADH2 HcGB (Example 10B) were fermented in a New Brunswick Scientific 10 liter Microferm. The cGB-PDE cDNA insert in plasmid YEpADH2 HcGB extends from the NcoI site at nucleotide 12 of SEQ ID NO: 22 to the XhoI site at the 3' end of SEQ ID NO: 22. An inoculum of 4×10**[text missing or illegible when filed]**cells was added to 8 liters of media containing SC-leu, 5% glucose, trace metals, and trace vitamins. Fermentation was maintained at 26° C., agitated at 600 rpm with the standard microbial impeller, and aerated with compressed air at 10 volumes per minute. When glucose done to 0.3% at 24 hours post-inoculation the culture was infused with 2 liters of 5×YEP media containing 15% glycerol. At 66 hours post-inoculation the yeast from the ferment was harvested by centrifugation at 4,000×g for 30 minutes at 4° C. Total yield of biomass from this fermentation approached 350 g wet weight.

[0121] Human cGB-PDE enzyme was purified from the yeast cell pellet. Assays for PDE activity using 1 mM cGMP as substituted was employed to follow the chromatography of the enzyme. All chromatographic manipulations were performed at 4° C.

[0122] Yeast (29 g wet weight) were resuspended in 70 ml of buffer A (25 mM Tris pH 8.0, 0.25 mM DTT, 5 mM $MgCl_2$, 10 μM $ZnSO^4$, 1 mM benzamidine) and lysed by passing through a microfluidizer at 22-24,000 psi. The lysate was centrifuged at 10,000×g for 30 minutes and the supernatant was applied to a 2.6×28 cm column containing Pharmacia Fast Flow Q anion exchange resin equilibrated with buffer B containing 20 mM BisTris-propane pH 6.8, 0.25 mM DTT, 1 mM $MgCl_2$, and 10 μM $ZnSO_4$. The column was washed with 5 column volumes of buffer B containing 0.125M NaCl and then developed with a linear gradient from 0.125 to 1.0M NaCl. Fractions containing the enzyme were pooled and applied directly to a 5×20 cm column of ceramic hydroxyapatite (BioRad) equilibrated in buffer C containing 20 mM BisTris-propane pH 6.8, 0.25 mM DTT, 0.25 MKCl, 1 mM $MgCl_2$, and 10 μM $ZnSO_4$.

The column was washed with 5 column volumes of buffer C and eluted with a linear gradient from 0 to 250 mM potassium phosphate in buffer C. The pooled enzyme was concentrated 8-fold by ultrafiltration (YM30 membrane, Amicon). The concentrated enzyme was chromatographed on a 2.6×90 cm column of Pharmacia Sephacryl S300 (Piscataway, N.J.) equilibrated in 25 mM BisTris-propane pH 6.8, 0.25 mM DTT, 0.25M NaCl, 1 mM $MgCl_2$, and 20 μM $ZnSO_4$. Approximately 4 mg of protein was obtained. The recombinant human cGB-PDE enzyme accounted for approximately 90% of protein obtained a judged by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining.

[0123] The purified protein was used as an antigen to raise monoclonal antibodies. Each of 19 week old Balb/c mice (Charles River Biotechnical Services, Inc., Wilmington, Mass.) was immunized sub-cutaneously with 50 ug purified human cGB-PDE enzyme in a 200 ul emulsion consisting of 50% Freund's complete adjuvant (Sigma Chemical Co.). Subsequent boosts on day 20 and day 43 were administered in incomplete Freund's adjuvant. A pre-fusion boost was done on day 86 using 50 ug enzyme in FBS. The fusion was performed on day 90.

[0124] The spleen from mouse #1817 was removed sterilely and placed in 10 ml serum form RPMI 1640. A single-cell suspension was formed and filtered through sterile 70 mesh Nitex cell strainer (Becton Dickinson, Parsippany, N.J.), and washed twice by centrifuging at 200 g for 5 minutes and resuspending the pellet in 20 ml serum free RPMI. Thymocytes taken from 3 naive Balb/c mice were prepared in a similar manner.

[0125] NS-1 myeloma cells, kept in log phase in RPMI with 11% Fetalclone (FBS) Hyclone Laboratories, Inc., Logan, Utah) for three days prior to fusion, were centrifuged 200 g for 5 minutes, and the pellet was washed twice as described in the foregoing paragraph. After washing each cell suspension was brought to a final volume of 10 ml in serum free RPMI, and 20 μl was diluted 1:50 in 1 ml serum free RPMI: 20 μl of each dilution was removed, mixed with 20 μl 0.4% trypan blue sun in 0.85% saline (Gibco), loaded onto a hemocytometer (Baxter Healthcare Corp., Deerfield, Ill.) and counted.

[0126] Two×10**[text missing or illegible when filed]**spleen cells were combined with 4.0×10^7 NS-1 cells, centrifuged and the supernatant was aspirated. The cell pellet was dislodged by tapping the tube and 2 ml of 37° C. PEG 1500 (50% in 75 mM Hepes, pH 8.0) (Boehringer Mannheim) was added with stirring over the course of 1 minute, followed by adding 14 ml of serum free RPMI over 7 minutes. An additional 16 ml RPMI was added and the cells were centrifuged at 200 g for 10 minutes. After discarding the supernatant, the pellet was resuspended in 200 ml RPMI containing 15% FBS, 100 μM sodium hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine (HAT) (Gibco), 25 units/ml IL-6 (Boehringer Mannheim) and 1.5×10**[text missing or illegible when filed]**thymocytes/ml. The suspension was first placed in a T225 flask (Corning, United Kingdom) at 37° C. for two hours before being dispensed into ten 96 well flat bottom tissue culture plates (Corning, United Kingdom) at 200 μl/well. Cells in plates were fed on days 3, 4, 5 post fusion day by aspirating approximately 100 μl from each well with an 20 G needle (Becton Dickinson), and adding 100 μl/well plating medium described above except containing 10 units/ml IL-6 and lacking thymocytes.

[0127] The fusion was screened initially by ELISA. Immulon 4 plates (Dynatech) were coated at 4° C. overnight with purified recombinant human cGB-PDE enzyme (100 ng/well in 50 mM carbonate buffer pH9.6). The plates were washed 3× with PBS containing 0.05% Tween 20 (PBST). The supernatants from the individual hybridoma wells were added to the enzyme coated wells (50 μl/well). After incubation at 37° C. for 30 minutes, and washing as above, 50 μl of horseradish peroxidase conjugated goat anti-mouse IgG(fc) (Jackson ImmunoResearch, West Grove, Pa.) diluted 1:3500 in PBST was added. Plates were incubated as above, washed 4× with PBST and 100 μl substrate consisting of 1 mg/ml o-phenylene diamine (Sigma) and 0.1 μl/ml 30% H_2O_2 in 100 mM citrate, pH 4.5, was added. The color reaction was stopped in 5 minutes with the addition of 50 μl of 15% H_2SO_4. A_{490} was read on a plate reader (Dynatech).

[0128] Wells C5G, **[text missing or illegible when filed]**4D, F1G, F9H, F11G, J4A, and J5D were picked and renamed 102A, 102B, 102C, 102D, 102E, 102F, and 102G respectively, cloned two or three times, successively, by doubling dilution in RPMI, 15% FBS, 100 μM sodium hypoxanthine, 16 μM thymidine, and 10 units/ml IL-6. Wells of clone plates were scored visually after 4 days and the number of colonies in the least dense wells were recorded. Selected wells of the each cloning were tested by ELISA.

[0129] The monoclonal antibodies produced by above hybridomas were isotyped in an ELISA assay. Results showed that monoclonal antibodies 102A to 102E were IgG1, 102F was IgG2b and 102G was IgG2a.

[0130] All seven monoclonal antibodies reacted with human cGS-PDE as determined by Western analysis.

EXAMPLE 12

[0131] Developing modulators of the biological activities of specific PDEs requires differentiating PDE isozymes present in a particular assay preparation. The classical enzymological approach of isolating PDEs from natural tissue sources and studying each new isozyme is hampered by the limits of purification techniques and the inability to definitively assess whether complete resolution of a isozyme has been achieved. Another approach has been to identify assay conditions which might favor the contribution of one isozyme and minimize the contribution of others in a preparation. Still another approach has been the separation of PDEs by immunological means. Each of the foregoing approaches for differentiating PDE isozymes is time consuming and technically difficult. As a result many attempts to develop selective PDE modulators have been performed with preparations containing more than one isozyme. Moreover, PDE preparations from natural tissue sources am susceptible to limited proteolysis and may contain mixtures of active proteolytic products that have different kinetic, regulatory and physiological properties than the full length PDEs.

[0132] Recombinant cGB-PDE polypeptide products of the invention greatly facilitate the development of new and specific cGB-PDE modulators. The use of human recombinant enzymes for screening for modulators has many inherent advantages. The need for purification of an isozyme can be avoided by expressing it recombinantly in a host cell that lacks endogenous phosphodiesterase activity (e.g. yeast swain YKS45 deposited as ATCC 74225). Screening compounds against human protein avoids complications that often arise from screening against non-human protein where a compound optimized on a non-human protein may fail to be specific for or react with the human protein. For example, a single amino acid difference between the human and rodent $5HT_{1B}$ serotonin receptors accounts for the difference in binding of a compound to the receptors [See Oskenberg

et al., *Nature,* 360: 161-163 (1992)]. Once a compound that modulates the activity of the cGB-PDE is discovered, its selectivity can be evaluated by comparing its activity on the cGB-PDE to its activity on other PDE isozymes. Thus, the combination of the recombinant cGB-PDE products of the invention with other recombinant PDE products in a series of independent assays provides a system for developing selective modulators of cGB-PDE. Selective modulators many include, for example, antibodies and other proteins or peptides which specifically bind to the cGB-PDE or cGB-PDE nucleic acid, oligonucleotides which specifically bind to the cGB-PDE (so Patent Cooperation Treaty International Publication No. WO93/05182 published Mar. 18, 1993 which describes methods for selecting oligonucleotides which selectively bind to target biomolecules) or cGB-PDE nucleic acid (e.g., antisense oligonucleotides) and other non-peptide natural or synthetic compounds which specifically bind to the cGB-PDE or cGB-PDE nucleic acid. Mutant forms of the cGB-PDE which alter the enzymatic activity of the cGB-PDE or its localization in a cell are also contemplated. Crystallization of recombinant cGB-PDE alone and bound to a modulator, analysis of atomic structure by X-ray crystallography, and computer modulating of those structures are methods useful for designing and optimizing non-peptide selective modulators. See, for example, Erickson et al., *Ann. Rep. Med. Chem.* 27: 271-289 (1992) for a general review of structure-based drug design.

[0133] Targets for the development of selective modulators include, for example: (1) the regions of the cGB-PDE which contact other proteins and/or localize the cGB-PDE within a cell, (2) the regions of the cGB-PDE which bind substrate, (3) the allosteric cGMP-binding site(s) of cGB-PDE, (4) the metal-binding regions of the cGB-PDE, (5) the phosphorylation site(s) of cGB-PDE and (6) the regions of the cGB-PDE which are involved in dimerization of cGB-PDE subunits.

[0134] While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those skilled in the art. Accordingly, only such limitations as appear in the appended claims should be placed on the invention.

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55                  60                  65                  70

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cca acc agg aag atc tct gcc tct gaa ttc gat cgg ccg ctt aga ccc      404
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135                 140                 145                 150

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            170                 175                 180

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Arg Ser Leu Leu Cys Thr Pro Ile Lys Asn Gly Lys Lys Asn Lys Val
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Glu Val Leu Cys Lys Trp Ile Leu Ser Val Lys Lys Asn Tyr Arg Lys
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Asn Val Ala Tyr His Asn Trp Arg His Ala Phe Asn Thr Ala Gln Cys
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Met Phe Ala Ala Leu Lys Ala Gly Lys Ile Gln Lys Arg Leu Thr Asp
615                 620                 625                 630

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gct cag ctc tac tgc cat tca atc atg gag cat cat cat ttt gat cag     2132
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tgc ctg atg atc ctt aat agt cct ggc aat cag att ctc agt ggc ctc     2180
Cys Leu Met Ile Leu Asn Ser Pro Gly Asn Gln Ile Leu Ser Gly Leu
    680                 685                 690

tcc att gaa gag tat aag acc acc ctg aag atc atc aag caa gct att     2228
Ser Ile Glu Glu Tyr Lys Thr Thr Leu Lys Ile Ile Lys Gln Ala Ile
695                 700                 705                 710

tta gcc aca gac cta gca ctg tac ata aag aga cga gga gaa ttt ttt     2276
Leu Ala Thr Asp Leu Ala Leu Tyr Ile Lys Arg Arg Gly Glu Phe Phe
                715                 720                 725

gaa ctt ata atg aaa aat caa ttc aat ttg gaa gat cct cat caa aag     2324
Glu Leu Ile Met Lys Asn Gln Phe Asn Leu Glu Asp Pro His Gln Lys
            730                 735                 740

gag ttg ttt tta gcg atg ctg atg aca gct tgt gat ctt tct gca att     2372
Glu Leu Phe Leu Ala Met Leu Met Thr Ala Cys Asp Leu Ser Ala Ile
        745                 750                 755

aca aaa ccc tgg cct att caa caa cgg ata gca gaa ctt gtt gcc act     2420
Thr Lys Pro Trp Pro Ile Gln Gln Arg Ile Ala Glu Leu Val Ala Thr
    760                 765                 770

gaa ttt ttt gac caa gga gat aga gag agg aaa gaa ctc aac ata gag     2468
Glu Phe Phe Asp Gln Gly Asp Arg Glu Arg Lys Glu Leu Asn Ile Glu
775                 780                 785                 790

ccc gct gat cta atg aac cgg gag aag aaa aac aaa atc cca agt atg     2516
Pro Ala Asp Leu Met Asn Arg Glu Lys Lys Asn Lys Ile Pro Ser Met
                795                 800                 805

caa gtt gga ttc ata gat gcc atc tgc ttg caa ctg tat gag gcc ttg     2564
Gln Val Gly Phe Ile Asp Ala Ile Cys Leu Gln Leu Tyr Glu Ala Leu
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            810                 815                 820

acc cat gtg tcg gag gac tgt ttc cct ttg ctg gac ggc tgc aga aag     2612
Thr His Val Ser Glu Asp Cys Phe Pro Leu Leu Asp Gly Cys Arg Lys
        825                 830                 835

aac agg cag aaa tgg cag gct ctt gca gaa cag cag gag aag aca ctg     2660
Asn Arg Gln Lys Trp Gln Ala Leu Ala Glu Gln Gln Glu Lys Thr Leu
    840                 845                 850

atc aat ggt gaa agc agc cag acc aac cga cag caa cgg aat tcc gtt     2708
Ile Asn Gly Glu Ser Ser Gln Thr Asn Arg Gln Gln Arg Asn Ser Val
855                 860                 865                 870

gct gtc ggg aca gtg tagccaggtg tatcagatga gtgagtgtgt gctcagctca     2763
Ala Val Gly Thr Val
                875

gtcctctgca acaccatgaa gctaggcatt ccagcttaat tcctgcagtt gactttaaaa   2823

aactggcata aagcactagt cagcatctag ttctagcttg accagtgaag agtagaacac   2883

caccacagtc agggtgcaga gcagttggca gtctcctttc gaacccagac tggtgaattt   2943

aaagaagagc agtcgtcgtt tatatctctg tcttttccta agcggggtgt ggaatctcta   3003

agaggagaga gagatctgga ccacaggtcc aatgcgctct gtcctctcag ctgcttcccc   3063

cactgtgctg tgacctctca atctgagaaa cgtgtaagga aggtttcagc gaattccctt   3123

taaaatgtgt cagacagtag cttcttgggc cgggttgttc ccgcagctcc ccatctgttt   3183

gttgtctatc ttggctgaaa gaggctttgc tgtacctgcc acactctcct ggatccctgt   3243

ccagtagctg atcaaaaaaa aggatgtgaa attctcgtgt gacttttag aaaaggaaag   3303

tgaccccgag gatcggtgtg gattcactag ttgtccacag atgatctgtt tagtttctag   3363

aattttccaa gatgatacac tcctccctag tctaggggtc agaccctgta tggtggctgt   3423

gacccttgag gaacttctct ctttgcatga cattagccat agaactgttc ttgtccaaat   3483

acacagctca tatgcagctt gcaggaaaca ctttaaaaac acaactatca cctatgttat   3543

tctgattaca gaagttatcc ctactcactg taaacataaa caaagccccc caaacttcaa   3603

atagttgtgt gtggtgagaa actgcaagtt ttcatctcca gagatagcta taggtaataa   3663

gtgggatgtt tctgaaactt ttaaaaataa tcttttacat atatgttaac tgttttctta   3723

tgagcactat ggtttgtttt ttttttttt tgctctgctt tgacttgccc ttttcactca   3783

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cagtgttcca tgctgtgagg gcaccatcgt gtattaaatc agttccctgg tcacacatag   3903

gtgagctggt tggaaatttt taccattaaa aaaccacttt cccacattga tgctttctaa   3963

tctggcacag gatgcttctt tttttccccc ttttctctgt ttaattattg gaaatgggat   4023

ctgtgggatc ctcgttccct ggcacctagc tgctctcaac gtggcctgtg gccagcagca   4083

ttggctagac ctgggggctt gttgggaacg gagaccctct gccctgcccc tggcctgctg   4143

acaaggacct gcattttgct gagctcccag tgaccctggt gtttaattgt taaccattga   4203

aaaaaatcaa actatagttt atttacaatg ttgtgttaat ttcgggtgta cagcaaagtg   4263

actcagtggt caagtacatt taaaacactg ggcatactct ctccctctcc ttgtgtacct   4323

ggttggtatt tccagaaacc atgctcttgt ctgtcctgta gttttggaag cgctttctct   4383

ttgaagactg ccttctctcc tctgtctgcc ctacatggac tagttcgttt attgtcctac   4443

atggctttgc ttccatgttc ctctcaactt t                                  4474
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<210> SEQ ID NO 10
<211> LENGTH: 875
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 10

Met Glu Arg Ala Gly Pro Gly Cys Arg Ala Ala Ala Thr Ala Met Gly
1 5 10 15

Pro Gly Leu Gly Arg Ser Val Ala Gly Arg Ser Leu Gly Leu Tyr Leu
20 25 30

Leu Tyr Phe Val Arg Lys Gly Thr Arg Glu Met Val Asn Ala Trp Phe
35 40 45

Ala Glu Arg Val His Thr Ile Pro Val Cys Lys Glu Gly Ile Lys Gly
50 55 60

His Thr Glu Ser Cys Ser Cys Pro Leu Gln Pro Ser Pro Arg Ala Glu
65 70 75 80

Ser Ser Val Pro Gly Thr Pro Thr Arg Lys Ile Ser Ala Ser Glu Phe
85 90 95

Asp Arg Pro Leu Arg Pro Ile Val Ile Lys Asp Ser Glu Gly Thr Val
100 105 110

Ser Phe Leu Ser Asp Ser Asp Lys Lys Glu Gln Met Pro Leu Thr Ser
115 120 125

Pro Arg Phe Asp Asn Asp Glu Gly Asp Gln Cys Ser Arg Leu Leu Glu
130 135 140

Leu Val Lys Asp Ile Ser Ser His Leu Asp Val Thr Ala Leu Cys His
145 150 155 160

Lys Ile Phe Leu His Ile His Gly Leu Ile Ser Ala Asp Arg Tyr Ser
165 170 175

Leu Phe Leu Val Cys Glu Asp Ser Ser Asn Asp Lys Phe Leu Ile Ser
180 185 190

Arg Leu Phe Asp Val Ala Glu Gly Ser Thr Leu Glu Glu Ala Ser Asn
195 200 205

Asn Cys Ile Arg Leu Glu Trp Asn Lys Gly Ile Val Gly His Val Ala
210 215 220

Ala Phe Gly Glu Pro Leu Asn Ile Lys Asp Ala Tyr Glu Asp Pro Arg
225 230 235 240

Phe Asn Ala Glu Val Asp Gln Ile Thr Gly Tyr Lys Thr Gln Ser Ile
245 250 255

Leu Cys Met Pro Ile Lys Asn His Arg Glu Glu Val Val Gly Val Ala
260 265 270

Gln Ala Ile Asn Lys Lys Ser Gly Asn Gly Gly Thr Phe Thr Glu Lys
275 280 285

Asp Glu Lys Asp Phe Ala Ala Tyr Leu Ala Phe Cys Gly Ile Val Leu
290 295 300

His Asn Ala Gln Leu Tyr Glu Thr Ser Leu Leu Glu Asn Lys Arg Asn
305 310 315 320

Gln Val Leu Leu Asp Leu Ala Ser Leu Ile Phe Glu Glu Gln Gln Ser
325 330 335

Leu Glu Val Ile Leu Lys Lys Ile Ala Ala Thr Ile Ile Ser Phe Met
340 345 350

Gln Val Gln Lys Cys Thr Ile Phe Ile Val Asp Glu Asp Cys Ser Asp
355 360 365

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Ser Phe Ser Ser Val Phe His Met Glu Cys Glu Glu Leu Glu Lys Ser
    370                 375                 380

Ser Asp Thr Leu Thr Arg Glu Arg Asp Ala Asn Arg Ile Asn Tyr Met
385                 390                 395                 400

Tyr Ala Gln Tyr Val Lys Asn Thr Met Glu Pro Leu Asn Ile Pro Asp
                405                 410                 415

Val Ser Lys Asp Lys Arg Phe Pro Trp Thr Asn Glu Asn Met Gly Asn
            420                 425                 430

Ile Asn Gln Gln Cys Ile Arg Ser Leu Leu Cys Thr Pro Ile Lys Asn
        435                 440                 445

Gly Lys Lys Asn Lys Val Ile Gly Val Cys Gln Leu Val Asn Lys Met
    450                 455                 460

Glu Glu Thr Thr Gly Lys Val Lys Ala Phe Asn Arg Asn Asp Glu Gln
465                 470                 475                 480

Phe Leu Glu Ala Phe Val Ile Phe Cys Gly Leu Gly Ile Gln Asn Thr
                485                 490                 495

Gln Met Tyr Glu Ala Val Glu Arg Ala Met Ala Lys Gln Met Val Thr
            500                 505                 510

Leu Glu Val Leu Ser Tyr His Ala Ser Ala Ala Glu Glu Glu Thr Arg
        515                 520                 525

Glu Leu Gln Ser Leu Ala Ala Ala Val Val Pro Ser Ala Gln Thr Leu
    530                 535                 540

Lys Ile Thr Asp Phe Ser Phe Ser Asp Phe Glu Leu Ser Asp Leu Glu
545                 550                 555                 560

Thr Ala Leu Cys Thr Ile Arg Met Phe Thr Asp Leu Asn Leu Val Gln
                565                 570                 575

Asn Phe Gln Met Lys His Glu Val Leu Cys Lys Trp Ile Leu Ser Val
            580                 585                 590

Lys Lys Asn Tyr Arg Lys Asn Val Ala Tyr His Asn Trp Arg His Ala
        595                 600                 605

Phe Asn Thr Ala Gln Cys Met Phe Ala Ala Leu Lys Ala Gly Lys Ile
    610                 615                 620

Gln Lys Arg Leu Thr Asp Leu Glu Ile Leu Ala Leu Leu Ile Ala Ala
625                 630                 635                 640

Leu Ser His Asp Leu Asp His Arg Gly Val Asn Asn Ser Tyr Ile Gln
                645                 650                 655

Arg Ser Glu His Pro Leu Ala Gln Leu Tyr Cys His Ser Ile Met Glu
            660                 665                 670

His His His Phe Asp Gln Cys Leu Met Ile Leu Asn Ser Pro Gly Asn
        675                 680                 685

Gln Ile Leu Ser Gly Leu Ser Ile Glu Glu Tyr Lys Thr Thr Leu Lys
    690                 695                 700

Ile Ile Lys Gln Ala Ile Leu Ala Thr Asp Leu Ala Leu Tyr Ile Lys
705                 710                 715                 720

Arg Arg Gly Glu Phe Phe Glu Leu Ile Met Lys Asn Gln Phe Asn Leu
                725                 730                 735

Glu Asp Pro His Gln Lys Glu Leu Phe Leu Ala Met Leu Met Thr Ala
            740                 745                 750

Cys Asp Leu Ser Ala Ile Thr Lys Pro Trp Pro Ile Gln Gln Arg Ile
        755                 760                 765
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Ala Glu Leu Val Ala Thr Glu Phe Phe Asp Gln Gly Asp Arg Glu Arg
    770                 775                 780

Lys Glu Leu Asn Ile Glu Pro Ala Asp Leu Met Asn Arg Glu Lys Lys
785                 790                 795                 800

Asn Lys Ile Pro Ser Met Gln Val Gly Phe Ile Asp Ala Ile Cys Leu
                805                 810                 815

Gln Leu Tyr Glu Ala Leu Thr His Val Ser Glu Asp Cys Phe Pro Leu
            820                 825                 830

Leu Asp Gly Cys Arg Lys Asn Arg Gln Lys Trp Gln Ala Leu Ala Glu
        835                 840                 845

Gln Gln Glu Lys Thr Leu Ile Asn Gly Glu Ser Ser Gln Thr Asn Arg
    850                 855                 860

Gln Gln Arg Asn Ser Val Ala Val Gly Thr Val
865                 870                 875


<210> SEQ ID NO 11
<211> LENGTH: 2060
<212> TYPE: DNA
<213> ORGANISM: human cDNA

<400> SEQUENCE: 11

gcggccgcgc tccggccgct ttgtcgaaag ccggcccgac tggagcagga cgaaggggga     60

gggtctcgag gccgagtcct gttcttctga gggacggacc ccagctgggg tggaaaagca    120

gtaccagaga gcctccgagg cgcgcggtgc caaccatgga gcgggccggc cccagcttcg    180

ggcagcagcg acagcagcag cagccccagc agcagaagca gcagcagagg gatcaggact    240

cggtcgaagc atggctggac gatcactggg actttacctt ctcatacttt gttagaaaag    300

ccaccagaga aatggtcaat gcatggtttg ctgagagagt tcacaccatc cctgtgtgca    360

aggaaggtat cagaggccac accgaatctt gctcttgtcc cttgcagcag agtcctcgtg    420

cagataacag tgtccctgga acaccaacca ggaaaatctc tgcctctgaa tttgaccggc    480

ctcttagacc cattgttgtc aaggattctg agggaactgt gagcttcctc tctgactcag    540

aaaagaagga acagatgcct ctaacccctc caaggtttga tcatgatgaa ggggaccagt    600

gctcaagact cttggaatta gtgaaggata tttctagtca tttggatgtc acagccttat    660

gtcacaaaat tttcttgcat atccatggac tgatatctgc tgaccgctat tccctgttcc    720

ttgtctgtga agacagctcc aatgacaagt ttcttatcag ccgcctcttt gatgttgctg    780

aaggttcaac actggaagaa gtttcaaata actgtatccg cttagaatgg aacaaaggca    840

ttgtgggaca tgtggcagcg cttggtgagc ccttgaacat caaagatgca tatgaggatc    900

ctcggttcaa tgcagaagtt gaccaaatta caggctacaa gacacaaagc attctttgta    960

tgccaattaa gaatcatagg gaagaggttg ttggtgtagc ccaggccatc aacaagaaat   1020

caggaaacgg tgggacattt actgaaaaag atgaaaagga ctttgctgct tatttggcat   1080

tttgtggtat tgttcttcat aatgctcagc tctatgagac ttcactgctg gagaacaaga   1140

gaaatcaggt gctgcttgac cttgctagtt taatttttga agaacaacaa tcattagaag   1200

taattttgaa gaaaatagct gccactatta tctctttcat gcaagtgcag aaatgcacca   1260

ttttcatagt ggatgaagat tgctccgatt ctttttctag tgtgtttcac atggagtgtg   1320

aggaattaga aaaatcatct gatacattaa caagggaaca tgatgcaaac aaaatcaatt   1380

acatgtatgc tcagtatgtc aaaaatacta tggaaccact ttatatccca gatgtcagta   1440
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aggataaaag atttccctgg acaactgaaa atacaggaaa tgtaaaccag cagtgcatta 1500

gaagtttgct ttgtacacct ataaaaaatg gaaagaagaa taaagttata ggggtttgcc 1560

aacttgttaa taagatggag gagaatactg gcaaggttaa gcctttcaac cgaaatgacg 1620

aacagtttct ggaagctttt gtcatctttt gtggcttggg gatccagaac acgcagatgt 1680

atgaagcagt ggagagagcc atggccaagc aaatggtcac attggaggtt ctgtcgtatc 1740

atgcttcagc agcagaggaa gaaacaagag agctacagtc gttagcggct gctgtggtgc 1800

catctgccca gacccttaaa attactgact ttagcttcag tgactttgag ctgtctgatc 1860

tggaaacagc actgtgtaca attcggatgt ttactgacct caaccttgtg cagaacttcc 1920

agatgaaaca tgaggttctt tgcagatgga ttttaagtgt taagaagaat tatcggaaga 1980

atgttgccta tcataattgg agacatgcct ttaatacagc tcagtgcatg tttgctgctc 2040

taaaagcagg caaaattcag 2060

<210> SEQ ID NO 12
<211> LENGTH: 1982
<212> TYPE: DNA
<213> ORGANISM: human cDNA

<400> SEQUENCE: 12

acaaaatttt cttgcatatc catggactga tatctgctga ccgctattcc ctgttccttg 60

tctgtgaaga cagctccaat gacaagtttc ttatcagccg cctctttgat gttgctgaag 120

gttcaacact ggaagaagtt tcaaataact gtatccgctt agaatggaac aaaggcattg 180

tgggacatgt ggcagcgctt ggtgagccct tgaacatcaa agatgcatat gaggatcctc 240

ggttcaatgc agaagttgac caaattacag gctacaagac acaaagcatt ctttgtatgc 300

caattaagaa tcatagggaa gaggttgttg gtgtagccca ggccatcaac aagaaatcag 360

gaaacggtgg gacatttact gaaaaagatg aaaaggactt tgctgcttat ttggcatttt 420

gtggtattgt tcttcataat gctcagctct atgagacttc actgctggag aacaagagaa 480

atcaggtgct gcttgacctt gctagtttaa tttttgaaga acaacaatca ttagaagtaa 540

ttttgaagaa aatagctgcc actattatct ctttcatgca agtgcagaaa tgcaccattt 600

tcatagtgga tgaagattgc tccgattctt tttctagtgt gtttcacatg gagtgtgagg 660

aattagaaaa atcatctgat acattaacaa gggaacatga tgcaaacaaa atcaattaca 720

tgtatgctca gtatgtcaaa aatactatgg aaccacttaa tatcccagat gtcagtaagg 780

ataaaagatt tccctggaca actgaaaata caggaaatgt aaaccagcag tgcattagaa 840

gtttgctttg tacacctata aaaaatggaa agaagaataa agttataggg gtttgccaac 900

ttgttaataa gatggaggag aatactggca aggttaagcc tttcaaccga aatgacgaac 960

agtttctgga agcttttgtc atcttttgtg gcttggggat ccagaacacg cagatgtatg 1020

aagcagtgga gagagccatg gccaagcaaa tggtcacatt ggaggttctg tcgtatcatg 1080

cttcagcagc agaggaagaa acaagagagc tacagtcgtt agcggctgct gtggtgccat 1140

ctgcccagac ccttaaaatt actgacttta gcttcagtga ctttgagctg tctgatctgg 1200

aaacagcact gtgtacaatt cggatgttta ctgacctcaa ccttgtgcag aacttccaga 1260

tgaaacatga ggttctttgc agatggattt taagtgttaa gaagaattat cggaagaatg 1320

ttgcctatca taattggaga catgccttta atacagctca gtgcatgttt gctgctctaa 1380

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aagcaggcaa aattcagaac aagctgactg acctggagat acttgcattg ctgattgctg 1440

cactaagcca cgatttggat caccgtggtg tgaataactc ttacatacag cgaagtgaac 1500

atccacttgc ccagctttac tgccattcaa tcatggaaca ccatcatttt gaccagtgcc 1560

tgatgattct taatagtcca ggcaatcaga ttctcagtgg cctctccatt gaagaatata 1620

agaccacgtt gaaaataatc aagcaagcta ttttagctac agacctagca ctgtacatta 1680

agaggcgagg agaatttttt gaacttataa gaaaaaatca attcaatttg gaagatcctc 1740

atcaaaagga gttgtttttg gcaatgctga tgacagcttg tgatctttct gcaattacaa 1800

aaccctggcc tattcaacaa cggatagcag aacttgtagc aactgaattt tttgatcaag 1860

gagacagaga gagaaaagaa ctcaacatag aacccactga tctaatgaac agggagaaga 1920

aaaacaaaat cccaagtatg caagttgggt tcatagatgc catctgcttg caactgtatg 1980

ag 1982

<210> SEQ ID NO 13
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 13

gccaccagag aaatggtc 18

<210> SEQ ID NO 14
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 14

acaatgggtc taagaggc 18

<210> SEQ ID NO 15
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 15

tcagtgcatg tttgctgc 18

<210> SEQ ID NO 16
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 16

tacaaacatg ttcatcag 18

<210> SEQ ID NO 17
<211> LENGTH: 1107
<212> TYPE: DNA
<213> ORGANISM: human cDNA

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<400> SEQUENCE: 17

gagacatgcc tttaatacag ctcagtgcat gtttgctgct ctaaaagcag gcaaaattca 60

gaacaagctg actgacctgg agatacttgc attgctgatt gctgcactaa gccacgattt 120

ggatcaccgt ggtgtgaata actcttacat acagcgaagt gaacatccac ttgcccagct 180

ttactgccat tcaatcatgg aacaccatca ttttgaccag tgcctgatga ttcttaatag 240

tccaggcaat cagattctca gtggcctctc cattgaagaa tataagacca cgttgaaaat 300

aatcaagcaa gctattttag ctacagacct agcactgtac attaagaggc gaggagaatt 360

ttttgaactt ataagaaaaa atcaattcaa tttggaagat cctcatcaaa aggagttgtt 420

tttggcaatg ctgatgacag cttgtgatct ttctgcaatt acaaaaccct ggcctattca 480

acaacggata gcagaacttg tagcaactga atttttgat caaggagaca gagagagaaa 540

agaactcaac atagaaccca ctgatctaat gaacagggag aagaaaaaca aaatcccaag 600

tatgcaagtt gggttcatag atgccatctg cttgcaactg tatgaggccc tgacccacgt 660

gtcagaggac tgtttccctt tgctagatgg ctgcagaaag aacaggcaga aatggcaggc 720

ccttgcagaa cagcaggaga agatgctgat taatggggaa agcggccagg ccaagcggaa 780

ctgagtggcc tatttcatgc agagttgaag tttacagaga tggtgtgttc tgcaatatgc 840

ctagtttctt acacactgtc tgtatagtgt ctgtatttgg tatatacttt gccactgctg 900

tatttttatt tttgcacaac ttttgagagt atagcatgaa tgtttttaga ggactattac 960

atatttttttg tatatttgtt ttatgctact gaactgaaag gatcaacaac atccactgtt 1020

agcacattga taaaagcatt gtttgtgata tttcgtgtac tgcaaagtgt atgcagtatt 1080

cttgcactga ggtttttttg cttgggg 1107

<210> SEQ ID NO 18
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 18

tttggaagat cctcatca 18

<210> SEQ ID NO 19
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 19

atgtctcgag tcagttccgc ttggcctg 28

<210> SEQ ID NO 20
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 20

tacagaattc tgaccatgga gcgggccggc 30

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<210> SEQ ID NO 21
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 21

cattctaagc ggatacag 18

<210> SEQ ID NO 22
<211> LENGTH: 2645
<212> TYPE: DNA
<213> ORGANISM: human cDNA
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (12)..(2636)

<400> SEQUENCE: 22

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gaattctgac c atg gag cgg gcc ggc ccc agc ttc ggg cag cag cga cag      50
             Met Glu Arg Ala Gly Pro Ser Phe Gly Gln Gln Arg Gln
             1               5                   10

cag cag cag ccc cag cag cag aag cag cag cag agg gat cag gac tcg       98
Gln Gln Gln Pro Gln Gln Gln Lys Gln Gln Gln Arg Asp Gln Asp Ser
    15                  20                  25

gtc gaa gca tgg ctg gac gat cac tgg gac ttt acc ttc tca tac ttt      146
Val Glu Ala Trp Leu Asp Asp His Trp Asp Phe Thr Phe Ser Tyr Phe
30                  35                  40                  45

gtt aga aaa gcc acc aga gaa atg gtc aat gca tgg ttt gct gag aga      194
Val Arg Lys Ala Thr Arg Glu Met Val Asn Ala Trp Phe Ala Glu Arg
                50                  55                  60

gtt cac acc atc cct gtg tgc aag gaa ggt atc aga ggc cac acc gaa      242
Val His Thr Ile Pro Val Cys Lys Glu Gly Ile Arg Gly His Thr Glu
            65                  70                  75

tct tgc tct tgt ccc ttg cag cag agt cct cgt gca gat aac agt gtc      290
Ser Cys Ser Cys Pro Leu Gln Gln Ser Pro Arg Ala Asp Asn Ser Val
        80                  85                  90

cct gga aca cca acc agg aaa atc tct gcc tct gaa ttt gac cgg cct      338
Pro Gly Thr Pro Thr Arg Lys Ile Ser Ala Ser Glu Phe Asp Arg Pro
    95                  100                 105

ctt aga ccc att gtt gtc aag gat tct gag gga act gtg agc ttc ctc      386
Leu Arg Pro Ile Val Val Lys Asp Ser Glu Gly Thr Val Ser Phe Leu
110                 115                 120                 125

tct gac tca gaa aag aag gaa cag atg cct cta acc cct cca agg ttt      434
Ser Asp Ser Glu Lys Lys Glu Gln Met Pro Leu Thr Pro Pro Arg Phe
                130                 135                 140

gat cat gat gaa ggg gac cag tgc tca aga ctc ttg gaa tta gtg aag      482
Asp His Asp Glu Gly Asp Gln Cys Ser Arg Leu Leu Glu Leu Val Lys
            145                 150                 155

gat att tct agt cat ttg gat gtc aca gcc tta tgt cac aaa att ttc      530
Asp Ile Ser Ser His Leu Asp Val Thr Ala Leu Cys His Lys Ile Phe
        160                 165                 170

ttg cat atc cat gga ctg ata tct gct gac cgc tat tcc ctg ttc ctt      578
Leu His Ile His Gly Leu Ile Ser Ala Asp Arg Tyr Ser Leu Phe Leu
    175                 180                 185

gtc tgt gaa gac agc tcc aat gac aag ttt ctt atc agc cgc ctc ttt      626
Val Cys Glu Asp Ser Ser Asn Asp Lys Phe Leu Ile Ser Arg Leu Phe
190                 195                 200                 205
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gat gtt gct gaa ggt tca aca ctg gaa gaa gtt tca aat aac tgt atc      674
Asp Val Ala Glu Gly Ser Thr Leu Glu Glu Val Ser Asn Asn Cys Ile
                210                 215                 220

cgc tta gaa tgg aac aaa ggc att gtg gga cat gtg gca gcg ctt ggt      722
Arg Leu Glu Trp Asn Lys Gly Ile Val Gly His Val Ala Ala Leu Gly
            225                 230                 235

gag ccc ttg aac atc aaa gat gca tat gag gat cct cgg ttc aat gca      770
Glu Pro Leu Asn Ile Lys Asp Ala Tyr Glu Asp Pro Arg Phe Asn Ala
        240                 245                 250

gaa gtt gac caa att aca ggc tac aag aca caa agc att ctt tgt atg      818
Glu Val Asp Gln Ile Thr Gly Tyr Lys Thr Gln Ser Ile Leu Cys Met
    255                 260                 265

cca att aag aat cat agg gaa gag gtt gtt ggt gta gcc cag gcc atc      866
Pro Ile Lys Asn His Arg Glu Glu Val Val Gly Val Ala Gln Ala Ile
270                 275                 280                 285

aac aag aaa tca gga aac ggt ggg aca ttt act gaa aaa gat gaa aag      914
Asn Lys Lys Ser Gly Asn Gly Gly Thr Phe Thr Glu Lys Asp Glu Lys
                290                 295                 300

gac ttt gct gct tat ttg gca ttt tgt ggt att gtt ctt cat aat gct      962
Asp Phe Ala Ala Tyr Leu Ala Phe Cys Gly Ile Val Leu His Asn Ala
            305                 310                 315

cag ctc tat gag act tca ctg ctg gag aac aag aga aat cag gtg ctg     1010
Gln Leu Tyr Glu Thr Ser Leu Leu Glu Asn Lys Arg Asn Gln Val Leu
        320                 325                 330

ctt gac ctt gct agt tta att ttt gaa gaa caa caa tca tta gaa gta     1058
Leu Asp Leu Ala Ser Leu Ile Phe Glu Glu Gln Gln Ser Leu Glu Val
    335                 340                 345

att ttg aag aaa ata gct gcc act att atc tct ttc atg caa gtg cag     1106
Ile Leu Lys Lys Ile Ala Ala Thr Ile Ile Ser Phe Met Gln Val Gln
350                 355                 360                 365

aaa tgc acc att ttc ata gtg gat gaa gat tgc tcc gat tct ttt tct     1154
Lys Cys Thr Ile Phe Ile Val Asp Glu Asp Cys Ser Asp Ser Phe Ser
                370                 375                 380

agt gtg ttt cac atg gag tgt gag gaa tta gaa aaa tca tct gat aca     1202
Ser Val Phe His Met Glu Cys Glu Glu Leu Glu Lys Ser Ser Asp Thr
            385                 390                 395

tta aca agg gaa cat gat gca aac aaa atc aat tac atg tat gct cag     1250
Leu Thr Arg Glu His Asp Ala Asn Lys Ile Asn Tyr Met Tyr Ala Gln
        400                 405                 410

tat gtc aaa aat act atg gaa cca ctt aat atc cca gat gtc agt aag     1298
Tyr Val Lys Asn Thr Met Glu Pro Leu Asn Ile Pro Asp Val Ser Lys
    415                 420                 425

gat aaa aga ttt ccc tgg aca act gaa aat aca gga aat gta aac cag     1346
Asp Lys Arg Phe Pro Trp Thr Thr Glu Asn Thr Gly Asn Val Asn Gln
430                 435                 440                 445

cag tgc att aga agt ttg ctt tgt aca cct ata aaa aat gga aag aag     1394
Gln Cys Ile Arg Ser Leu Leu Cys Thr Pro Ile Lys Asn Gly Lys Lys
                450                 455                 460

aat aaa gtt ata ggg gtt tgc caa ctt gtt aat aag atg gag gag aat     1442
Asn Lys Val Ile Gly Val Cys Gln Leu Val Asn Lys Met Glu Glu Asn
            465                 470                 475

act ggc aag gtt aag cct ttc aac cga aat gac gaa cag ttt ctg gaa     1490
Thr Gly Lys Val Lys Pro Phe Asn Arg Asn Asp Glu Gln Phe Leu Glu
        480                 485                 490

gct ttt gtc atc ttt tgt ggc ttg ggg atc cag aac acg cag atg tat     1538
Ala Phe Val Ile Phe Cys Gly Leu Gly Ile Gln Asn Thr Gln Met Tyr
    495                 500                 505
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gaa gca gtg gag aga gcc atg gcc aag caa atg gtc aca ttg gag gtt     1586
Glu Ala Val Glu Arg Ala Met Ala Lys Gln Met Val Thr Leu Glu Val
510                 515                 520                 525

ctg tcg tat cat gct tca gca gca gag gaa gaa aca aga gag cta cag     1634
Leu Ser Tyr His Ala Ser Ala Ala Glu Glu Glu Thr Arg Glu Leu Gln
                530                 535                 540

tcg tta gcg gct gct gtg gtg cca tct gcc cag acc ctt aaa att act     1682
Ser Leu Ala Ala Ala Val Val Pro Ser Ala Gln Thr Leu Lys Ile Thr
            545                 550                 555

gac ttt agc ttc agt gac ttt gag ctg tct gat ctg gaa aca gca ctg     1730
Asp Phe Ser Phe Ser Asp Phe Glu Leu Ser Asp Leu Glu Thr Ala Leu
        560                 565                 570

tgt aca att cgg atg ttt act gac ctc aac ctt gtg cag aac ttc cag     1778
Cys Thr Ile Arg Met Phe Thr Asp Leu Asn Leu Val Gln Asn Phe Gln
    575                 580                 585

atg aaa cat gag gtt ctt tgc aga tgg att tta agt gtt aag aag aat     1826
Met Lys His Glu Val Leu Cys Arg Trp Ile Leu Ser Val Lys Lys Asn
590                 595                 600                 605

tat cgg aag aat gtt gcc tat cat aat tgg aga cat gcc ttt aat aca     1874
Tyr Arg Lys Asn Val Ala Tyr His Asn Trp Arg His Ala Phe Asn Thr
                610                 615                 620

gct cag tgc atg ttt gct gct cta aaa gca ggc aaa att cag aac aag     1922
Ala Gln Cys Met Phe Ala Ala Leu Lys Ala Gly Lys Ile Gln Asn Lys
            625                 630                 635

ctg act gac ctg gag ata ctt gca ttg ctg att gct gca cta agc cac     1970
Leu Thr Asp Leu Glu Ile Leu Ala Leu Leu Ile Ala Ala Leu Ser His
        640                 645                 650

gat ttg gat cac cgt ggt gtg aat aac tct tac ata cag cga agt gaa     2018
Asp Leu Asp His Arg Gly Val Asn Asn Ser Tyr Ile Gln Arg Ser Glu
    655                 660                 665

cat cca ctt gcc cag ctt tac tgc cat tca atc atg gaa cac cat cat     2066
His Pro Leu Ala Gln Leu Tyr Cys His Ser Ile Met Glu His His His
670                 675                 680                 685

ttt gac cag tgc ctg atg att ctt aat agt cca ggc aat cag att ctc     2114
Phe Asp Gln Cys Leu Met Ile Leu Asn Ser Pro Gly Asn Gln Ile Leu
                690                 695                 700

agt ggc ctc tcc att gaa gaa tat aag acc acg ttg aaa ata atc aag     2162
Ser Gly Leu Ser Ile Glu Glu Tyr Lys Thr Thr Leu Lys Ile Ile Lys
            705                 710                 715

caa gct att tta gct aca gac cta gca ctg tac att aag agg cga gga     2210
Gln Ala Ile Leu Ala Thr Asp Leu Ala Leu Tyr Ile Lys Arg Arg Gly
        720                 725                 730

gaa ttt ttt gaa ctt ata aga aaa aat caa ttc aat ttg gaa gat cct     2258
Glu Phe Phe Glu Leu Ile Arg Lys Asn Gln Phe Asn Leu Glu Asp Pro
    735                 740                 745

cat caa aag gag ttg ttt ttg gca atg ctg atg aca gct tgt gat ctt     2306
His Gln Lys Glu Leu Phe Leu Ala Met Leu Met Thr Ala Cys Asp Leu
750                 755                 760                 765

tct gca att aca aaa ccc tgg cct att caa caa cgg ata gca gaa ctt     2354
Ser Ala Ile Thr Lys Pro Trp Pro Ile Gln Gln Arg Ile Ala Glu Leu
                770                 775                 780

gta gca act gaa ttt ttt gat caa gga gac aga gag aga aaa gaa ctc     2402
Val Ala Thr Glu Phe Phe Asp Gln Gly Asp Arg Glu Arg Lys Glu Leu
            785                 790                 795

aac ata gaa ccc act gat cta atg aac agg gag aag aaa aac aaa atc     2450
Asn Ile Glu Pro Thr Asp Leu Met Asn Arg Glu Lys Lys Asn Lys Ile
        800                 805                 810
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cca agt atg caa gtt ggg ttc ata gat gcc atc tgc ttg caa ctg tat     2498
Pro Ser Met Gln Val Gly Phe Ile Asp Ala Ile Cys Leu Gln Leu Tyr
    815                 820                 825

gag gcc ctg acc cac gtg tca gag gac tgt ttc cct ttg cta gat ggc     2546
Glu Ala Leu Thr His Val Ser Glu Asp Cys Phe Pro Leu Leu Asp Gly
830                 835                 840                 845

tgc aga aag aac agg cag aaa tgg cag gcc ctt gca gaa cag cag gag     2594
Cys Arg Lys Asn Arg Gln Lys Trp Gln Ala Leu Ala Glu Gln Gln Glu
                850                 855                 860

aag atg ctg att aat ggg gaa agc ggc cag gcc aag cgg aac tgactcgag   2645
Lys Met Leu Ile Asn Gly Glu Ser Gly Gln Ala Lys Arg Asn
            865                 870                 875


<210> SEQ ID NO 23
<211> LENGTH: 875
<212> TYPE: PRT
<213> ORGANISM: human cDNA

<400> SEQUENCE: 23

Met Glu Arg Ala Gly Pro Ser Phe Gly Gln Gln Arg Gln Gln Gln Gln
1               5                   10                  15

Pro Gln Gln Gln Lys Gln Gln Gln Arg Asp Gln Asp Ser Val Glu Ala
            20                  25                  30

Trp Leu Asp Asp His Trp Asp Phe Thr Phe Ser Tyr Phe Val Arg Lys
        35                  40                  45

Ala Thr Arg Glu Met Val Asn Ala Trp Phe Ala Glu Arg Val His Thr
    50                  55                  60

Ile Pro Val Cys Lys Glu Gly Ile Arg Gly His Thr Glu Ser Cys Ser
65                  70                  75                  80

Cys Pro Leu Gln Gln Ser Pro Arg Ala Asp Asn Ser Val Pro Gly Thr
                85                  90                  95

Pro Thr Arg Lys Ile Ser Ala Ser Glu Phe Asp Arg Pro Leu Arg Pro
            100                 105                 110

Ile Val Val Lys Asp Ser Glu Gly Thr Val Ser Phe Leu Ser Asp Ser
        115                 120                 125

Glu Lys Lys Glu Gln Met Pro Leu Thr Pro Pro Arg Phe Asp His Asp
    130                 135                 140

Glu Gly Asp Gln Cys Ser Arg Leu Leu Glu Leu Val Lys Asp Ile Ser
145                 150                 155                 160

Ser His Leu Asp Val Thr Ala Leu Cys His Lys Ile Phe Leu His Ile
                165                 170                 175

His Gly Leu Ile Ser Ala Asp Arg Tyr Ser Leu Phe Leu Val Cys Glu
            180                 185                 190

Asp Ser Ser Asn Asp Lys Phe Leu Ile Ser Arg Leu Phe Asp Val Ala
        195                 200                 205

Glu Gly Ser Thr Leu Glu Glu Val Ser Asn Asn Cys Ile Arg Leu Glu
    210                 215                 220

Trp Asn Lys Gly Ile Val Gly His Val Ala Ala Leu Gly Glu Pro Leu
225                 230                 235                 240

Asn Ile Lys Asp Ala Tyr Glu Asp Pro Arg Phe Asn Ala Glu Val Asp
                245                 250                 255

Gln Ile Thr Gly Tyr Lys Thr Gln Ser Ile Leu Cys Met Pro Ile Lys
            260                 265                 270

Asn His Arg Glu Glu Val Val Gly Val Ala Gln Ala Ile Asn Lys Lys
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        275                 280                 285

Ser Gly Asn Gly Gly Thr Phe Thr Glu Lys Asp Glu Lys Asp Phe Ala
    290                 295                 300

Ala Tyr Leu Ala Phe Cys Gly Ile Val Leu His Asn Ala Gln Leu Tyr
305                 310                 315                 320

Glu Thr Ser Leu Leu Glu Asn Lys Arg Asn Gln Val Leu Leu Asp Leu
                325                 330                 335

Ala Ser Leu Ile Phe Glu Glu Gln Gln Ser Leu Glu Val Ile Leu Lys
            340                 345                 350

Lys Ile Ala Ala Thr Ile Ile Ser Phe Met Gln Val Gln Lys Cys Thr
        355                 360                 365

Ile Phe Ile Val Asp Glu Asp Cys Ser Asp Ser Phe Ser Ser Val Phe
    370                 375                 380

His Met Glu Cys Glu Glu Leu Glu Lys Ser Ser Asp Thr Leu Thr Arg
385                 390                 395                 400

Glu His Asp Ala Asn Lys Ile Asn Tyr Met Tyr Ala Gln Tyr Val Lys
                405                 410                 415

Asn Thr Met Glu Pro Leu Asn Ile Pro Asp Val Ser Lys Asp Lys Arg
            420                 425                 430

Phe Pro Trp Thr Thr Glu Asn Thr Gly Asn Val Asn Gln Gln Cys Ile
        435                 440                 445

Arg Ser Leu Leu Cys Thr Pro Ile Lys Asn Gly Lys Lys Asn Lys Val
    450                 455                 460

Ile Gly Val Cys Gln Leu Val Asn Lys Met Glu Glu Asn Thr Gly Lys
465                 470                 475                 480

Val Lys Pro Phe Asn Arg Asn Asp Glu Gln Phe Leu Glu Ala Phe Val
                485                 490                 495

Ile Phe Cys Gly Leu Gly Ile Gln Asn Thr Gln Met Tyr Glu Ala Val
            500                 505                 510

Glu Arg Ala Met Ala Lys Gln Met Val Thr Leu Glu Val Leu Ser Tyr
        515                 520                 525

His Ala Ser Ala Ala Glu Glu Glu Thr Arg Glu Leu Gln Ser Leu Ala
    530                 535                 540

Ala Ala Val Val Pro Ser Ala Gln Thr Leu Lys Ile Thr Asp Phe Ser
545                 550                 555                 560

Phe Ser Asp Phe Glu Leu Ser Asp Leu Glu Thr Ala Leu Cys Thr Ile
                565                 570                 575

Arg Met Phe Thr Asp Leu Asn Leu Val Gln Asn Phe Gln Met Lys His
            580                 585                 590

Glu Val Leu Cys Arg Trp Ile Leu Ser Val Lys Lys Asn Tyr Arg Lys
        595                 600                 605

Asn Val Ala Tyr His Asn Trp Arg His Ala Phe Asn Thr Ala Gln Cys
    610                 615                 620

Met Phe Ala Ala Leu Lys Ala Gly Lys Ile Gln Asn Lys Leu Thr Asp
625                 630                 635                 640

Leu Glu Ile Leu Ala Leu Leu Ile Ala Ala Leu Ser His Asp Leu Asp
                645                 650                 655

His Arg Gly Val Asn Asn Ser Tyr Ile Gln Arg Ser Glu His Pro Leu
            660                 665                 670

Ala Gln Leu Tyr Cys His Ser Ile Met Glu His His His Phe Asp Gln
        675                 680                 685
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Cys Leu Met Ile Leu Asn Ser Pro Gly Asn Gln Ile Leu Ser Gly Leu
    690                 695                 700

Ser Ile Glu Glu Tyr Lys Thr Thr Leu Lys Ile Ile Lys Gln Ala Ile
705                 710                 715                 720

Leu Ala Thr Asp Leu Ala Leu Tyr Ile Lys Arg Arg Gly Glu Phe Phe
                725                 730                 735

Glu Leu Ile Arg Lys Asn Gln Phe Asn Leu Glu Asp Pro His Gln Lys
            740                 745                 750

Glu Leu Phe Leu Ala Met Leu Met Thr Ala Cys Asp Leu Ser Ala Ile
        755                 760                 765

Thr Lys Pro Trp Pro Ile Gln Gln Arg Ile Ala Glu Leu Val Ala Thr
    770                 775                 780

Glu Phe Phe Asp Gln Gly Asp Arg Glu Arg Lys Glu Leu Asn Ile Glu
785                 790                 795                 800

Pro Thr Asp Leu Met Asn Arg Glu Lys Lys Asn Lys Ile Pro Ser Met
                805                 810                 815

Gln Val Gly Phe Ile Asp Ala Ile Cys Leu Gln Leu Tyr Glu Ala Leu
            820                 825                 830

Thr His Val Ser Glu Asp Cys Phe Pro Leu Leu Asp Gly Cys Arg Lys
        835                 840                 845

Asn Arg Gln Lys Trp Gln Ala Leu Ala Glu Gln Gln Glu Lys Met Leu
    850                 855                 860

Ile Asn Gly Glu Ser Gly Gln Ala Lys Arg Asn
865                 870                 875


<210> SEQ ID NO 24
<211> LENGTH: 235
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 24

Phe Gln Met Lys His Glu Val Leu Cys Lys Trp Ile Leu Ser Val Lys
1               5                   10                  15

Lys Asn Tyr Arg Lys Asn Val Ala Tyr His Asn Trp Arg His Ala Phe
            20                  25                  30

Asn Thr Ala Gln Cys Met Phe Ala Ala Leu Lys Ala Gly Lys Ile Gln
        35                  40                  45

Lys Arg Leu Thr Asp Leu Glu Ile Leu Ala Leu Leu Ile Ala Ala Leu
    50                  55                  60

Ser His Asp Leu Asp His Arg Gly Val Asn Asn Ser Tyr Ile Gln Arg
65                  70                  75                  80

Ser Glu His Pro Leu Ala Gln Leu Tyr Cys His Ser Ile Met Glu His
                85                  90                  95

His His Phe Asp Gln Cys Leu Met Ile Leu Asn Ser Pro Gly Asn Gln
            100                 105                 110

Ile Leu Ser Gly Leu Ser Ile Glu Glu Tyr Lys Thr Thr Leu Lys Ile
        115                 120                 125

Ile Lys Gln Ala Ile Leu Ala Thr Asp Leu Ala Leu Tyr Ile Lys Arg
    130                 135                 140

Arg Gly Glu Phe Phe Glu Leu Ile Met Lys Asn Gln Phe Asn Leu Glu
145                 150                 155                 160

Asp Pro His Gln Lys Glu Leu Phe Leu Ala Met Leu Met Thr Ala Cys
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                165                 170                 175

Asp Leu Ser Ala Ile Thr Lys Pro Trp Pro Ile Gln Gln Arg Ile Ala
            180                 185                 190

Glu Leu Val Ala Thr Glu Phe Phe Asp Gln Gly Asp Arg Glu Arg Lys
        195                 200                 205

Glu Leu Asn Ile Glu Pro Ala Asp Leu Met Asn Arg Glu Lys Lys Asn
    210                 215                 220

Lys Ile Pro Ser Met Gln Val Gly Phe Ile Asp
225                 230                 235


<210> SEQ ID NO 25
<211> LENGTH: 244
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 25

Phe His Ile Pro Gln Glu Ala Leu Val Arg Phe Met Tyr Ser Leu Ser
1               5                   10                  15

Lys Gly Tyr Arg Arg Ile Thr Tyr His Asn Trp Arg His Gly Phe Asn
            20                  25                  30

Val Gly Gln Thr Met Phe Ser Leu Leu Val Thr Gly Lys Leu Lys Arg
        35                  40                  45

Tyr Phe Thr Asp Leu Glu Ala Leu Ala Met Val Thr Ala Ala Phe Cys
    50                  55                  60

His Asp Ile Asp His Arg Gly Thr Asn Asn Leu Tyr Gln Met Lys Ser
65                  70                  75                  80

Gln Asn Pro Leu Ala Lys Leu His Gly Ser Ser Ile Leu Glu Arg His
                85                  90                  95

His Leu Glu Phe Gly Lys Thr Leu Leu Arg Asp Glu Ser Leu Asn Ile
            100                 105                 110

Phe Gln Asn Leu Asn Arg Arg Gln His Glu His Ala Ile His Met Met
        115                 120                 125

Asp Ile Ala Ile Ile Ala Thr Asp Leu Ala Leu Tyr Cys Lys Lys Arg
    130                 135                 140

Thr Met Phe Gln Lys Ile Val Asp Gln Ser Lys Thr Tyr Glu Thr Gln
145                 150                 155                 160

Gln Glu Trp Thr Gln Tyr Met Met Leu Asp Gln Thr Arg Lys Glu Ile
                165                 170                 175

Val Met Ala Met Met Met Thr Ala Cys Asp Leu Ser Ala Ile Thr Lys
            180                 185                 190

Pro Trp Glu Val Gln Ser Lys Val Ala Leu Leu Val Ala Ala Glu Phe
        195                 200                 205

Trp Glu Gln Gly Asp Leu Glu Arg Thr Val Leu Gln Gln Asn Pro Ile
    210                 215                 220

Pro Met Met Asp Arg Asn Lys Ala Asp Glu Leu Pro Lys Leu Gln Val
225                 230                 235                 240

Gly Phe Ile Asp


<210> SEQ ID NO 26
<211> LENGTH: 244
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 26
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Phe Gln Ile Pro Gln Glu Val Leu Val Arg Phe Leu Phe Ser Val Ser
1               5                   10                  15

Lys Gly Tyr Arg Arg Ile Thr Tyr His Asn Trp Arg His Gly Phe Asn
            20                  25                  30

Val Ala Gln Thr Met Phe Thr Leu Leu Met Thr Gly Lys Leu Lys Ser
        35                  40                  45

Tyr Tyr Thr Asp Leu Glu Ala Phe Ala Met Val Thr Ala Gly Leu Cys
    50                  55                  60

His Asp Ile Asp His Arg Gly Thr Asn Asn Leu Tyr Gln Met Lys Ser
65                  70                  75                  80

Gln Asn Pro Leu Ala Lys Leu His Gly Ser Ser Ile Leu Glu Arg His
                85                  90                  95

His Leu Glu Phe Gly Lys Phe Leu Leu Ser Glu Glu Thr Leu Asn Ile
            100                 105                 110

Tyr Gln Asn Leu Asn Arg Arg Gln His Glu His Val Ile His Leu Met
        115                 120                 125

Asp Ile Ala Ile Ile Ala Thr Asp Leu Ala Leu Tyr Phe Lys Lys Arg
    130                 135                 140

Thr Met Phe Gln Lys Ile Val Asp Glu Ser Lys Asn Tyr Glu Asp Arg
145                 150                 155                 160

Lys Ser Trp Val Glu Tyr Leu Ser Leu Glu Thr Thr Arg Lys Glu Ile
                165                 170                 175

Val Met Ala Met Met Met Thr Ala Cys Asp Leu Ser Ala Ile Thr Lys
            180                 185                 190

Pro Trp Glu Val Gln Ser Lys Val Ala Leu Leu Val Ala Ala Glu Phe
        195                 200                 205

Trp Glu Gln Gly Asp Leu Glu Arg Thr Val Leu Asp Gln Gln Pro Ile
    210                 215                 220

Pro Met Met Asp Arg Asn Lys Ala Ala Glu Leu Pro Lys Leu Gln Val
225                 230                 235                 240

Gly Phe Ile Asp


<210> SEQ ID NO 27
<211> LENGTH: 244
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 27

Phe Lys Val Pro Val Glu Val Leu Thr Arg Trp Met Thr Tyr Val Arg
1               5                   10                  15

Lys Gly Tyr Arg Ala Val Thr Tyr His Asn Trp Arg His Gly Phe Asn
            20                  25                  30

Val Gly Gln Thr Met Phe Thr Leu Leu Met Thr Gly Arg Leu Lys Lys
        35                  40                  45

Tyr Tyr Thr Asp Leu Glu Ala Phe Ala Met Leu Ala Ala Ala Phe Cys
    50                  55                  60

His Asp Ile Asp His Arg Gly Thr Asn Asn Leu Tyr Gln Met Lys Ser
65                  70                  75                  80

Thr Ser Pro Leu Ala Arg Leu His Gly Ser Ser Ile Leu Glu Arg His
                85                  90                  95

His Leu Glu Tyr Ser Lys Thr Leu Leu Gln Asp Glu Ser Leu Asn Ile
            100                 105                 110
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Phe Gln Asn Leu Asn Lys Arg Gln Tyr Glu Thr Val Ile His Leu Phe
        115                 120                 125

Glu Val Ala Ile Ile Ala Thr Asp Leu Ala Leu Tyr Phe Lys Lys Arg
    130                 135                 140

Thr Met Phe Gln Lys Ile Val Asp Ala Cys Glu Lys Met Glu Thr Glu
145                 150                 155                 160

Glu Glu Ala Ile Lys Tyr Val Thr Ile Asp Pro Thr Lys Lys Glu Ile
                165                 170                 175

Ile Met Ala Met Met Met Thr Ala Cys Asp Leu Ser Ala Ile Thr Lys
            180                 185                 190

Pro Trp Glu Val Gln Ser Gln Val Ala Leu Leu Val Ala Asn Glu Phe
        195                 200                 205

Trp Glu Gln Gly Asp Leu Glu Arg Thr Val Leu Gln Gln Gln Pro Ile
    210                 215                 220

Pro Met Met Asp Arg Asn Lys Lys Asp Glu Leu Pro Lys Leu Gln Val
225                 230                 235                 240

Gly Phe Ile Asp


<210> SEQ ID NO 28
<211> LENGTH: 233
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 28

Tyr Lys Ile Asp Cys Pro Thr Leu Ala Arg Phe Cys Leu Met Val Lys
1               5                   10                  15

Lys Gly Tyr Arg Asp Pro Pro Tyr His Asn Trp Met His Ala Phe Ser
            20                  25                  30

Val Ser His Phe Cys Tyr Leu Leu Tyr Lys Asn Leu Glu Leu Thr Asn
        35                  40                  45

Tyr Leu Glu Asp Met Glu Ile Phe Ala Leu Phe Ile Ser Cys Met Cys
    50                  55                  60

His Asp Leu Asp His Arg Gly Thr Asn Asn Ser Phe Gln Val Ala Ser
65                  70                  75                  80

Lys Ser Val Leu Ala Ala Leu Tyr Ser Ser Glu Gly Ser Val Met Glu
                85                  90                  95

Arg His His Phe Ala Gln Ala Ile Ala Ile Leu Asn Thr His Gly Cys
            100                 105                 110

Asn Ile Phe Asp His Phe Ser Arg Lys Asp Tyr Gln Arg Met Leu Asp
        115                 120                 125

Leu Met Arg Asp Ile Ile Leu Ala Thr Asp Leu Ala His His Leu Arg
    130                 135                 140

Ile Phe Lys Asp Leu Gln Lys Met Ala Glu Val Gly Tyr Asp Arg Thr
145                 150                 155                 160

Asn Lys Gln His His Ser Leu Leu Leu Cys Leu Leu Met Thr Ser Cys
                165                 170                 175

Asp Leu Ser Asp Gln Thr Lys Gly Trp Lys Thr Thr Arg Lys Ile Ala
            180                 185                 190

Glu Leu Ile Tyr Lys Glu Phe Phe Ser Gln Gly Asp Leu Glu Lys Ala
        195                 200                 205

Met Gly Asn Arg Pro Met Glu Met Met Asp Arg Glu Lys Ala Tyr Ile
    210                 215                 220
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Pro Glu Leu Gln Ile Ser Phe Met Glu
225 230

<210> SEQ ID NO 29
<211> LENGTH: 230
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 29

Phe Lys Ile Pro Val Ser Cys Leu Ile Ala Phe Ala Glu Ala Leu Glu
1 5 10 15

Val Gly Tyr Ser Lys Tyr Lys Asn Pro Tyr His Asn Leu Ile His Ala
20 25 30

Ala Asp Val Thr Gln Thr Val His Tyr Ile Met Leu His Thr Gly Ile
35 40 45

Met His Trp Leu Thr Glu Leu Glu Ile Leu Ala Met Val Phe Ala Ala
50 55 60

Ala Ile His Asp Tyr Glu His Ile Gly Thr Thr Asn Asn Phe His Ile
65 70 75 80

Gln Thr Arg Ser Asp Val Ala Ile Leu Tyr Asn Asp Arg Ser Val Leu
85 90 95

Glu Asn His His Val Ser Ala Ala Tyr Arg Leu Met Gln Glu Glu Glu
100 105 110

Met Asn Val Leu Ile Asn Leu Ser Lys Asp Asp Trp Arg Asp Leu Arg
115 120 125

Asn Leu Val Ile Glu Met Val Leu Ser Thr Asp Met Ser Gly His Phe
130 135 140

Gln Gln Ile Lys Asn Ile Arg Asn Ser Leu Gln Gln Pro Glu Gly Leu
145 150 155 160

Asp Lys Ala Lys Thr Met Ser Leu Ile Leu His Ala Ala Asp Ile Ser
165 170 175

His Pro Ala Lys Ser Trp Lys Leu His His Arg Trp Thr Met Ala Leu
180 185 190

Met Glu Glu Phe Phe Leu Gln Gly Asp Lys Glu Ala Glu Leu Gly Leu
195 200 205

Pro Phe Ser Pro Leu Cys Asp Arg Lys Ser Thr Met Val Ala Gln Ser
210 215 220

Gln Ile Gly Phe Ile Asp
225 230

<210> SEQ ID NO 30
<211> LENGTH: 230
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 30

Phe Lys Ile Pro Thr Val Phe Leu Met Thr Phe Leu Asp Ala Leu Glu
1 5 10 15

Thr Gly Tyr Gly Lys Tyr Lys Asn Pro Tyr His Asn Gln Ile His Ala
20 25 30

Ala Asp Val Thr Gln Thr Val His Cys Phe Leu Leu Arg Thr Gly Met
35 40 45

Val His Cys Leu Ser Glu Ile Glu Val Leu Ala Ile Ile Phe Ala Ala
50 55 60

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```
Ala Ile His Asp Tyr Glu His Ile Gly Thr Thr Asn Ser Phe His Ile
65                  70                  75                  80

Gln Thr Lys Ser Glu Gln Ala Ile Leu Tyr Asn Asp Arg Ser Val Leu
                85                  90                  95

Glu Asn His His Ile Ser Ser Val Phe Arg Met Met Gln Asp Asp Glu
            100                 105                 110

Met Asn Ile Phe Ile Asn Leu Thr Lys Asp Glu Phe Val Glu Leu Arg
        115                 120                 125

Ala Leu Val Ile Glu Met Val Leu Ala Thr Asp Met Ser Cys His Phe
    130                 135                 140

Gln Gln Val Lys Ser Met Lys Thr Ala Leu Gln Gln Leu Glu Arg Ile
145                 150                 155                 160

Asp Lys Ser Lys Ala Leu Ser Leu Leu Leu His Ala Ala Asp Ile Ser
                165                 170                 175

His Pro Thr Lys Gln Trp Ser Val His Ser Arg Trp Thr Lys Ala Leu
            180                 185                 190

Met Glu Glu Phe Phe Arg Gln Gly Asp Lys Glu Ala Glu Leu Gly Leu
        195                 200                 205

Pro Phe Ser Pro Leu Cys Asp Arg Thr Ser Thr Leu Val Ala Gln Ser
    210                 215                 220

Gln Ile Gly Phe Ile Asp
225                 230


<210> SEQ ID NO 31
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: rat

<400> SEQUENCE: 31

Phe Gln Ile Pro Ala Asp Thr Leu Leu Arg Tyr Leu Leu Thr Leu Glu
1               5                   10                  15

Gly His Tyr His Ser Asn Val Ala Tyr His Asn Ser Ile His Ala Ala
            20                  25                  30

Asp Val Val Gln Ser Ala His Val Leu Leu Gly Thr Pro Ala Leu Glu
        35                  40                  45

Ala Val Phe Thr Asp Leu Glu Val Leu Ala Ala Ile Phe Ala Cys Ala
    50                  55                  60

Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln Phe Leu Ile Asn
65                  70                  75                  80

Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser Ser Val Leu Glu
                85                  90                  95

Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln Gly Glu Asn Cys
            100                 105                 110

Asp Ile Phe Gln Asn Leu Ser Thr Lys Gln Lys Leu Ser Leu Arg Arg
        115                 120                 125

Met Val Ile Asp Met Val Leu Ala Thr Asp Met Ser Lys His Met Ser
    130                 135                 140

Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys Lys Val Thr Ser
145                 150                 155                 160

Leu Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln Val Leu
                165                 170                 175

Gln Ser Leu Val His Cys Ala Asp Leu Ser Asn Pro Ala Lys Pro Leu
```

-continued

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            180                 185                 190

Pro Leu Tyr Arg Gln Trp Thr Glu Arg Ile Met Ala Glu Phe Phe Gln
        195                 200                 205

Gln Gly Asp Arg Glu Arg Glu Ser Gly Leu Asp Ile Ser Pro Met Cys
    210                 215                 220

Asp Lys His Thr Ala Ser Val Glu Lys Ser Gln Val Gly Phe Ile Asp
225                 230                 235                 240


<210> SEQ ID NO 32
<211> LENGTH: 239
<212> TYPE: PRT
<213> ORGANISM: drosophila

<400> SEQUENCE: 32

Met Ile Pro Pro Lys Thr Phe Leu Asn Phe Met Ser Thr Leu Glu Asp
1               5                   10                  15

His Tyr Val Lys Asp Asn Pro Phe His Asn Ser Leu His Ala Ala Asp
            20                  25                  30

Val Thr Gln Ser Thr Asn Val Leu Leu Asn Thr Pro Ala Leu Glu Gly
        35                  40                  45

Val Phe Thr Pro Leu Glu Val Gly Gly Ala Leu Phe Ala Ala Cys Ile
    50                  55                  60

His Asp Val Asp His Pro Gly Leu Thr Asn Gln Phe Leu Val Asn Ser
65                  70                  75                  80

Ser Ser Glu Leu Ala Leu Met Tyr Asn Asp Glu Ser Val Leu Glu Asn
                85                  90                  95

His His Leu Ala Val Ala Phe Lys Leu Leu Gln Asn Gln Gly Cys Asp
            100                 105                 110

Ile Phe Cys Asn Met Gln Lys Lys Gln Arg Gln Thr Leu Arg Lys Met
        115                 120                 125

Val Ile Asp Ile Val Leu Ser Thr Asp Met Ser Lys His Met Ser Leu
    130                 135                 140

Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys Lys Val Ala Gly Ser
145                 150                 155                 160

Gly Val Leu Leu Leu Asp Asn Tyr Thr Asp Arg Ile Gln Val Leu Glu
                165                 170                 175

Asn Leu Val His Cys Ala Asp Leu Ser Asn Pro Thr Lys Pro Leu Pro
            180                 185                 190

Leu Tyr Lys Arg Trp Val Ala Leu Leu Met Glu Glu Phe Phe Leu Gln
        195                 200                 205

Gly Asp Lys Glu Arg Glu Ser Gly Met Asp Ile Ser Pro Met Cys Asp
    210                 215                 220

Arg His Asn Ala Thr Ile Glu Lys Ser Gln Val Gly Phe Ile Asp
225                 230                 235


<210> SEQ ID NO 33
<211> LENGTH: 385
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 33

Leu Leu Glu Leu Val Lys Asp Ile Ser Ser His Leu Asp Val Thr Ala
1               5                   10                  15

Leu Cys His Lys Ile Phe Leu His Ile His Gly Leu Ile Ser Ala Asp
```

-continued

```
            20                  25                  30

Arg Tyr Ser Leu Phe Leu Val Cys Glu Asp Ser Ser Asn Asp Lys Phe
        35                  40                  45

Leu Ile Ser Arg Leu Phe Asp Val Ala Glu Gly Ser Thr Leu Glu Glu
    50                  55                  60

Ala Ser Asn Asn Cys Ile Arg Leu Glu Trp Asn Lys Gly Ile Val Gly
65                  70                  75                  80

His Val Ala Ala Phe Gly Glu Pro Leu Asn Ile Lys Asp Ala Tyr Glu
                85                  90                  95

Asp Pro Arg Phe Asn Ala Glu Val Asp Gln Ile Thr Gly Tyr Lys Thr
            100                 105                 110

Gln Ser Ile Leu Cys Met Pro Ile Lys Asn His Arg Glu Glu Val Val
        115                 120                 125

Gly Val Ala Gln Ala Ile Asn Lys Lys Ser Gly Asn Gly Gly Thr Phe
    130                 135                 140

Thr Glu Lys Asp Glu Lys Asp Phe Ala Ala Tyr Leu Ala Phe Cys Gly
145                 150                 155                 160

Ile Val Leu His Asn Ala Gln Leu Tyr Glu Thr Ser Leu Leu Glu Asn
                165                 170                 175

Lys Arg Asn Gln Val Leu Leu Asp Leu Ala Ser Leu Ile Phe Glu Glu
            180                 185                 190

Gln Gln Ser Leu Glu Val Ile Leu Lys Lys Ile Ala Ala Thr Ile Ile
        195                 200                 205

Ser Phe Met Gln Val Gln Lys Cys Thr Ile Phe Ile Val Asp Glu Asp
    210                 215                 220

Cys Ser Asp Ser Phe Ser Ser Val Phe His Met Glu Cys Glu Glu Leu
225                 230                 235                 240

Glu Lys Ser Ser Asp Thr Leu Thr Arg Glu Arg Asp Ala Asn Arg Ile
                245                 250                 255

Asn Tyr Met Tyr Ala Gln Tyr Val Lys Asn Thr Met Glu Pro Leu Asn
            260                 265                 270

Ile Pro Asp Val Ser Lys Asp Lys Arg Phe Pro Trp Thr Asn Glu Asn
        275                 280                 285

Met Gly Asn Ile Asn Gln Gln Cys Ile Arg Ser Leu Leu Cys Thr Pro
    290                 295                 300

Ile Lys Asn Gly Lys Lys Asn Lys Val Ile Gly Val Cys Gln Leu Val
305                 310                 315                 320

Asn Lys Met Glu Glu Thr Thr Gly Lys Val Lys Ala Phe Asn Arg Asn
                325                 330                 335

Asp Glu Gln Phe Leu Glu Ala Phe Val Ile Phe Cys Gly Leu Gly Ile
            340                 345                 350

Gln Asn Thr Gln Met Tyr Glu Ala Val Glu Arg Ala Met Ala Lys Gln
        355                 360                 365

Met Val Thr Leu Glu Val Leu Ser Tyr His Ala Ser Ala Ala Glu Glu
    370                 375                 380

Glu
385


<210> SEQ ID NO 34
<211> LENGTH: 353
<212> TYPE: PRT
<213> ORGANISM: bovine
```

-continued

<400> SEQUENCE: 34

Ile Leu Gln Leu Cys Gly Glu Leu Tyr Asp Leu Asp Ala Ser Ser Leu
1 5 10 15

Gln Leu Lys Val Leu Gln Tyr Leu Gln Gln Glu Thr Gln Ala Ser Arg
20 25 30

Cys Cys Leu Leu Leu Val Ser Glu Asp Asn Leu Gln Leu Ser Cys Lys
35 40 45

Val Ile Gly Asp Lys Val Leu Glu Glu Glu Ile Ser Phe Pro Leu Thr
50 55 60

Thr Gly Arg Leu Gly Gln Val Val Glu Asp Lys Lys Ser Ile Gln Leu
65 70 75 80

Lys Asp Leu Thr Ser Glu Asp Met Gln Gln Leu Gln Ser Met Leu Gly
85 90 95

Cys Glu Val Gln Ala Met Leu Cys Val Pro Val Ile Ser Arg Ala Thr
100 105 110

Asp Gln Val Val Ala Leu Ala Cys Ala Phe Asn Lys Leu Gly Gly Asp
115 120 125

Leu Phe Thr Asp Gln Asp Glu His Val Ile Gln His Cys Phe His Tyr
130 135 140

Thr Ser Thr Val Leu Thr Ser Thr Leu Ala Phe Gln Lys Glu Gln Lys
145 150 155 160

Leu Lys Cys Glu Cys Gln Ala Leu Leu Gln Val Ala Lys Asn Leu Phe
165 170 175

Thr His Leu Asp Asp Val Ser Val Leu Leu Gln Glu Ile Ile Thr Glu
180 185 190

Ala Arg Asn Leu Ser Asn Ala Glu Ile Cys Ser Val Phe Leu Ile Asp
195 200 205

Gln Asn Glu Leu Val Ala Lys Val Phe Asp Gly Gly Val Leu Glu Asp
210 215 220

Glu Ser Tyr Glu Ile Arg Ile Pro Ala Asp Gln Gly Ile Ala Gly His
225 230 235 240

Val Ala Thr Thr Gly Gln Ile Leu Asn Ile Pro Asp Ala Tyr Ala His
245 250 255

Pro Leu Phe Tyr Arg Gly Val Asp Asp Ser Thr Gly Arg Phe Thr Arg
260 265 270

Asn Ile Leu Cys Phe Pro Ile Lys Asn Glu Asn Gln Glu Val Ile Gly
275 280 285

Val Ala Glu Leu Val Asn Lys Ile Asn Gly Pro Trp Phe Ser Lys Phe
290 295 300

Asp Glu Asp Leu Ala Thr Ala Phe Ser Ile Tyr Cys Gly Ile Ser Ile
305 310 315 320

Ala His Ser Leu Leu Tyr Lys Lys Val Asn Glu Ala Gln Tyr Arg Ser
325 330 335

His Leu Ala Asn Glu Met Met Met Tyr His Met Lys Val Ser Asp Asp
340 345 350

Glu

<210> SEQ ID NO 35
<211> LENGTH: 402
<212> TYPE: PRT
<213> ORGANISM: bovine

-continued

<400> SEQUENCE: 35

```
Leu Leu Glu Val Leu Leu Glu Glu Ala Gly Ser Val Glu Leu Ala Ala
1               5                  10                  15

His Arg Ala Leu Gln Arg Leu Ala Gln Leu Leu Gln Ala Asp Arg Cys
            20                  25                  30

Ser Met Phe Leu Cys Arg Ala Arg Asn Gly Thr Pro Glu Val Ala Ser
        35                  40                  45

Lys Leu Leu Asp Val Thr Pro Thr Ser Lys Phe Glu Asp Asn Leu Val
    50                  55                  60

Val Pro Asp Arg Glu Ala Val Phe Pro Leu Asp Val Gly Ile Val Gly
65                  70                  75                  80

Trp Val Ala His Thr Lys Lys Thr Phe Asn Val Pro Asp Val Lys Lys
                85                  90                  95

Asn Ser His Phe Ser Asp Phe Met Asp Lys Gln Thr Gly Tyr Val Thr
            100                 105                 110

Arg Asn Leu Leu Ala Thr Pro Ile Val Met Gly Lys Glu Val Leu Ala
        115                 120                 125

Val Phe Met Ala Val Asn Lys Val Asp Ala Ser Glu Phe Ser Lys Gln
    130                 135                 140

Asp Glu Glu Val Phe Ser Lys Tyr Leu Ser Phe Val Ser Ile Ile Leu
145                 150                 155                 160

Lys Leu His His Thr Asn Tyr Leu Tyr Asn Ile Glu Ser Arg Arg Ser
                165                 170                 175

Gln Ile Leu Met Trp Ser Ala Asn Lys Val Phe Glu Glu Leu Thr Asp
            180                 185                 190

Val Glu Arg Gln Phe His Lys Ala Leu Tyr Thr Val Arg Thr Tyr Leu
        195                 200                 205

Asn Cys Glu Arg Tyr Ser Ile Gly Leu Leu Asp Met Thr Lys Glu Lys
    210                 215                 220

Glu Phe Tyr Asp Glu Trp Pro Val Lys Pro Gly Glu Val Glu Pro Tyr
225                 230                 235                 240

Lys Gly Pro Lys Thr Pro Asp Gly Arg Glu Val Ile Phe Tyr Lys Ile
                245                 250                 255

Ile Asp Tyr Ile Leu His Gly Lys Glu Glu Ile Lys Val Ile Pro Thr
            260                 265                 270

Pro Pro Met Asp His Trp Thr Leu Ile Ser Gly Leu Pro Thr Tyr Val
        275                 280                 285

Ala Glu Asn Gly Phe Ile Cys Asn Met Leu Asn Ala Pro Ala Asp Glu
    290                 295                 300

Tyr Phe Thr Phe Gln Lys Gly Pro Val Asp Glu Thr Gly Trp Val Ile
305                 310                 315                 320

Lys Asn Val Leu Ser Leu Pro Ile Val Asn Lys Lys Glu Asp Ile Val
                325                 330                 335

Gly Val Ala Thr Phe Tyr Asn Arg Lys Asp Gly Lys Pro Phe Asp Glu
            340                 345                 350

Tyr Asp Glu His Ile Ala Glu Thr Leu Thr Gln Phe Leu Gly Trp Ser
        355                 360                 365

Leu Leu Asn Thr Asp Thr Tyr Glu Lys Met Asn Lys Leu Glu Asn Arg
    370                 375                 380

Lys Asp Ile Ala Gln Glu Met Leu Met Asn His Thr Lys Ala Thr Pro
```

-continued

385 390 395 400

Asp Glu

<210> SEQ ID NO 36
<211> LENGTH: 404
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 36

Leu Phe Glu Leu Val Gln Asp Met Gln Glu Asn Val Asn Met Glu Arg
1 5 10 15

Val Val Phe Lys Ile Leu Arg Arg Leu Cys Ser Ile Leu His Ala Asp
20 25 30

Arg Cys Ser Leu Phe Met Tyr Arg Gln Arg Asn Gly Val Ala Glu Leu
35 40 45

Ala Thr Arg Leu Phe Ser Val Gln Pro Asp Ser Val Leu Glu Asp Cys
50 55 60

Leu Val Pro Pro Asp Ser Glu Ile Val Phe Pro Leu Asp Ile Gly Val
65 70 75 80

Val Gly His Val Ala Gln Thr Lys Lys Met Val Asn Val Gln Asp Val
85 90 95

Met Glu Cys Pro His Phe Ser Ser Phe Ala Asp Glu Leu Thr Asp Tyr
100 105 110

Val Thr Arg Asn Ile Leu Ala Thr Pro Ile Met Asn Gly Lys Asp Val
115 120 125

Val Ala Val Ile Met Ala Val Asn Lys Leu Asp Gly Pro Cys Phe Thr
130 135 140

Ser Glu Asp Glu Asp Val Phe Leu Lys Tyr Leu Asn Phe Gly Thr Leu
145 150 155 160

Asn Leu Lys Ile Tyr His Tyr Ser Tyr Leu His Asn Cys Glu Thr Arg
165 170 175

Arg Gly Gln Val Leu Leu Trp Ser Ala Asn Lys Val Phe Glu Glu Leu
180 185 190

Thr Asp Ile Glu Arg Gln Phe His Lys Ala Phe Tyr Thr Val Arg Ala
195 200 205

Tyr Leu Asn Cys Asp Arg Tyr Ser Val Gly Leu Leu Asp Met Thr Lys
210 215 220

Glu Lys Glu Phe Phe Asp Val Trp Pro Val Leu Met Gly Glu Ala Gln
225 230 235 240

Ala Tyr Ser Gly Pro Arg Thr Pro Asp Gly Arg Glu Ile Leu Phe Tyr
245 250 255

Lys Val Ile Asp Tyr Ile Leu His Gly Lys Glu Asp Ile Lys Val Ile
260 265 270

Pro Ser Pro Pro Ala Asp His Trp Ala Leu Ala Ser Gly Leu Pro Thr
275 280 285

Tyr Val Ala Glu Ser Gly Phe Ile Cys Asn Ile Met Asn Ala Pro Ala
290 295 300

Asp Glu Met Phe Asn Phe Gln Glu Gly Pro Leu Asp Asp Ser Gly Trp
305 310 315 320

Ile Val Lys Asn Val Leu Ser Met Pro Ile Val Asn Lys Lys Glu Glu
325 330 335

Ile Val Gly Val Ala Thr Phe Tyr Asn Arg Lys Asp Gly Lys Pro Phe

-continued

```
            340                 345                 350

Asp Glu Gln Asp Glu Val Leu Met Glu Ser Leu Thr Gln Phe Leu Gly
        355                 360                 365

Trp Ser Val Leu Asn Thr Asp Thr Tyr Asp Lys Met Asn Lys Leu Glu
    370                 375                 380

Asn Arg Lys Asp Ile Ala Gln Asp Met Val Leu Tyr His Val Arg Cys
385                 390                 395                 400

Asp Arg Glu Glu


<210> SEQ ID NO 37
<211> LENGTH: 401
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 37

Leu Leu Arg Asp Phe Gln Asp Asn Leu Gln Ala Glu Lys Cys Val Phe
1               5                   10                  15

Asn Val Met Lys Lys Leu Cys Phe Leu Leu Gln Ala Asp Arg Met Ser
            20                  25                  30

Leu Phe Met Tyr Arg Ala Arg Asn Gly Ile Ala Glu Leu Ala Thr Arg
        35                  40                  45

Leu Phe Asn Val His Lys Asp Ala Val Leu Glu Glu Cys Leu Val Ala
    50                  55                  60

Pro Asp Ser Glu Ile Val Phe Pro Leu Asp Met Gly Val Val Gly His
65                  70                  75                  80

Val Ala Leu Ser Lys Lys Ile Val Asn Val Pro Asn Thr Glu Glu Asp
                85                  90                  95

Glu His Phe Cys Asp Phe Val Asp Thr Leu Thr Glu Tyr Gln Thr Lys
            100                 105                 110

Asn Ile Leu Ala Ser Pro Ile Met Asn Gly Lys Asp Val Val Ala Ile
        115                 120                 125

Ile Met Ala Val Asn Lys Val Asp Gly Pro His Phe Thr Glu Asn Asp
    130                 135                 140

Glu Glu Ile Leu Leu Lys Tyr Leu Asn Phe Ala Asn Leu Ile Met Lys
145                 150                 155                 160

Val Phe His Leu Ser Tyr Leu His Asn Cys Glu Thr Arg Arg Gly Gln
                165                 170                 175

Ile Leu Leu Trp Ser Gly Ser Lys Val Phe Glu Glu Leu Thr Asp Ile
            180                 185                 190

Glu Arg Gln Phe His Lys Ala Leu Tyr Thr Val Arg Ala Phe Leu Asn
        195                 200                 205

Cys Asp Arg Tyr Ser Val Gly Leu Leu Asp Met Thr Lys Gln Lys Glu
    210                 215                 220

Phe Phe Asp Val Trp Pro Val Leu Met Gly Glu Ala Pro Pro Tyr Ala
225                 230                 235                 240

Gly Pro Arg Thr Pro Asp Gly Arg Glu Ile Asn Phe Tyr Lys Val Ile
                245                 250                 255

Asp Tyr Ile Leu His Gly Lys Glu Asp Ile Lys Val Ile Pro Asn Pro
            260                 265                 270

Pro Pro Asp His Trp Ala Leu Val Ser Gly Leu Pro Thr Tyr Val Ala
        275                 280                 285

Gln Asn Gly Leu Ile Cys Asn Ile Met Asn Ala Pro Ser Glu Asp Phe
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    290                 295                 300

Phe Ala Phe Gln Lys Glu Pro Leu Asp Glu Ser Gly Trp Met Ile Lys
305                 310                 315                 320

Asn Val Leu Ser Met Pro Ile Val Asn Lys Lys Glu Glu Ile Val Gly
                325                 330                 335

Val Ala Thr Phe Tyr Asn Arg Lys Asp Gly Lys Pro Phe Asp Glu Met
            340                 345                 350

Asp Glu Thr Leu Met Glu Ser Leu Ala Gln Phe Leu Gly Trp Ser Val
        355                 360                 365

Leu Asn Pro Asp Thr Tyr Glu Leu Met Asn Lys Leu Glu Asn Arg Lys
    370                 375                 380

Asp Ile Phe Gln Asp Met Val Lys Tyr His Val Lys Cys Asp Asn Glu
385                 390                 395                 400

Glu
```

<210> SEQ ID NO 38
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 38

```
Glu Pro Leu Asn Ile Lys Asp Ala Tyr Glu Asp Pro Arg Phe Asn Ala
1               5                   10                  15

Glu Val Asp Gln Ile Thr Gly Tyr Lys Thr Gln Ser Ile Leu Cys Met
            20                  25                  30

Pro Ile Lys Met His Arg Glu Glu Val Val Gly Val Ala Gln Ala Ile
        35                  40                  45

Asn Lys Lys Ser Gly Asn Gly Gly Thr Phe Thr Glu Lys Asp Glu Lys
    50                  55                  60

Asp Phe Ala Ala Tyr Leu Ala Phe Cys Gly Ile Val Leu His Met Ala
65                  70                  75                  80

Gln Leu Tyr Glu
```

<210> SEQ ID NO 39
<211> LENGTH: 81
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 39

```
Lys Ile Val Asn Val Pro Asn Thr Glu Glu Asp Glu His Phe Cys Asp
1               5                   10                  15

Phe Val Asp Thr Leu Thr Glu Tyr Gln Thr Lys Asn Ile Leu Ala Ser
            20                  25                  30

Pro Ile Met Asn Gly Lys Asp Val Val Ala Ile Ile Met Ala Val Asn
        35                  40                  45

Lys Val Asp Gly Pro His Phe Thr Glu Asn Asp Glu Glu Ile Leu Leu
    50                  55                  60

Lys Tyr Leu Asn Phe Ala Asn Leu Ile Met Lys Val Phe His Leu Ser
65                  70                  75                  80

Tyr
```

<210> SEQ ID NO 40
<211> LENGTH: 81
<212> TYPE: PRT

-continued

<213> ORGANISM: bovine

<400> SEQUENCE: 40

Lys Met Val Asn Val Gln Asp Val Met Glu Cys Pro His Phe Ser Ser
1 5 10 15

Phe Ala Asp Glu Leu Thr Asp Tyr Val Thr Arg Asn Ile Leu Ala Thr
20 25 30

Pro Ile Met Asn Gly Lys Asp Val Val Ala Val Ile Met Ala Val Asn
35 40 45

Lys Leu Asp Gly Pro Cys Phe Thr Ser Glu Asp Glu Asp Val Phe Leu
50 55 60

Lys Tyr Leu Asn Phe Gly Thr Leu Asn Leu Lys Ile Tyr His Leu Ser
65 70 75 80

Tyr

<210> SEQ ID NO 41
<211> LENGTH: 81
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 41

Lys Thr Phe Asn Val Pro Asp Val Lys Lys Asn Ser His Phe Ser Asp
1 5 10 15

Phe Met Asp Lys Gln Thr Gly Tyr Val Thr Arg Asn Ile Leu Ala Thr
20 25 30

Pro Ile Val Met Gly Lys Glu Val Leu Ala Val Phe Met Ala Val Asn
35 40 45

Lys Val Asp Ala Ser Glu Phe Ser Lys Gln Asp Glu Glu Val Phe Ser
50 55 60

Lys Tyr Leu Ser Phe Val Ser Ile Ile Leu Lys Leu His His Thr Asn
65 70 75 80

Tyr

<210> SEQ ID NO 42
<211> LENGTH: 81
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 42

Lys Ser Ile Gln Leu Lys Asp Leu Thr Ser Glu Asp Met Gln Gln Leu
1 5 10 15

Gln Ser Met Leu Gly Cys Glu Val Gln Ala Met Leu Cys Val Pro Val
20 25 30

Ile Ser Arg Ala Thr Asp Gln Val Val Ala Leu Ala Cys Ala Phe Asn
35 40 45

Lys Leu Gly Gly Asp Leu Phe Thr Asp Gln Asp Glu His Val Ile Gln
50 55 60

His Cys Phe His Tyr Thr Ser Thr Val Leu Thr Ser Thr Leu Ala Phe
65 70 75 80

Gln

<210> SEQ ID NO 43
<211> LENGTH: 91
<212> TYPE: PRT
<213> ORGANISM: bovine

-continued

<400> SEQUENCE: 43

Glu Pro Leu Asn Ile Pro Asp Val Ser Lys Asp Lys Arg Phe Pro Trp
1 5 10 15

Thr Asn Glu Asn Met Gly Asn Ile Asn Gln Gln Cys Ile Arg Ser Leu
20 25 30

Leu Cys Thr Pro Ile Lys Asn Gly Lys Lys Asn Lys Val Ile Gly Val
35 40 45

Cys Gln Leu Val Asn Lys Met Glu Glu Thr Thr Gly Lys Val Lys Ala
50 55 60

Phe Asn Arg Asn Asp Glu Gln Phe Leu Glu Ala Phe Val Ile Phe Cys
65 70 75 80

Gly Leu Gly Ile Gln Asn Thr Gln Met Tyr Glu
85 90

<210> SEQ ID NO 44
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 44

Leu Ile Cys Asn Ile Met Asn Ala Pro Ser Glu Asp Phe Phe Ala Phe
1 5 10 15

Gln Lys Glu Pro Leu Asp Glu Ser Gly Trp Met Ile Lys Asn Val Leu
20 25 30

Ser Met Pro Ile Val Asn Lys Lys Glu Glu Ile Val Gly Val Ala Thr
35 40 45

Phe Tyr Asn Arg Lys Asp Gly Lys Pro Phe Asp Glu Met Asp Glu Thr
50 55 60

Leu Met Glu Ser Leu Ala Gln Phe Leu Gly Trp Ser Val Leu Asn Pro
65 70 75 80

Asp Thr Tyr Glu

<210> SEQ ID NO 45
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 45

Phe Ile Cys Asn Ile Met Asn Ala Pro Ala Asp Glu Met Phe Asn Phe
1 5 10 15

Gln Glu Gly Pro Leu Asp Asp Ser Gly Trp Ile Val Lys Asn Val Leu
20 25 30

Ser Met Pro Ile Val Asn Lys Lys Glu Glu Ile Val Gly Val Ala Thr
35 40 45

Phe Tyr Asn Arg Lys Asp Gly Lys Pro Phe Val Glu Gln Asp Glu Val
50 55 60

Leu Met Glu Ser Leu Thr Gln Phe Leu Gly Trp Ser Val Leu Asn Thr
65 70 75 80

Asp Thr Tyr Asp

<210> SEQ ID NO 46
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: bovine

-continued

<400> SEQUENCE: 46

```
Phe Ile Cys Asn Met Leu Asn Ala Pro Ala Asp Glu Tyr Phe Thr Phe
1               5                   10                  15

Gln Lys Gly Pro Val Asp Glu Thr Gly Trp Val Ile Lys Asn Val Leu
            20                  25                  30

Ser Leu Pro Ile Val Asn Lys Lys Glu Asp Ile Val Gly Val Ala Thr
        35                  40                  45

Phe Tyr Asn Arg Lys Asp Gly Lys Pro Phe Asp Glu Tyr Asp Glu His
    50                  55                  60

Ile Ala Glu Thr Leu Thr Gln Phe Leu Gly Trp Ser Leu Leu Asn Thr
65                  70                  75                  80

Asp Thr Tyr Glu
```

<210> SEQ ID NO 47
<211> LENGTH: 82
<212> TYPE: PRT
<213> ORGANISM: bovine

<400> SEQUENCE: 47

```
Gln Ile Leu Asn Ile Pro Asp Ala Tyr Ala His Pro Leu Phe Tyr Arg
1               5                   10                  15

Gly Val Asp Asp Ser Thr Gly Phe Arg Thr Arg Asn Ile Leu Cys Phe
            20                  25                  30

Pro Ile Lys Asn Glu Asn Gln Glu Val Ile Gly Val Ala Glu Leu Val
        35                  40                  45

Asn Lys Ile Asn Gly Pro Trp Phe Ser Lys Phe Asp Glu Asp Leu Ala
    50                  55                  60

Thr Ala Phe Ser Ile Tyr Cys Gly Ile Ser Ile Ala His Ser Leu Leu
65                  70                  75                  80

Tyr Lys
```

1. A purified and isolated polynucleotide encoding cGB-PDE.

2. The polynucleotide of claim 1 which is a DNA sequence.

3. The DNA sequence of claim 2 which is a cDNA sequence or a biological replica thereof.

4. The DNA sequence of claim 2 which is a genomic DNA sequence or a biological replica thereof.

5. An RNA transcript of the genomic DNA sequence of claim 4.

6. The DNA sequence of claim 2 which is a wholly or partially chemically synthesized DNA sequence or a biological replica thereof.

7. The DNA sequence of claim 4 further comprising an endogenous expression control DNA sequence.

8. A DNA vector comprising a DNA sequence according to claim 2.

9. The vector of claim 8 wherein said DNA sequence is operatively linked to an expression control DNA sequence.

10. A host cell stably transformed or transfected with a DNA sequence according to claim 7 in a manner allowing the expression in said host cell of cGB-PDE polypeptide possessing a ligand/receptor binding biological activity or immunological property specific to cGB-PDE.

11. A method for producing cGB-PDE polypeptide, said method comprising growing a host cell according to claim 10 in a suitable nutrient medium and isolating cGB-PDE polypeptide from said cell or the medium of its growth.

12. A polypeptide or peptide capable of specifically binding to cGB-PDE.

13. An antibody substance according to claim 12.

14. A monoclonal antibody according to claim 13.

15. A hybridoma cell line producing a monoclonal antibody according to claim 14.

16. A humanized antibody substance according to claim 13.

17. An antisense polynucleotide specific for a polynucleotide encoding cGB-PDE.

18. A DNA sequence encoding cGB-PDE and selected from the group consisting of:

(a) the DNA sequence set out in SEQ ID NO: 9 or 22;

(b) a DNA which hybridizes under stringent conditions to the DNA of (a); and

(c) a DNA sequence which, but for the redundancy of the generic code, would hybridize under stringent conditions to a DNA sequence of (a) or (b).

19. A method for modulating the enzymatic activity of cGB-PDE, comprising contacting cGB-PDE with an effective amount of an agent that binds CGB-PDE and activates or inhibits cGB-PDE.

20. The method of claim 19 wherein the agent that binds cGB-PDE is selected from the group consisting of antibodies, peptides, proteins, oligonucleotides, antisense molecules, non-peptide compounds, and small molecules.

21. The method of claim 20, wherein the agent that binds cGB-PDE is an anti-cGB-PDE antibody.

22. A method for identifying an agent that specifically binds to cGB-PDE comprising:

(a) contacting cGB-PDE with an effective amount of a test agent; and

(b) determining if the test agent specifically binds cGB-PDE.

23. A method for identifying an agent that specifically binds to cGB-PDE so as to modulate the enzymatic activity of cGB-PDE comprising:

(a) contacting cGB-PDE with an effective amount of a test agent;

(b) determining if the test agent specifically binds cGB-PDE; and

(c) testing for modulation of cGB-PDE activity.

24. The method of claim 23, wherein the cGB-PDE is recombinant cGB-PDE.

25. The method of claim 23, wherein the agent is selected from the group consisting of antibodies, peptides, proteins, oligonucleotides, antisense molecules, non-peptide compounds, and small molecules.

26. A method of using an agent that modulates the enzymatic activity of cGB-PDE for treating a condition that involves signal transduction pathways utilizing cyclic nucleotides as second messengers, comprising administering to a subject an agent that modulates the activity of cGB-PDE.

27. An agent identified by the method of claim 24.

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