DISSERTATION

CHARACTERIZATION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES IN THE TRANSCRIPTOME OF THE CRUSTACEAN MOLTING GLAND

Submitted by

Nada Mukhtar Rifai

Department of Biology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2019

Doctoral Committee:

Advisor: Donald L. Mykles

Deborah Garrity
Shane Kanatous
Santiago Di-Pietro

ABSTRACT

CHARACTERIZATION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES IN THE TRANSCRIPTOME OF THE CRUSTACEAN MOLTING GLAND

Molting in crustaceans is a complex physiological process that has to occur in order for the animal to grow. The old exoskeleton must be discarded and a new one to be formed from the inside out. Molting is coordinated and regulated mainly by two hormones; steroid hormones named ecdysteroids, which are synthesized and secreted from a pair of Y- organs (YOs) that are located in the cephalothorax and a neuropeptide hormone, the molt inhibiting hormone (MIH), which is secreted from the X-organ/sinus gland complex located in the eyestalks. Molting is induced when MIH is decreased in the blood (hemolymph) which in turn stimulates the YOs to produce and secrete ecdysteroids (molting hormones). There are four distinctive physiological states that the YO can be in throughout the molt cycle; the transition of the YO from the "basal" to the "activated" state happens when the animal enters premolt. During mid-premolt, the YO transitions to the "committed" state, in which the YO becomes insensitive to MIH. In this state, the circulating hemolymph contains high levels of ecdysteroids, which increase to a peak before the actual molt (ecdysis) happens. The YO transitions from the committed to the repressed state in late premolt. Finally, the YO returns back to the basal state in the postmolt stage. MIH binds to membrane receptors, activating a signal transduction pathway divided into "triggering" and "summation" phases. A transient increase in cAMP during the triggering phase leads to prolonged cGMP-dependent suppression of ecdysteroidogenesis during the summation phase. This allows for sustained inhibition of the YO between MIH pulses in the intermolt animal. Cyclic nucleotide phosphodiesterases (PDEs) play an important role by controlling cAMP and cGMP levels. PDEs

hydrolyze the phosphodiester bond in cAMP and cGMP to AMP and GMP, respectively. Mammals have 21 PDE genes that are categorized into 11 families, designated PDE1 to PDE11. Each PDE family has specific catalytic and biochemical properties and tissue distributions. Eight contigs encoding full-length PDE sequences were identified in the *G. lateralis* Y-organ transcriptome. Seven contigs encoding four full-length PDE sequences and three contigs encoding partial-length PDE were identified in the *Carcinus maenas* transcriptome. Multiple sequence alignments showed high sequence identities with orthologs from other species in catalytic (PDEase) and other conserved functional domains. Sequence analysis assigned the Gl-PDE sequences and Cm-PDE sequences to PDE1, PDE2, PDE3, PDE4, PDE5, PDE7, PDE8, PDE9, and PDE11 classes, indicating a high diversity of PDE genes in decapod crustaceans. The reduced sensitivity to MIH by the committed YO is associated with a large increase in PDE activity, which suggests that PDEs modulate the response to neuropeptide during the molt cycle. Non-hydrolyzable analogs of cAMP and cGMP inhibit YO ecdysteroid secretion *in-vitro*. Moreover, *C. maenas* YO ecdysteroidogenesis is inhibited by IBMX, a general PDE inhibitor, and Zaprinast, a specific PDE5 inhibitor. Rolipram, a specific PDE4 inhibitor, has no effect. These data suggest that PDE5 activity modulates the effect of MIH on YO ecdysteroidogenesis. RNA-seq data from MLA showed different mRNA levels for the different PDEs; PDE1 and PDE2 showed a similar pattern as they both increased in intermolt (IM) then decreased dramatically in early premolt (EP), mid premolt (MP), late premolt (LP), and post molt (PM). PDE4 increased in IM followed by a slight decrease and increase in EP and MP then a sharp decline in both LP and PM. Both PDE5 and PDE9 were similar in terms they increased in IM followed by a sharp decrease in EP, MP, LP and they differed as PDE5 increased slightly in PM whereas PDE9 remained decreased. PDE7 began with an increase in IM then a decline with a constant expression level in both EP and MP

followed by dramatic decline in LP and PM. PDE11 showed a typical pattern consistent with the ecdysteroid expression level as it began with a slight increase in IM followed by an increased in EP and reached a peak in MP then declined in a dramatic way in LP and continued decreasing in PM. Taken together, the data suggest that PDE5 and PDE11 play a role in regulating cyclic nucleotide levels in the YO.

ACKNOWLEDGEMENTS

First and foremost, Alhamdullah (means Thanks GOD in Arabic) for giving me everything I've dreamt of, and the strength and power required to finish an important milestone in my life. I could have never done this without the faith I have in Allah, the Almighty.

Then, my great thanks, sincere gratitude, and appreciation to my advisor Dr. Donald Mykles for his gentleness, motivation, endless support, infinite encouragement, and supervision throughout my years of study. I could not have imagined a better advisor and mentor as a PhD student. I also extend my thanks and appreciation for my committee members: Dr. Garrity, Dr. Shane, and Dr. Santiago for their time, help, and insightful comments.

My thanks are comprehensive to the Ministry of Higher Education in Libya for supplying me this study scholarship. I am grateful to have my husband, Khalid, and my lovely children, Saja and Serajaldeen, beside me and for their understanding, patience, and support all these years. My sincere respect and gratitude to my dear father for his support, encouragement and enthusiasm to continue my higher studies, to my lovely mother for her continuous prayers to succeed; and to my sisters (Nahla and Nuha), and brothers (Adam, Anwar, and Ayoub) simply for being awesome siblings; and to my family-in-law for their support; and family and friends in Libya. Last but not least, I would like to thank all former and current students in the crab lab for being such a great family.

DEDICATION

In loving memory of my brother "Ahmad" (1983-2013), may Allah gather us together in heaven.

TABLE OF CONTENTS

CHAPTER ONE

Introduction

Background and Rationale:

Although a crustacean grows, its exoskeleton (made from chitin and calcium) does not, so the animal must molt its old exoskeleton to accommodate its expanding body, which is essential for growth, metamorphosis, and reproduction (Skinner, 1962). Depending on the rigidness of the exoskeleton, the crustacean molt cycle is divided into five distinctive stages designated from A to E (Drach, 1939). The molt cycle duration varies depending on the species. The process of molting is hormonally controlled. In preparation for molting, the tissue layer under the exoskeleton detaches and secretes a new exoskeleton. At this stage, the animal has two skeletons – the outer and the inner exoskeleton. After the two outermost layers of the new skeleton are formed, the old skeleton detaches along specific weak points and the animal pulls out, leaving its old skeleton intact except for the split. Mobility is limited immediately after a molt because the exoskeleton is not rigid enough to keep the limbs stiff. Although crustaceans molt throughout their entire life, they molt less frequently with age (Chang et al., 1993).

The crustacean molt cycle is divided into five stages (A-E), based on changes in the exoskeleton. The actual shedding of the exoskeleton occurs at ecdysis (Stage E) and is followed by postmolt stages A and B, which are both marked by thickening and hardening of the new exoskeleton. Anecdysis, or Stage C_4, or intermolt stage, is the interval characterized by a hard exoskeleton that is tightly adhered to the epidermis and is the stage during which the animal feeds and reproduces. Stages $D_{1–4}$, known as premolt or proecdysis, is when the animal prepares for

molting by synthesizing the outermost layers of a new exoskeleton and regenerates lost appendages (Chang and Mykles, 2011).

Molting in crustaceans might be affected by various environmental factors, such as reproduction, nourishment, and migration. Moreover, the frequency and timing of molting in crustaceans can be impacted by some conditions such as salinity and temperature (Skinner, 1985).

The molting cycle in decapod crustaceans is controlled via two endocrine glands:

Molting can be stimulated or manipulated by eyestalk ablation (ESA), an acute method to induce molting in the lab, or autotomy of 5 or more walking legs (Multiple Leg Autotomy, MLA), which resembles the natural way. Molting is a very complicated process in which two glands contribute to complete this vital mission. The Y-organs (YOs) are a pair of molting glands located in the anterior body. The YOs secretes steroid molting hormones or ecdysteroids, which stimulate molting processes. The YOs are suppressed by molt-inhibiting hormone (MIH) and the crustacean hyperglycemic hormone (CHH), which are neuropeptides secreted by the X-organ -sinus gland complex found in the eyestalks (Lachaise et al., 1993, Covi et al., 2010).

MIH is a member of a novel neuropeptide family, which has been found only in arthropods. This neuropeptide family regulates a variety of functions including growth, molting, reproduction, and metabolism. MIH binds to hormone receptors on the membranes of YO cells and likely mediates its action via cyclic nucleotide second messengers (Covi et al., 2009). As shown in Fig. 1.1., MIH signaling usually involves an increase in cAMP, followed by a larger increase in cGMP. The delayed increase in cGMP suggests that MIH activates a soluble NO-sensitive guanylyl cyclase (GC-I), as activation of a membrane GC would result in an immediate increase in cGMP. Both cAMP and cGMP inhibit YO ecdysteroidogenesis (Covi et al., 2012). Phosphodiesterases

(PDEs) such as PDE1 and PDE5 hydrolyze cAMP and cGMP and thus control the responsiveness of YOs to MIH (Covi et al. 2012).

Molting is induced when MIH is decreased in the blood, which, in turn, stimulates the YO to produce and secrete ecdysteroids. The YO transitions from the "basal" to the "activated" state and the animal enters premolt. During mid-premolt, the YO transitions to the "committed" state, in which the YO becomes insensitive to MIH and CHH (Chang and Mykles 2011). The reduced sensitivity to the neuropeptide MIH is associated with a large increase in PDE activity (Fig1.2.) (Chang and Mykles 2011). This suggests that PDE activity controls the response to MIH and CHH during the molt cycle and may also explain the difference in the effect of IBMX, a universal PDE inhibitor, on the YOs of the two species.

Cyclic nucleotide phosphodiesterase's (PDEs) and their contribution in the MIH signaling pathway in the crustacean YO:

Cyclic nucleotide phosphodiesterases (PDEs) are prevalent enzymes that have been significant and valuable targets in medical and pharmacological fields due to their critical function in regulating the second messengers adenosine 3'5' cyclic monophosphate (cAMP) and/or guanosine 3'5' cyclic monophosphate (cGMP) in signal transduction pathways (Murthy and Mangot 2015). Upon binding of the ligand to its specific receptor on the cell membrane, the second messenger's concentrations will be changed, that alternatively will lead to the signal transmission within the cell. These second messengers are controlled by both the rate of synthesis, with the action of adenylyl/guanylyl cyclase on ATP/GTP and the rate of cAMP/cGMP degradation by the action of PDEs (Fajardo et al., 2014). So, PDEs are enzymes that hydrolyze the 3' cyclic phosphodiester bond in cAMP and cGMP to AMP and GMP, respectively. Mammals have 21 PDE genes that are categorized into 11 families, designated PDE1 to PDE11. Each PDE

family has specific catalytic and biochemical properties, protein sequences, inhibition tendency, and tissue distributions (Table 1.1) (Sandeep et al., 2008; Francis et al*.,* 2011). Mammalian class I PDEs have an HD domain in the C-terminal half and show high affinity for cAMP and/or cGMP. Protein domains involved in regulation of PDE enzymatic activity and sub-cellular localization are mainly present in the N-terminal half. Some PDEs have phosphorylation sites targeted by protein kinases and lipid modification sites. Approximately 270 aa in the C-terminal catalytic domain are conserved, with a sequence identity of 35% to 50% among different PDE families. Some PDE families are composed of 2 to 4 subfamily genes showing sequence identity of more than 70% and having identical protein domain organization. Multiple transcriptional products, which are generated from most PDE genes by alternative splicing or transcription from distinct promoters, have been identified or predicted in human genome databases (Francis et al*.,* 2011).

The number of PDE genes in crustaceans is unknown. YOs have PDE activity, which is inhibited by IBMX, 8MM-IBMX, and zaprinast, but not EHNA or rolipram (Nakatsuji et al., 2006). These data suggest that the YO has PDE1 and PDE5 activity, but not PDE2 or PDE4 activity. Interestingly, IBMX inhibits ecdysteroidogenesis in the green crab *Carcinus maenas* YO, but not the blackback land crab *Gecarcinus lateralis* YO, which suggests that there is a difference in cyclic nucleotide metabolism between the two species (Nakatsuji et al., 2009; Covi et al., 2008, 2009, 2012.

The purpose of this research project is to identify and characterize the different types of PDEs in the YO of the blackback land crab *Gecarcinus lateralis* and the green crab (*Carcinus maenas*) by using the power of transcriptomics, as well as conventional lab techniques, such as qPCR and *in-vitro* experiments. The hypothesis is based on the premise that the reduced sensitivity to MIH by the committed YO is associated with a large increase in PDE activity, which suggests that PDEs

modulate the response to the neuropeptide, MIH, during the molt cycle. Thus, increasing ecdysteroidogenesis.

This thesis addresses the identification and characterization of the PDEs in the land crab and green crab YO from the transcriptomics data, reports results from YO assays in both land crab and green crab, compares PDE gene expression in different tissues from both the land crab and green crab using qPCR, and reports the effects of MLA and ESA ± rapamycin on PDE gene expression using RNA-seq and qPCR. The thesis concludes with a chapter summarizing the results and their significance and recommending future directions.

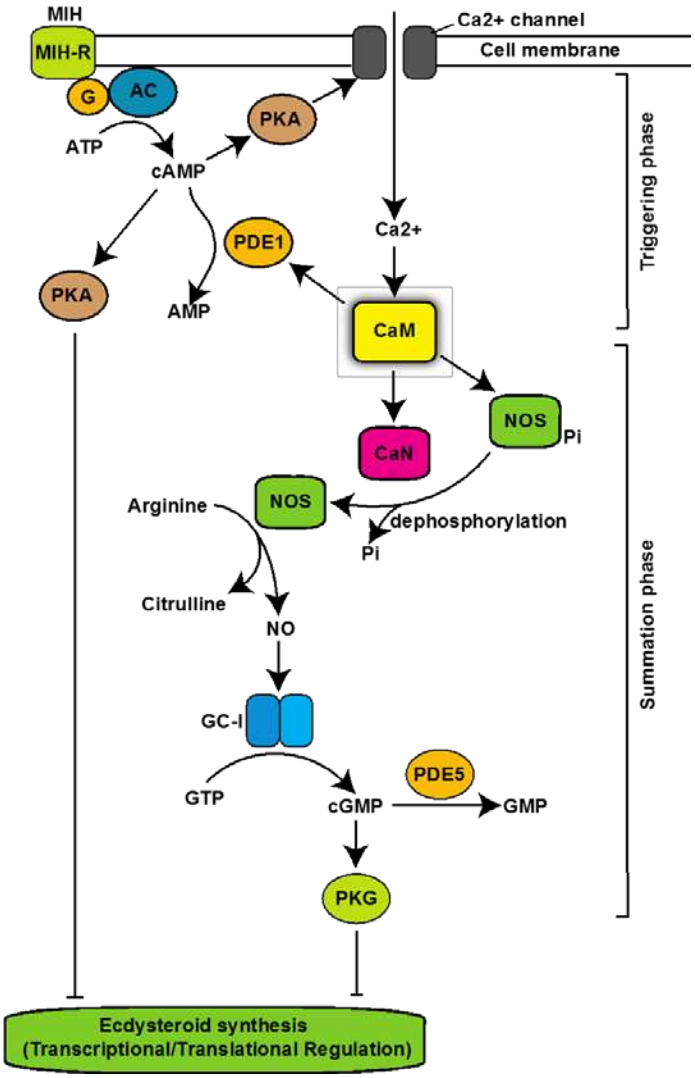


Figure 1.1. Proposed MIH signaling pathway regulating ecdysteroidogenesis in decapod crustacean molting gland. The "triggering" phase is initiated by binding of MIH to a G protein-coupled receptor (MIH-R) and activation of adenylyl cyclase (AC); cAMP increases intracellular Ca^{2+} via cAMP-dependent protein kinase (PKA) phosphorylation of Ca^{2+} channels. Sensitivity to MIH is determined by phosphodiesterase 1 (PDE1) activity, which varies during the molting cycle. The "summation" phase is mediated by NO and cGMP. Calmodulin (CaM) links the two phases by activating NO synthase (NOS) directly and indirectly via calcineurin (CaN). Dephosphorylation of NOS by CaM can potentially prolong the response to MIH. CaM can also activate PDE1 to inhibit the triggering phase (PDE1 can also hydrolyze cGMP, thus inhibiting the summation phase). cGMP-dependent protein kinase (PKG) inhibits ecdysteroidogenesis. Chronic activation of PKA may directly inhibit ecdysteroidogenesis. Our assumption is that YOs from all decapods are regulated by the same pathway but may differ in the sensitivity of the triggering and summation phases. Other abbreviations: G, G protein; GC-I, NO-sensitive guanylyl cyclase; PDE5, cGMP PDE. From Covi et al., (2012).

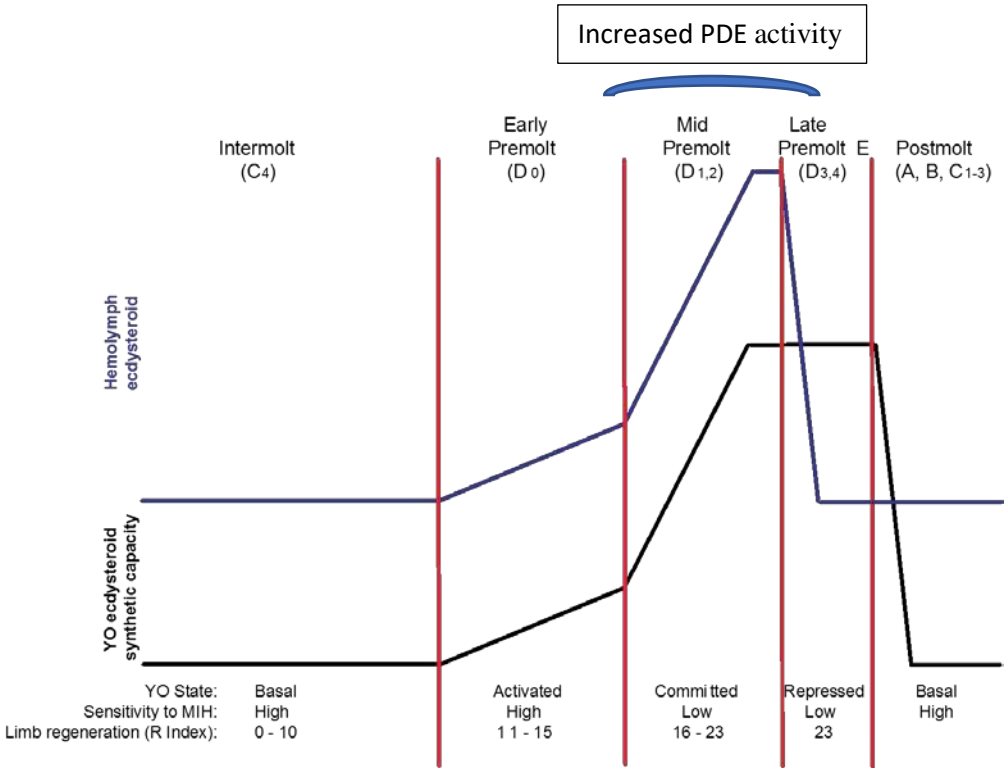


Figure 1. 2. Hormonal regulation of molting in the blackback land crab, *Gecarcinus lateralis*. Diagram shows the relationship between molt stage, YO state, YO sensitivity to MIH, limb regeneration (R index), YO ecdysteroid synthetic capacity, and hemolymph ecdysteroid titer. During postmolt (A, B, C_{1-3}), intermolt (C_4), early premolt (D_0), and mid premolt ($D_{1,2}$), hemolymph ecdysteroid titers are correlated with YO synthetic capacity; during late premolt ($D_{3,4}$), high ecdysteroid represses YO ecdysteroidogenesis and ecdysteroid titer falls. The YO transitions through four physiological states during the molt cycle: basal (B), activated (A), committed (C), and repressed (R). The B to A transition is triggered by a reduction in MIH; the YOs hypertrophy, but remain sensitive to MIH, as premolt is suspended by MIH injection or by limb bud autotomy (LBA). At the A to C transition, the animal becomes committed to molt, as the YO is less sensitive to MIH and premolt is not suspended by LBA; this transition may be triggered by an increase in MIH or an unidentified tropic factor. At the C to R transition, YO ecdysteroid synthetic capacity remains high, but high hemolymph ecdysteroid titer inhibits ecdysteroid secretion. Molting, or ecdysis (E), marks the R to B transition, during which the YO atrophies and becomes sensitive to MIH. From Chang and Mykles (2011).

Table1.1. Human PDE isozymes are divided into 11 families and differ according to substrate specificity, mechanisms of regulation, and sensitivity to inhibitors. ↑ represents an increase in catalytic activity, whereas ↓ represents a decrease in catalytic activity. * Number of isozymes refers to the number of distinct protein products derived from all genes within a given family that have been identified to date. From Fajardo et al., (2014).

Isozyme Family	Number of Genes	Putative Number of Isozymes *	Substrate Specificity	Regulators	Inhibitors
1	3	21	dual	Ca^{2+}-CaM: ↑ PKA: ↓	IC224, SH51866, 8-methoxymethyl-IBMX
2	1	3	dual	cGMP: ↑	EHNA, BAY 60-7550, PDP, IC933
3	2	4	dual	cGMP:↓ PKA: ↑	Milrinone, Tolafentrine, Cilostazol, Cilostamide, OPC-33540
4	4	31	cAMP	PKA: ↓	Rolipram, Cilomilast, Roflumilast, Ro20-1724, Denbufylline, AWD12281
5	1	3	cGMP	cGMP: ↑ PKG: ↑	Sildenafil, Zaprinast, Dipyridamole, Tadalafil, Vardenafil, DMPPO, E402, DA8159, 8-methoxymethyl-IBMX
6	3	3	cGMP	Transducin: ↑	Sildenafil, Dipyridamole, Zaprinast
7	2	7	cAMP	unknown	BRL 50481, IC242, Dipyridamole, Thiadiazoles
8	2	9	cAMP	unknown	Dipyridamole
9	1	2	cGMP	unknown	BAY73-669, SCH 51886, Zaprinast
10	1	10	dual	PKA: ↑	Papaverine, PF-2545920, PQ-10, Dipyridamole
11	1	4	dual	unknown	BC 11-38, Dipyridamole

CHAPTER TWO

IDENTIFICATION AND CHARACTERIZATION OF THE PHOSPHODIESTERASES (PDES) IN THE DECAPOD CRUSTACEAN'S Y-ORGAN USING TRANSCRIPTOMICS AND qPCR

Summary

Cyclic nucleotide signaling mediates the suppression of the crustacean molting gland (Y-organ or YO) by molt-inhibiting hormone (MIH). When MIH level drops the YO transitions from the basal to the activated state and the animal enters premolt. During mid-premolt, the YO transitions to the committed state, in which the YO becomes insensitive to MIH. Phosphodiesterases (PDEs) hydrolyze the phosphodiester bond in cAMP and cGMP to AMP and GMP, respectively, and thus can modulate the response of the YO to MIH. In some species, PDE inhibitors decrease molting hormone (ecdysteroid) biosynthesis by the YO *in-vitro*, indicating that PDE activity can keep cyclic nucleotide levels low. Increased PDE activity in the YO is correlated with a reduced sensitivity to MIH when the animal becomes committed to molt. In mammals, 21 PDE genes are organized into 11 families, designated PDE1 to PDE11. Each PDE family has specific catalytic and biochemical properties and tissue distributions. The number and types of PDE genes in crustaceans is unknown. A reference YO transcriptome from the blackback land crab (*Gecarcinus lateralis*), consisting of 3 biological replicates of intermolt animals, was analyzed for PDE sequences. Nine different contigs encoding seven full-length PDE sequences two partials were identified in *G. lateralis*. Seven contigs encoding four full-length PDE sequences

and three contigs encoding partial-length PDE were identified in the green shore crab (*Carcinus maenas)* transcriptome. Protein alignments and ClustalX analysis of the Gl-PDE sequences with orthogs from other species in the GenBank database showed that the sequences corresponded to PDE1, 2, 3 4, 5, 7, 8, 9, and 11. General and selective inhibitors were used to characterize the PDEs regulating ecdysteroid secretion in the green crab, *Carcinus maenas,* YO. IBMX, vinpocetine, EHNA and zaprinast ± rMIH significantly inhibited ecdysteroid secretion, while rolipram, dipyridamole, and BC11-38 did not. This suggests that PDE1, PDE2 and PDE5/11 are primarily responsible for regulating cAMP and cGMP levels. No effect on ecdysteroidogenesis was seen on the blackback land crab, *Gecarcinus lateralis*, YOs when exposed to the same PDE inhibitors *in-vitro*, indicating different regulatory metabolic machineries between the two species.

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes involved in the regulation of the intracellular concentrations of the second messengers cAMP and/or cGMP (Thompson and Appleman, 1971; Conti, 2000; Soderling and Beavo 2000). PDEs belong to a highly conserved family among all the phyla, and due to their importance in the clinical field, they have received high attention and interest (Levy et al., 2011; Ahmad et al., 2015). Because of the biochemical properties, as well as distinguishing features and complexness of the PDE system, many sophisticated and advanced approaches have been established to understand their role in regulating the cyclic nucleotides cAMP/cGMP in several signaling pathways. This has been linked to the basic pharmacological fact that regulating the degradation of any ligand or second messenger provides greater efficiency, in terms of a prompt and a greater alteration in their percentage concentration, than the regulation and modulation of their synthesis rate (Bender and Beavo, 2006; Halpin, 2008).

In mammalian systems, the PDE I superfamily consists of 11 distinct PDE families where each family is unique in having different isoforms and splice variants. PDEs are the only known enzymes that are capable of hydrolyzing or breaking down the phosphodiester bond in the second messengers cAMP/cGMP to their 5' inactive monophosphates, so their concentrations remain regulated throughout the cell at all times. PDEs are also effective in controlling the amplitude, spatial, and temporal duration of these cyclic nucleotides as well, so they are not located in unnecessary parts of a cell (Puzzo et al., 2008; Demirbas et al., 2013; Mittal et al., 2017).

Beside the crucial role of PDEs in mammals, PDEs have also been found to be pivotal in other species. For instance, the dunce gene, a cAMP PDE, in *Drosophila melanogaster*, fruit fly, is important in learning, memory and female fertility (Bellen et al., 1987; Yh et al., 1991; Day et al., 2005). Moreover, these PDEs, as the main players in the cAMP signaling pathway and the cGMP/NO signaling pathway, contribute indirectly in a variety of physiological processes; such as muscle relaxation, visual transduction, endocrine, neuronal, immune, and cardiovascular functions (Yan et al., 2016). Moreover, some PDEs will degrade only cAMP (PDE4, PDE7, PDE8), while others will only hydrolyze cGMP (PDE5, PDE6, PDE9). The rest of the PDE families, PDE1, PDE2, PDE3, PDE10, PDE11, are capable of catalyzing both second messengers as they have a dual specificity (Beavo and Brunton 2000; Mehats et al., 2002). It is thought that an invariant glutamine residue located at the catalytic core is responsible for the PDE cyclic nucleotide selectivity (Xu et al., 2000; Xu et al., 2004; Ke et al., 2011).

All eleven PDE families share a conserved catalytic domain on the carboxyl terminus and a variable regulatory domain on the amino terminus (Francis et al., 2011). About 270 conserved amino acids span the catalytic domain, and the sequence identity can reach high percentages of 35-50% between different PDE families (Houslay and Adams, 2003). This identity can be up to

70% within the same family. The PDE catalytic domain is an alpha helical region that is composed of 16 α-helices which in turn can be divided into three subdomains. These three subdomains form a deep hydrophobic pocket where a Zn^{++} binding site is located. Within the PDE catalytic domain, a glutamine switch (Q-switch) is made up of an invariant glutamine residue, which is important to control the selectivity of PDEs toward cAMP or cGMP or both cyclic nucleotides (Xu et al., 2000). On the other hand, regulatory domains differ among the PDE families and that is what makes each PDE family unique and special in terms of their mode of regulation and sensitivity to specific inhibitors. For instance, PDE1 was among the first discovered PDE families; it is particularly regulated by a Ca^{++}/Calmodulin (CaM) binding site. PDE3 contains a transmembrane domain and PDE4 is modified by upstream conserved regions (UCRs). PDE8 has two distinct regulatory domains: a response regulatory receiver (REC) and PAS. PDE7 and PDE9 lack specific domains on their amino termini (Omori and Kotera, 2007). On the other hand, about half of the PDE families (PDE2, PDE5, PDE 6, PDE10, PDE11) have tandem GAF domains that function in the dimerization of these PDEs, in addition to the cGMP binding region (Ho et al., 2000; Yausa et al., 2000). Moreover, human PDEs databases show that 21 genes produce transcriptional variants that arose from alternative splicing or transcription from various promoters (Omori and Kotera, 2007; Francis et al., 2011).

The number and types of PDE genes in crustaceans is unknown. Studies by Nakatsuji et al(2008) addressed the hypothesis that the responsiveness of crayfish *(Procambarus clarkii*) YOs to MIH may be caused by the alteration of the PDE activity throughout the molt cycle. Furthermore, YOs have PDE activity, which is inhibited by IBMX, 8MM-IBMX, and zaprinast, but not EHNA or Ro-20-1724 (Nakatsuji, 2006). These data suggest that the YO has PDE1 and PDE5 activity, but not PDE2 or PDE4 activity. Interestingly, IBMX inhibits ecdysteroidogenesis

in the green shore crab *Carcinus maenas* YO, but not the *G. lateralis* YO, which suggests that there is a difference in cyclic nucleotide metabolism between the two species (Nakatsuji, 2009; Covi et al., 2008. 2009, 2012)

This study was conducted to identify and characterize the different PDEs in the crustacean YO. For this purpose, a reference YO transcriptome from the blackback land crab (*Gecarcinus lateralis*), consisting of 3 biological replicates of intermolt animals, was analyzed for PDE sequences that are essential elements in the cAMP/cGMP signaling pathways. Two different transcriptome databases of the green shore crab (*Carcinus maenas*) were used to extract different PDE families. Multiple alignments, phylogeny of PDEs from a variety of orthologs and a comparison between PDEs in both *G. lateralis* and *H. sapiens* are exhibited in this chapter. *In-vitro* YO assays from both *G. lateralis* and *C. maenas* were performed to determine the effects of the different PDE inhibitors on ecdysteriodogenesis. Real-time PCR was conducted to compare the PDE gene expression from different tissues from the both studied decapod species.

Materials and Methods

Animals:

Adult male *Gecarcinus lateralis* (blackback land crabs) were collected from the Dominican Republic and shipped to Colorado, USA by commercial air cargo. The animals were adapted and acclimated to the new conditions by maintaining them at 27 ˚C and a relative humidity of ~80%. Intermolt crabs were kept in plastic cages with aspen bedding moistened with 5 p.p.t. Instant Ocean (Aquarium Systems, Mentor, OH). Crabs were maintained in an environmental chamber in a 12 hrs light:12 hrs dark cycle and were fed iceberg lettuce, carrots, and raisins twice a week (Covi et al., 2010). Blackback land crabs molt about once a year. Our other model species, *Carcinus maenas* (green shore crab) was collected from Bodega Bay Harbor in California. Animals were

kept in their optimal conditions either in Bodega Marine Laboratory or when they were shipped to Colorado. At CSU, green crabs were fed chicken liver once a week and were maintained in aerated tanks less than half filled with 30 p.p.t Instant Ocean at 20 ˚C. The water was changed twice a week unless it became cloudy or a death happened in a tank (Lee et al., 2007).

Transcriptomics:

Gecarcinus lateralis and *Carcinus maenas* lack a fully sequenced genome. Therefore, a reference YO transcriptome from the blackback land crab (*G. lateralis*), consisting of 3 biological replicates of intermolt animals, was assembled from RNA-seq data. For this purpose, the fiddler crab (*Uca pugnas)* limb bud transcriptome was used as a query to extract the different PDEs in the land crab's YO transcriptome (Das et al., 2016). *G lateralis* PDEs served as queries to extract different PDEs from two *C. maenas* transcriptome databases (Tepolt &Palumbi 2015; Verbruggen et al., 2015). By using the software perfectBlast, the PDE nucleotide sequences were extracted. Upon extraction, each nucleotide sequence was translated by using the translate tool, EXPASY (https://web.expasy.org/translate/) and the appropriate Open Reading Frame (OPR) was chosen. Then, a standard protein BLAST, blastp,

(https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch), as well as SMARTBLAST (https://blast.ncbi.nlm.nih.gov/smartblast/smartBlast.cgi) was used to find out the corresponded PDE and the possible similar orthologs. Also, a conserved domain search from the Conserved Domain Database (CDD) was used to locate the different PDE domains, (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). InterPro was used for protein sequence analysis and classification (https://www.ebi.ac.uk/interpro/)

In-vitro YO assays:

Hemolymph was withdrawn from each animal prior to dissecting. 100 µl of hemolymph was combined with 300 µl methanol (MeOH 100%). Green crabs were dissected directly on the assigned date, but the case was a bit different for the land crabs as they were dissected on day 3 post ESA to allow the YOs to be stimulated and activated to secrete ecdysteroids. For both species, one YO of each pair was incubated in 500 µl of crab saline with the appropriate vehicle (as the control), whereas the other pair was incubated in 500 µl of crab saline with the experimental chemical agent, a PDE inhibitor (as the experimental). The incubation time for both control and experimental samples was 4.5 hours, then 200 µl of media was added to 600 µl Methanol (MeOH 100%). Hemolymph and media samples were sent to Bodega Marine Laboratory for ELISA to evaluate the ecdysteroid levels.

RNA isolation, cDNA synthesis and PCR:

Different tissues (B, CM, ESG, G, H, HG, HP, MG, T, TG, YO) were harvested from intermolt land crabs and green crabs. All tissues were placed immediately in RNA-later after cleaning the tissues in crab saline. Tissues were kept overnight in 4°C, then transferred to -20 °C until the time of RNA purification. Total RNA was isolated from crab tissues using TRIzol reagent (Life Technologies, Carlsbad, CA) as described by (Covi et al., 2010). YO tissues (50-200 mg) were homogenized by using a micro-tube homogenizer system, while all the other tissues were homogenized by a Qiagen TissueLyser ll for two minutes at a frequency of 30 revolutions per second. One ml TRIzol was added to the samples, then centrifuged at 12,000 g for 15 min at 4 ºC. Supernatants were phenol-chloroform extracted and RNA in the aqueous phase was precipitated using isopropanol (0.75 ml per 1 ml TRIzol reagent). RNA was treated with DNase I (Life Technologies), extracted twice with phenol: chloroform:isoamyl alcohol (25:24:1), precipitated

with isopropanol, washed twice with 70% ethanol in DEPC water, and resuspended in nuclease-free water. A nanodrop spectrophotometer was used to verify the purity of RNA. cDNA was synthesized using 2 µl total RNA in a 20 µl total reaction with SuperScript III reverse transcriptase (Life Technologies) and oligo-dT (20) VN primer (50 µmol/l; IDT, Coralville, IA) as described (Covi et al., 2010). RNA was treated with RNase H (Fisher Scientific, Pittsburgh, PA) and stored at -80 ºC.

End-point PCR was used to amplify the desired product and to increase the yield of each PDE gene as well as making external standards of the different genes to be used later in qPCR. Sequence-specific primers (Table 2.2.) were utilized to detect the different PDE products in both land crab and green crab. Each PCR reaction contained 3 µl DI H_2O, 5 µl Master Mix, 1 µl cDNA template, and 0.5 µl of each forward and reverse primers. The concentration of the primers was 20 µM. cDNA was amplified in a thermocycler where denaturation occurred at 94 °C for 3 minutes to initiate the process, then followed by 30-35 cycles of 30 seconds at 94 °C, 30 seconds at the lowest annealing temperature (see Table 2.1.), 30 seconds at 72 °C. Final elongation was set for 7 minutes at 72 °C. PCR products were then separated on 1% agarose gel that contained TAE buffer (composed of 40 mM Tris acetate and 2 mM EDTA with an 8.5 pH). Ethidium bromide was applied to stain the gel and a UV light was used to visualize the gel.

Tissue expression of G. lateralis and C. maenas PDEs:

Real-time PCR (RT-PCR) was used to quantify the expression of the different PDEs in the following tissue; Brain, Claw Muscle, Eyestalk Ganglia, Gill, Heart, Hind Gut, Hepatopancreas, Mid Gut, Testis, Thoracic Ganglion, and Y-organ to display a panel comparison between two crustacean models; the blackback land crab *G. lateralis* and the green shore crab *C. maenas*. Animals from both species were adult intermolt male crabs.

cDNA was synthesized as indicated previously, and a LightCycler 480 thermocycler (Roche Applied Science, Indianapolis, IN) was used to quantify the mRNA transcripts of PDE1, PDE 2, PDE4, PDE7, PDE9, and PDE11 for *G. lateralis* and PDE4, PDE5, PDE9, and PDE11 for *C. maenas*. Each reaction consisted of 1 µl cDNA or standard, 5 µl SYBR Green I Master mix (Roche Applied Science), 3 µl nuclease-free water, and 0.5 µl each of 10 mM forward and reverse primers (Table 2.3). PCR conditions were as follows: an initial denaturation at 95 ºC for 5 min, followed by 45 cycles of denaturation at 95 ºC for 10 s, annealing at 62 ºC for 20 s, and extensions at 72 ºC for 20 s, followed by melting curve analysis of the PCR product. Concentrations of mRNA transcripts were determined by the LightCycler 480 software (Roche, version 1.5) using a serial dilution of standards of the PCR product for each gene of interest. The amounts of mRNA transcript in copy numbers per µg of total RNA in the cDNA synthesis reaction were calculated based on the standard curve and the calculated molecular weight of dsDNA products.

Statistical Analysis and software:

Multiple sequence alignments were generated by utilizing ClustalX version 2.0.12 (Thompson et al., 1997) using the amino acid sequences. A phylogenetic tree was constructed by using PhyML 3.0 (Dereeper et al., 2008; Guindon et al., 2010; Anisimova and Gascuel 2010) and iTOL (Letunic and Bork 2016). A schematic diagram compared the domain organization between the *G. lateralis* and *Homo sapiens* PDEs. For *in-vitro* YO assays, a paired t-test was used to compare the means of ecdyseroid levels secreted from both control and experimental YOs. Primers were designed by IDT. Means for mRNA transcript abundance were compared using an analysis of variance (ANOVA) for tissue distribution versus log copy number. Sigma plot 12.5 software (Systat Software, Inc., Chicago, IL, USA) was used to produce and build up the graphs and figures. The Tukey test was used to determine significance among the means.

Results

Characterization and identification of PDEs from the G. lateralis YO transcriptome and C. maenas cardiac transcriptome:

A *de novo* transcriptome was used to characterize and identify the different PDE families located in the YO. The extraction resulted in a total of nine PDE contigs, including seven full length sequences and two partial sequences (Table 2.1). A *de novo* cardiac/assembly of *C. maenas* transcriptome was utilized to characterize and identify the different PDE families. The extraction resulted in a total of seven PDE contigs, four with full length sequences and three with partial sequences (Table 2.2.). ClustalX analysis assigned the *G. lateralis* PDEs to PDE1, PDE2, PDE3, PDE4, PDE5, PDE7, PDE8, PDE9, and PDE11. Multiple sequence alignments showed high sequence identities with orthologs from other species in the catalytic and other conserved functional domains. The conserved catalytic domain of all PDEs contained the initiating YHN (or FHN in PDE9) motif, as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N).

Characterization of Ca^{+2}/CaM PDE Gl-PDE1:

A full sequence length, ~ 3037 bp, of PDE1 contig was found in the YO transcriptome through RNA sequencing (RNA-seq). *Gl-PDE1* corresponds to the Ca^{+2}/ calmodulin PDE in other orthologs, as it contains the following domains: a conserved catalytic domain at the carboxyl terminus and a regulatory domain specifically coding to a Ca^{+2}/ calmodulin domain proximal to the amino terminus. Both the DNA and the deduced amino acid sequences are shown in Fig 2.1. Multiple sequence alignments of the *G. lateralis* PDE1 and other orthologs from different species

showed high levels of sequence identity/similarity (Fig 2.2). Identity to the fruit fly PDE1C was 55%, whereas identity to the human PDE1C was 44%.

Characterization of the GAF-PDEs: Gl-PDE2, Gl-PDE5, Cm-PDE5, Gl-PDE11, Cm-PDE11 and the specific cGMP Gl-PDE9 and Cm-PDE9:

Three GAF-PDEs with full length sequences were established in the *G. lateralis* YO transcriptome, as they corresponded to *PDE2*, *PDE5*, and *PDE11* orthologs. All these genes were composed of the conserved catalytic domain and either one or two GAF regulatory domains. Whereas *Gl-PDE2*, a PDE dual substrate (4729 bp) contained only one GAF domain, namely GAF B (Fig. 2.3), Gl-PDE5, a cGMP binding PDE (6761 bp), and *Gl-PDE11*, a dual specificity PDE (5752 bp) had two tandem GAF domains (GAF A and GAF B) (Fig. 2.8 & Fig. 2. 18). *Cm-PDE5* (2996 bp) and *Cm-PDE11* (5839 bp) were demonstrated in Figs. 2.9 &. 2.19. Multiple sequence alignments showed high sequence identity/similarity. For instance, Gl-PDE2 shared 56% identity with human PDE2A3 and 38% identity with *C. elegans* PDE2 (Fig. 2.4). Gl-PDE5 shared 89% identity with Cm-PDE5 and 42% identity with *H. sapiens* PDE5A (Fig. 2.10). The dual PDE, Gl-PDE11, shared 97% identity with Cm-PDE11 and about 72% with *D. melanogaster* PDE11C, whereas it shared 50% identity with the human PDE11A (Fig. 2.20). Moreover, Gl-PDE2, 5, & 11 and Cm-PDE5 & 11 contained the conserved motif sequence, NKFDE, in their GAF domains. Although *Gl-PDE9* (4111 bp) and *Cm-PDE9* (4356 bp) are cGMP-specific PDEs, they both lack the GAF domains. The DNA and amino acid sequences are presented in Fig. 2.15 and Fig. 2.16. Multiple alignments showed that Gl-PDE9 has an identity of 93% with Cm-PDE9, 52% with Dm PDE9B and 55% with Hs-PDE9A (Fig. 2.17).

Characterization of cAMP-specific PDEs: Gl-PDE4, Cm-PDE4, Gl-PDE7 and Cm-PDE8:

cAMP-specific PDE, *Gl-PDE4*, and high affinity cAMP-specific PDE, *Gl-PDE7*, were found in the YO transcriptome as full length contigs (6130 bp and 7531 bp, respectively). Both PDEs had the conserved catalytic domain near the carboxyl terminus. Two upstream conserved regions (UCRs) have been detected in the regulatory domain in the *Gl-PDE4* gene (Fig. 2.5). *Cm-PDE4* was found in the cardiac transcriptome as a full length contig (6122 bp) with a conserved catalytic domain proximal to the COOH terminus. One UCR was observed in the regulatory domain region of Cm-PDE4 (Fig. 2. 6). Gl-PDE4 has an identity of 91% with Cm-PDE4, 64% with the *D. melanogaster* dunce gene *PDE4D* and 60% with *H. sapiens* PDE4D3 (Fig. 2.7). The DNA and deduced amino acid sequences of *Gl-PDE7* are shown in Fig. 2.11. Moreover, Gl-PDE7 has an identity of 46% to both PDE7A in *H. sapiens* and *D. rerio* (Fig. 2.12). High affinity cAMP specific and IBMX insensitive PDE, *Cm-PDE8*, has been revealed in the cardiac transcriptome of the green shore crab as a full length contig (3007 bp). *Cm-PDE8* contains a conserved catalytic domain near the carboxy termini and the PAS regulatory domain in the amino terminus (Fig. 2.13). Multiple alignment of Cm-PDE8 with ortholog species showed identity of 44% with Dm-PDE8O and 36% with Hs-PDE8B (Fig. 2.14).

Phylogeny of G. lateralis and C. maenas PDEs with different orthologs:

A phylogenetic tree was constructed using the software iTOL (Interactive Tree Of Life), (https://itol.embl.de/itol.cgi). 102 PDE orthologs from different invertebrate and vertebrate species were compared to the nine *G. lateralis* PDE contigs from the YO transcriptome and seven *C. maenas* PDE contigs from the green shore crab cardiac transcriptome. All PDEs from *G. lateralis* and *C. maenas* were closely related. Moreover, all sixteen PDEs clustered with their corresponding orthologs (Letunic and Bork 2016) (Fig. 2.21).

Comparison between G. lateralis and H. sapiens PDEs:

As illustrated in Fig. 2.22, all nine PDEs from the blackback land crab were compared with the corresponding human PDE. The domain organization with their interval lengths are shown as well. Catalytic domains are located near the carboxyl terminus in both species. This was the case for all PDEs except for Gl-PDE7 and Gl-PDE9, as their catalytic domains seemed distal to the carboxyl terminus and were not organized with Hs-PDE7 and Hs-PDE9. Moreover, Gl-PDE2 contained only one GAF domain, which contrasted with Hs-PDE2, which has two GAF domains. Moreover, Gl-PDE3 and Gl-PDE8 were found as partial contigs, so no regulatory domains were present to match them to the corresponding human PDEs. PDE1, PDE4, PDE5, and PDE11 were similar in both species regarding their lengths and domain localization.

In-vitro Y-Organ assays in G. lateralis and C. maenas:

Y-organs in both studied species had undergone *in-vitro* assays to observe the effects of different PDE inhibitors on ecdysteroid synthesis and synthesis. In the green shore crab, *C maenas*, PDE inhibitors IBMX, vinpocetine, EHNA, and zaprinast inhibited YO ecdysteroid secretion (Table 2.4), which suggests that PDE1, PDE2, and PDE5/11 play major roles in controlling cyclic nucleotide levels in the YO of *C. maenas.* Ecdysteroidogenesis was not affected by either rolipram or dipyridamole, which selectively inhibit PDE4 and PDE7 (Table 2.4). In contrast, YOs from the blackback land crab, *G. lateralis*, showed no effect when exposed to the same PDE inhibitors (Table 2.5).

PDE gene expression in different tissues in both G. lateralis and C. maenas:

Quantitative-PCR (qPCR) was used to quantify the mRNA of the nine *PDEs* in eleven tissues: Brain (B), Claw Muscle (CM), Eyestalk Ganglia (ESG), Gill (G), Heart (H), Hindgut (HG), Hepatopancreas (HP), Midgut (MG), Testis (T), Thoracic Ganglion (TG), and Y-Organ (YO) in the blackback land crab and the green shore crab.

G. lateralis PDE1 was expressed in low levels in all tissues, but still there was a significant difference between the heart and YO, as well as the claw muscle, to the YO and midgut. *G. lateralis PDE2* and *PDE5* showed relatively high expression in all the tissues with the highest mRNA level in the heart, which contrasted with *PDE11*, which was expressed at lower levels in the same tissues. *Gl-PDE2* showed a significant increase of ~ 100-fold in the heart compared to hepatopancreas and hindgut. Also, the YO demonstrated considerable increase difference (~15-fold change) when compared to the hepatopancreas. *Gl-PDE5* displayed slight significant increases between the YO and midgut (6.4-fold change), and among the heart versus hepatopancreas and midgut (~ 10-fold change). The testes showed the highest *Gl-PDE11* expression and this tissue was different from the claw muscle and hindgut (~8-fold change). An inconsiderable difference was exhibited when the YO was compared to the hindgut (Figs. 2.23 a, b, c, d). Gl-PDE 4,7, and 9 were expressed in such extremely low levels that they could be barely detected. There was no statistical significance among the tested tissues**.**

In contrast, *C maenas PDE11* was expressed at high levels in all the tissues except in the HG. A significant difference of mRNA expression of *Cm-PDE11* was seen in the thoracic ganglion versus each of midgut, ~ $15*10^4$ -fold increase, and claw muscle, ~$5*10^3$ fold increase. Also, the eyestalk ganglia were significantly different from the midgut by ~ $15*10^3$ -fold increase. *Cm-PDE4, Cm-PDE8*, and *Cm-PDE9* were expressed in very low levels in all tissues. *Cm-PDE4*

displayed a minor difference when comparing the claw muscle to both thoracic ganglion and the YO. Moreover, a small statistical significance was noted between the testis and the YO. *Cm-PDE8* demonstrated a slight statistical significance observed in the brain versus both the midgut and the thoracic ganglion, also there was a small change between the eyestalk ganglion and thoracic ganglion. Finally, *Cm-PDE9* was expressed with minor differences in the thoracic ganglion versus both midgut and hindgut, and between the YO and hindgut (Figs. 2.24 a, b, c, d).

Discussion

PDEs are important and crucial players in both cAMP and cGMP signaling pathways. They act as negative regulators that breakdown/hydrolyze these second messengers (Thompson and Appleman, 1971; Conti, 2000; Soderling and Beavo 2000). Since the numbers and types of PDEs are still unknown in crustaceans, a *de novo* transcriptome (Das et al., 2016) was used to identify and characterize these enzymes. In this novel study, nine PDE contigs were extracted from the YO transcriptome. Sequence analysis assigned the *Gl-PDE* sequences to seven full length *PDEs*: *PDE1, PDE2, PDE4, PDE5, PDE7, PDE9*, and *PDE11* and two partial *PDEs*: *PDE3* and *PDE8*, thus indicating a high diversity of PDE genes found in decapod crustaceans. By similarity and specificity, this group comprises three cAMP-PDEs, two cGMP-PDEs, and four dual-specific PDEs. Moreover, seven PDE contigs were extracted from a *de novo* assembly of the *C. maenas* cardiac transcriptome (Tepolt & Palumbi 2015; Verbruggen et al., 2015). Sequence analysis assigned Cm-PDEs to four full-length *PDEs: PDE4, PDE8, PDE9,* and *PDE11* and three partial *PDEs*: *PDE1*, *PDE5* and *PDE3*. This prevalent expression of the assorted PDE genes in the YO of the blackback land crab and green shore crab might suggest a vital function in controlling the physiological differences that occur in the molting gland throughout the molt stages.

As a comparison to other arthropod models, the fruit fly, *Drosophila melanogaster*, genome encodes six different PDEs (Day et al., 2005), including the famous *dunce* gene that has been connected to several psychological issues because of its potential role in memory and learning (Walter & Kiger 1984; Bolger et al., 1993).

By examining the deduced DNA and amino acid sequences for Gl-PDEs, as well as the pairwise comparison between Gl-PDEs and Hs-PDEs, the following features were noted: the conserved catalytic domain in all PDE families (which is located in the carboxyl region of the protein and accounts for about 270 amino acids) shares high identity and is composed of a dense alpha-helical structure composed of 16 α-helices which in turn is divided into three subdomains. These α-helices form the active site, which is highly conserved among all PDE families (Charbonnea et al., 1986; Francis et al., 2011). Different affinities toward different cyclic nucleotide substrates are assumed to occur because of the slight variation in the catalytic domain for each PDE family (Manallack et al., 2005). Moreover, the active site is organized in two regions: the hydrolysis center and the hydrophobic recognition pocket (Wang et al.,2003; Liu et al., 2007). All PDEs have a similar structure regarding the hydrolysis center as they follow the general hydrolysis mechanism in breaking down and hydrolyzing cAMP/cGMP (Liu et al., 2007). The deep hydrophobic pocket, which can bind to either the substrate or the inhibitor, contains the glutamine switch (Q-switch). The Q-switch has a very important role in PDE nucleotide selectivity, as well as two metal binding sites important in catalytic activity, mainly divalent cations such as Zn^{2+} and possibly Mg^{2+}. Histidine residues are essential in chelating such metal ions (Xu et al., 2000; Houslay 2001; Richter et al., 2001; Liu et al., 2007; Ke et al., 2011). The basis of the glutamine switch is to either orient to hydrolyze cAMP or cGMP and, in that case, it will be constrained tightly with hydrogen bonds with the selective cyclic nucleotide. But in the

case of a dual PDE, the Q-switch rotates freely in either direction, depending on which substrate (cAMP/cGMP) needs to be hydrolyzed (Ke et al., 2011).

Interestingly, *G. lateralis* PDEs contained the conserved catalytic domain proximate to their C-terminus resembling other species. The length of the conserved catalytic domain in all studied Gl-PDEs ranged between 200-270 amino acids, which was comparable with previously characterized PDEs. Furthermore, the catalytic domain was initiated by the conserved signature sequence motif (YHN) in all PDEs, except in PDE9 which started with (FHN) and this was consistent with other orthologs from different invertebrate and vertebrate species (Broderick et al., 2003 and Wang et al., 2003). The metal binding motif with the specific sequence signature (HDX_2HX_4N), which has about 11 invariant amino acids (Manallack et al., 2005), aligned nicely and was identical among *G. lateralis* PDEs and PDEs from other species.

In contrast, the N-terminal regulatory domain, which borders the catalytic domain, is unique and distinct for each PDE family. N-terminal regulatory domains regulate the enzymatic activity of PDEs. Also, regions that autoinhibit the catalytic domains or regulate PDE subcellular localization are found in the N-terminal region (Omori & Kotera 2007; Azevedo et al., 2014).

The PDE1 family, a dual PDE, is the only family that depends exclusively on the influx of calcium ions to stimulate enzyme activity. This is an example of cross-talk between the cAMP and Ca^{2+} signal transduction pathways. Mammalian PDE1 has two Ca^{2+}/calmodulin (Ca^{2+}/CaM) regulatory domains that contain binding sites for the Ca^{2+}/CaM complex (Gross & Clark 1977; Meeker & Harden 1983; Gooraya et al., 2004; Gooraya & Cooper 2005). In contrast to the human PDE1C, Gl-PDE1 contained only one Ca^{2+}/CaM domain, which is similar to the *Drosophila* PDE1, suggesting that arthropods might not require both binding sites (Sonnenburg et al., 1995; Day et al., 2005).

The GAF domain represents a highly conserved sequence that has been conserved throughout more than 2 billion years of evolution. GAF got its name from the first three proteins in which the sequence was reported: c**G**MP-specific cyclic nucleotide PDEs, cyanobacterial *Anabaena* **A**denylyl cyclase, and *E. coli* transcription factor **F**hlA. Although PDE GAF domains bind cGMP, other proteins that contain these sequences do not bind cGMP (Martinez et al., 2002). Mammalian GAF-PDEs contain two tandem GAF domains, GAF-A and GAF-B, in the N-terminal region. Catalytic activation requires at least one of the GAF domains to bind a cGMP. For instance, PDE2 in mammalian systems contain two GAF domains, and GAF-B binds cGMP, whereas in PDE5, GAF-A binds this cyclic nucleotide (Lin et al., 2002; Francis 2005). Such binding causes a conformational change, allowing PKG to phosphorylate a nearby serine, which increases the catalytic activity. PDE11, the most recent PDE discovered and the one most related to PDE5, is still poorly understood, but it contains homologous regulatory domains as in PDE2 and PDE5. All GAF PDEs produce multiple variants via alternative splicing and different initiation sites.

Three GAF-PDEs, PDE2, 5, and 11 have been characterized in this study and they shared features and characteristics with other analogs, such as the conserved signature motif ($N(K/R)X_nFX_3DE$) specific to the GAF domains. Gl-PDE2 aligned nicely with *C. elegans* and *H.sapiens* with identical regions in the catalytic and regulatory domain. In contrast to Hs-PDE2, which contained two complete GAF domains, Gl-PDE2 contained only the GAF-B domain similar to the protozoan parasite *Trypanosoma brucei* Tb PDE2A and Tb PDE2B (Zoraghi & Seebeck, 2002). Only one GAF domain binds to cGMP or cAMP (Martins et al., 1982; Heikaus et al., 2009). The presence of a single GAF domain in Gl-PDE2 might be enough to bind small molecules such as cAMP/cGMP in an allosteric manner to stimulate the catalytic core domain or might bind a different small molecule, since it is originated from a different ancestor than PDE5 and PDE11.

Due to the fact that Gl-PDE2 is a dual enzyme, it might serve and mediate a cross-talk between cAMP and cGMP signaling pathways (Houslay 2001; Yuasa et al., 2001). Gl-PDE5, Cm-PDE5, Gl-PDE11, and Cm-PDE11 contained two complete tandem GAF domains homologous to the *Homo sapiens* PDEs; Hs-PDE5A and Hs-PDE11A4, respectively. The two GAF domains are linked by a variable region of amino acids which was consistent with another GAF PDEs (Yuasa et al., 2001; Makhlouf et al., 2006).

Gl-PDE9 and Cm-PDE9, cGMP-specific PDEs, contained the conserved catalytic domain which aligned with other ortholog PDEs; however, no other regulatory domains were detected. Mammalian PDE9 has a high affinity for cGMP, and it is 20-100 fold higher than PDE5 and PDE6, respectively. This might indicate that PDE9 will be distributed in cells with low titers of cGMP. Moreover, PDE9 contains the most variant catalytic domain when compared to the other PDE family members. It is likely that cGMP binds to the catalytic domain since it lacks the GAF domain (Fisher et al., 1998; Omori & Cotori 2007; Liu et al., 2008).

Gl-PDE4, Cm-PDE4 and Gl-PDE7 both hydrolyze cAMP and are highly similar to orthologs from other species (Figs. 2.6 & 2.10). In addition to the catalytic domain, Gl-PDE4 contains two Upstream Conserved Regions (UCRs) in its N-terminal region (Fig. 2.5) in a similar location to where GAF domains are found in PDE2, PDE5, and PDE11. This arrangement of domains is equivalent to *dunce* PDE, Dm-PDE4 and the human PDE, Hs-PDE4D. The regulatory domains, UCR1 and UCR2, are separated by sequences with less homology. Although the specific function of PDE4 is still open to assessment, studies on *Drosophila* revealed that a mutated *dunce* locus leads to impaired learning and memory in the fly (Bolger et al., 1993). In contrast, Cm-PDE4 contained only one UCR regulatory domain (UCR2) in its N-terminal region (Fig. 2.6). Human PDE4 isoforms exhibit both long forms (two UCR domains) and short forms (lack UCR1). This

complexity in PDE4 variants defines subcellular localization differences between the short and long forms (Xie et al., 2014). Gl-PDE7 is a PDE that hydrolyzes the second messenger, cAMP; it contained the conserved catalytic core domain and shared high similarities with its orthologs. Interestingly, the ORF of Gl-PDE7 displayed an extremely long sequence and ran to ~1039 AA when compared to either other *G. lateralis* PDEs or orthologs from different species. The 3' untranslated region showed a similar pattern and had an extended sequence ~ 7531 bp (Fig. 2.11). Such an observation might be the first of a kind to be seen in any PDE. Thus, it might have an importance in post-transcriptional regulation that is essential in mammalian cells (Matoulkova et al., 2012). Moreover, comparable to other studied PDEs to date, no specific regulatory domains were identified. Cm-PDE8 has a high affinity and specificity to hydrolyze cAMP. But is the only cAMP PDE that is insensitive to IBMX (Omori and Kotera, 2007). Cm-PDE8 displayed similar features compared to orthologs from other species in terms of the conserved catalytic domain and PAS regulatory domain. PAS is an acronym from the first three proteins in which in which the sequence was reported: **P**eriodic circadian protein, **A**ryl hydrocarbon receptor nuclear translocator protein, and **S**ingle-minded protein. The PAS domain is a molecular Velcro that binds small proteins and molecules (Tsai & Beavo, 2012). Unlike other PAS proteins, the regulation of PDE8 through PAS is still unknown (Demirbas et al., 2013). Previous studies on PDE8 family stated that it is a regulator in steroidogenesis in Leydig cells, as well as in adrenal steroidogenesis (Tsai & Beavo, 2012). Such findings might indicate the role of Cm-PDE8 on regulating the molting gland's ecdysteroidogenesis.

As shown in Figure 2. 21, phylogenetic analysis showed that Gl-PDEs and Cm-PDEs clustered with orthologs from invertebrate and vertebrate species indicating high homology and identity. The only exception observed was with PDE5. Invertebrate and vertebrate PDE5s

clustered in two divergent groups, which might indicate these PDE genes have two different ancestral origins. PDE1, 3, 4, 5, 8, 9, and 11 in both *G. lateralis* and *C. maenas* were closely related suggesting similar physiological systems.

YO *in-vitro* assays for both of our model species, green crab and backblack land crab, investigated the sensitivity to PDE inhibitors. Green crab YOs were sensitive to IBMX (3-isobutyl-l-methylxanthine), a potent non-selective PDE inhibitor (Table 2.4). Similar results have been reported in previous studies; IBMX significantly inhibits PDE in the crayfish *P. clarkii* (Nakatsuji et al., 2006). Moreover, in experiments done on *Manduca* larvae, Smith (1993) reported that the incubation of prothoracic glands (which are counterpart to crustacean YOs) with the molting hormone (PTTH) and IBMX *in-vitro,* blocked the degradation of cAMP but not cGMP. Also, PDE activity was seen in the absence of IBMX in the previous study (Smith 1993). Inhibition of PDEs, except PDE8 and PDE9, by IBMX caused an elevation in cAMP activity in the human adrenal gland (Beavo et al., 1970; Tomes et al., 1993). Vinpocetine, a selective PDE1 inhibitor extensively used on rats in studies on neurodegenerative diseases as in the case of Alzheimer's showed inhibition of PDE1 (Ahn et al., 1989; Deshmuch et al., 2010). Likewise, memory is enhanced in rodents and humans by vinpocetine, which increases cAMP levels (Deshmukh et al., 2011; Medina et al., 2011). Our results showed that green crab YOs were inhibited by vinpocetine, which might indicate a potential role of PDE1 in controlling ecdysteroid synthesis. EHNA, a specific PDE2 inhibitor, inhibited ecdysteroid secretion by the green crab YOs. Similar effects were also observed in mammalian nervous tissues but was not effective in pharmacological tests (Bessodes et al., 1982; Gomez & Breitenbucher 2013). Zaprinast, a selective PDE5/PDE11 inhibitor, inhibited the green crab YOs. Zaprinast, the precursor of sildenafil (Viagra), inhibits PDE5 and PDE11 with different affinities. Zaprinast inhibited ecdysteroidogenesis in YOs of green crabs especially at

higher concentrations. Similar observations were seen in previous studies: *In-vitro* YO assays in crayfish, *P. clarkia,* demonstrated that Zaprinast partially inhibited PDE activity (Nakatsuji et al., 2006). Experiments on *Drosophila* showed that Zaprinast suppressed PDE6, the closest morphologically to PDE5, in Malpighian (renal) tubules (Broderick et al., 2004; Day et al., 2005).

Interestingly, rolipram (selective-PDE4 inhibitor), dipyridamole (selective-PDE7 inhibitor), and BC11-38 (potent PDE11 inhibitor) had no effect on the ecdysteroid secretion in all *in-vitro* YO assays of the green crab. These results differ from previously published studies on other species: *in-vivo* administration of rolipram showed that there were effective neurodegenerative and neuroprotective impacts on the spinal cord of rat embryos (Richer et al., 2001). Moreover, rolipram inhibited PDE4 in *Drosophila* and mice in therapeutic experiments which were done to find a cure for Fragile-X syndrome (Nikulina et al., 2004; Choi et al., 2015). Dipyridamole was used as an anti-inflammatory agent to inhibit PDE7. This chemical was used in specific locations where PDE7 is normally distributed, such as T-cells, B-cells, skeletal and cardiac muscles (Gresele et al., 2011). This might be the reason for the lack of inhibition of Gl-PDE7 in the YO. The novel PDE11 inhibitor, BC 11-38, was used in mice to inhibit PDE11A4 isoform (homologous to Gl-PDE11) in the brain, particularly the hippocampus, and showed tremendous effects as a therapeutic target for mood and depressive disorders (Kelly 2017). Even though this drug is known to have a high membrane permeability, it is still under investigation because of lack of information concerning whether it can cross the blood-brain barrier. Thus, might be the reason that YO ecdyseroidogenesis was not affected when exposed to the potent PDE11 inhibitor, BC11-38. (Ceyhan et al., 2012; Kelly 2017). Another reason might be, the shortage of adequate information of the nature of PDE11 and how it selects specific inhibitors (Weeks et al., 2009). *G.*

lateralis YOs were insensitive to all tested PDE inhibitors in this work and that might point to the difference of the YOs regulatory systems between the two species (Table 2.5).

As illustrated in figure 2.23, a tissue distribution panel was constructed to compare the expression of the different PDEs in the YO with other tissues. Gl-PDE2 and Gl-PDE5 were expressed at their highest levels in heart tissue. Mammalian cardiomyocytes express PDE2, but not PDE5 (Weber et al., 2017). PDE2 was expressed at reasonably high levels compared to its expression in other tissues. Remarkably, Gl-PDE11 was highly expressed in the testis compared to other examined tissues. That pattern is parallel to the mammalian system in which PDE11 is expressed in the prostate and testis, as it is thought to function in spermatogenesis (Franscis 2005). Moreover, low expression of Gl-PDEs in the YO was not surprising as these molting glands were harvested from intermolt animals in which results were anticipated. Fig 2.24 shows the tissue expression of Cm-PDEs; Cm-PDE4, 8, and 9 were expressed in very low levels. Cm-PDE11 was expressed in high levels which might indicate a powerful function of this dual PDE in a variety of tissues, especially in the thoracic ganglion, eyestalk ganglia, and YO. Once again, the different gene expression patterns observed in the two studied species might be due of different metabolic systems and variable modes of regulation.

Conclusions

Nine contigs encoding seven full-length PDE sequences and two contigs encoding partial-length PDE were identified in the *Gecarcinus lateralis* Y-organ transcriptome. Seven contigs encoding four full-length PDE sequences and three contigs encoding partial-length PDE were identified in the *Carcinus maenas* transcriptome. Multiple sequence alignments showed high sequence identities with orthologs from other species mainly in the catalytic (PDEase) domain and in the regulatory domains. Sequence analysis assigned the Gl-PDE and Cm-PDEs sequences to

PDE1, PDE2, PDE3, PDE4, PDE5, PDE7, PDE8, PDE9, and PDE11 classes, indicating a high diversity of PDE genes are found in decapod crustaceans. PDE inhibitors IBMX, vinpocetine, EHNA, and Zaprinast inhibited YO ecdysteroid secretion, which suggests that PDE1, PDE2, and PDE5/11 play major roles in controlling cyclic nucleotide levels in the YO of green crab, *C. maenas.* The backblack land crab, *G. lateralis*, YOs showed no response when incubated in the same PDE inhibitors. Cm-PDE11, a dual PDE, was expressed at high levels in different tissues. This might indicate a crucial role of PDE11 in cAMP/cGMP signaling pathways; especially the MIH signaling pathway in the molting gland.

Table 2.1: Shows the nine PDE contigs extracted from the *G. lateralis* YO transcriptome. PDE1, PDE2, PDE4, PDE5, PDE7, PDE9, and PDE11 are full lengths, PDE3 and PDE8 are partial lengths. Abbreviations; AA: Amino Acid; bp: base pair; Ca^{+2}/CaM: calcium/calmodulin; cAMP: cyclic adenosine 3′,5′-monophosphate; cGMP: cyclic guanosine 3′,5′-monophosphate; PKA: protein kinase A; PKG: protein kinase G.

PDE Family	Contig #	Contig length	ORF length	Descriptive Name	Substrate Specificity cAMP	Substrate Specificity cGMP	Regulator
PDE1	c251477_g2_i1	3037 bp	690 AA	CaM-dependent PDE	+	+	Ca^{+2}/CaM
PDE2	c263693_g1_i1	4729 bp	940 AA	cGMP-stimulated PDE	+	+	cGMP
PDE3	c243404_g2_i1	1235 bp	275 AA	cGMP-inhibited PDE	+	+	cGMP
PDE4	c226920_g1_i2	6130 bp	804 AA	cAMP-specific PDE	+	-	PKA
PDE5	c269402_g1_i2	6761 bp	861 AA	cGMP-binding PDE	-	+	PKG
PDE7	c266596_g1_i3	7531 bp	1039 AA	High affinity cAMP-specific PDE	+	-	N/A
PDE8	c263162_g1_i1	1675 bp	350 AA	High affinity cAMP-specific and IBMX-insensitive PDE	+	-	N/A
PDE9	c244059_g1_i2	4111 bp	699 AA	High affinity cGMP-specific PDE	+	-	N/A
PDE11	c263764_g1_i1	5752 bp	864 AA	Dual specificity PDE	+	+	N/A

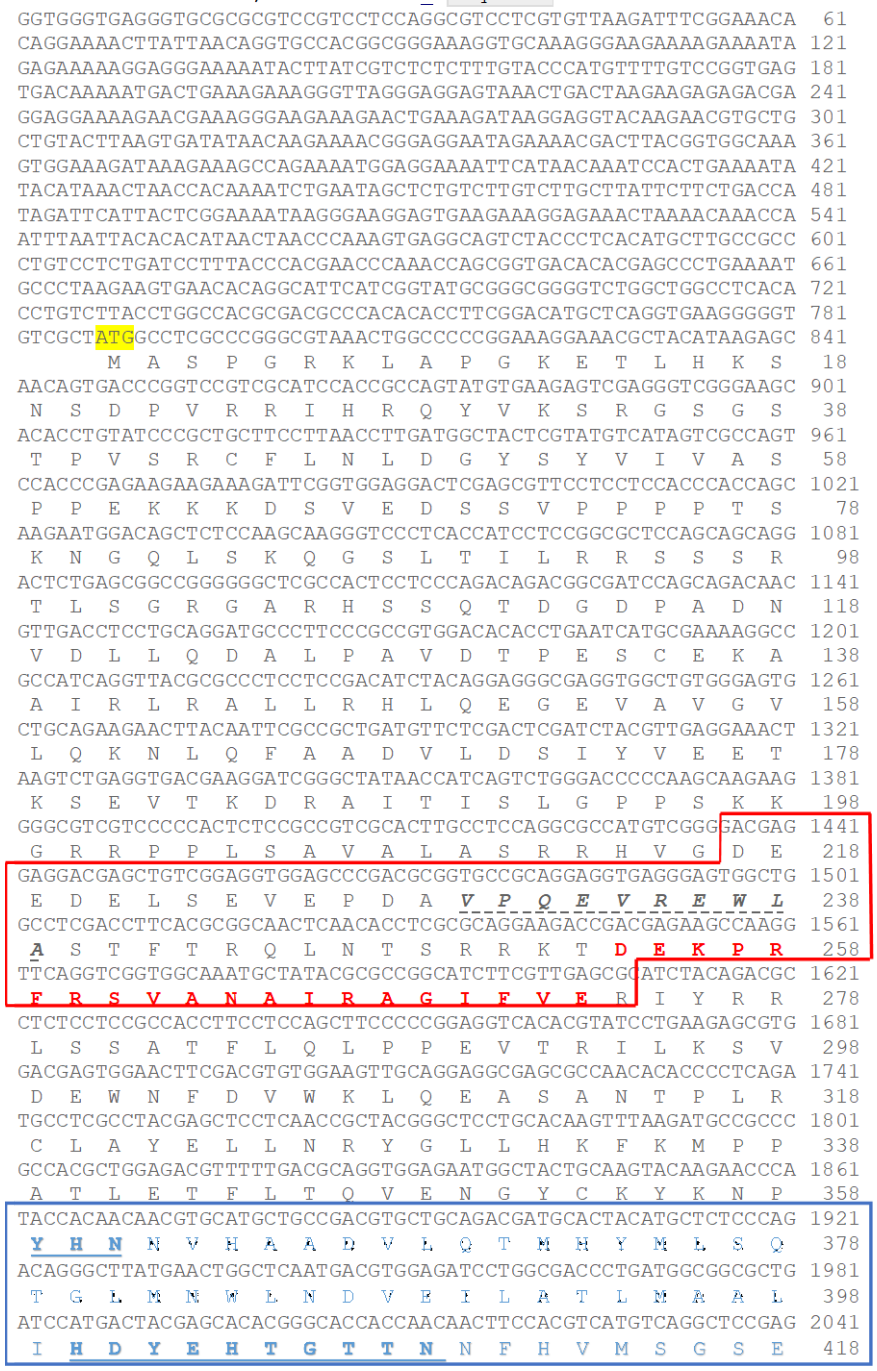
Table 2.2: Shows the seven PDE contigs extracted from the *C. maenas* transcriptomes. PDE4, PDE8, PDE9, and PDE11 are full lengths, PDE1, PDE3 and PDE5 are partial lengths. Abbreviations; AA: Amino Acid; bp: base pair; Ca^{+2}/CaM: calcium/calmodulin; cAMP: cyclic adenosine 3′,5′-monophosphate; cGMP: cyclic guanosine 3′,5′-monophosphate; PKA: protein kinase A; PKG: protein kinase G.

PDE Family	Contig #	Contig length	ORF length	Descriptive Name	Substrate Specificity cAMP	cGMP	Regulator
PDE1	comp81969_c0_seq2	2014bp	355 AA	CaM dependent PDE	+	+	Ca^{+2}/CaM
PDE3	comp74103_c0_seq1	1168 bp	256 AA	cGMP-inhibited PDE	+	+	cGMP
PDE4	lcl|Cmaenas_contig_7516	6122 bp	787 AA	cAMP-specific PDE	+	-	PKA
PDE5	comp86676_c0_seq7	2996 bp	861 AA	cGMP-binding PDE	-	+	PKG
PDE8	lcl|Cmaenas_contig_4936	3007 bp	975 AA	High affinity cAMP-specific and IBMX-insensitive PDE	+	-	N/A
PDE9	lcl|Cmaenas_contig_6558	4356 bp	620 AA	High affinity cGMP-specific PDE	+	-	N/A
PDE11	lcl|Cmaenas_contig_16769	5839 bp	859 AA	Dual specificity PDE	+	+	N/A

Table 2.3. Oligonucleotide primers used in qPCR to identify gene expression of Gl-PDEs and Cm-PDEs in different tissues. Abbreviations: Gl, *Gecarcinus lateralis*; Cm, *Carcinus maenas*; F, Forward; R, Reverse, PDE, cyclic nucleotide phosphodiesterase; Numbers (1,2,4,5,7,8,9,11), PDE family.

Primer name	Primer sequence (5'-3')	Amplicon Product (bp)	Annealing Temp.
Gl-PDE1 -F2	GGTGGCAAAGTGGAAAGATAAAG	226	62 C°
Gl-PDE1 -R2	CCTCCTCGTCTCTCTTCTTAGT		62 C°
Gl-PDE2 -F2	GGTGGTAGTGGCACGTTTAT	301	62 C°
Gl-PDE2 -R2	TCCCTCTTTCCTTCCTCTTCT		62 C°
Gl-PDE5 -F2	CAGACCACCGGATGCTTATT	316	62 C°
Gl-PDE5 -R1	TCCTCGACCCGATTCTATGT		62 C°
Gl-PDE11-F2	GACTCCAGACTTGGTTCTTTCC	322	62 C°
Gl-PDE11-R2	CGACTGATGTCACTTGCATATC		62 C°
Cm-PDE4-F1	TTACCTATGGCGGCGAATG	103	62 C°
Cm-PDE4-R1	TGGGTCTGAATGGTTTCCAG		62 C°
Cm-PDE8-F1	GATATGTTTGATGCGTGGGATG	103	62 C°
Cm-PDE8-R1	TCTGTTCCTGTTCTTCCTGTTC		62 C°
Cm-PDE9-F1	GAAGTGTTTCGCCGCTTTC	101	62 C°
Cm-PDE9-R1	CCATACATCATCTGGGTCACG		62 C°
Cm-PDE11-F1	TTTCTGGATCTGGATCTGATTGG	102	62 C°
Cm-PDE11-R1	ATGATAGGTCACGTTGCGATAG		62 C°

GlPDE1 3037 nucleotides, structure: C sequence



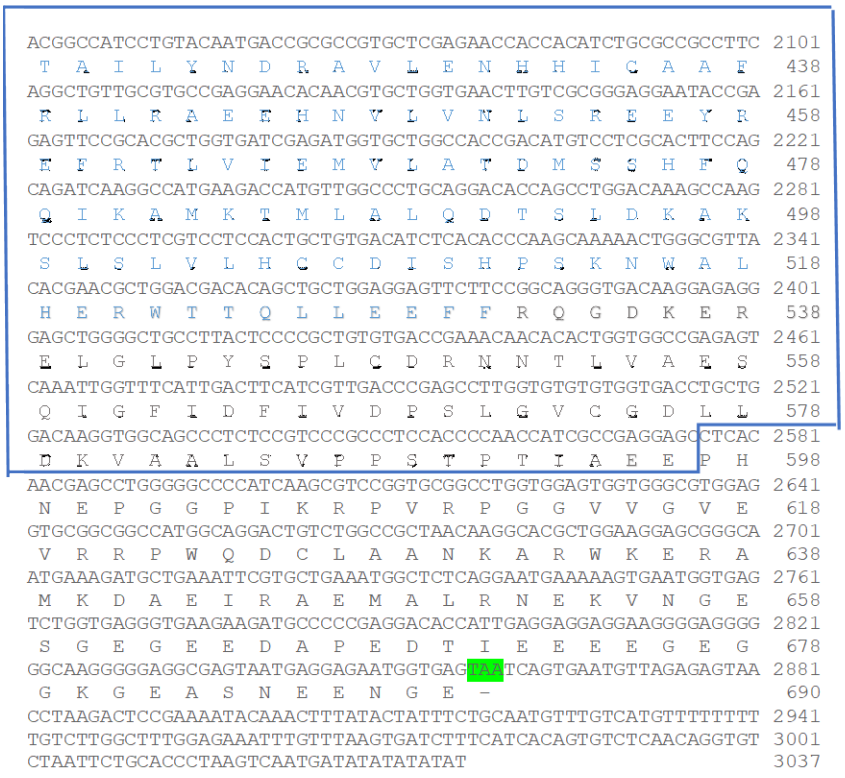
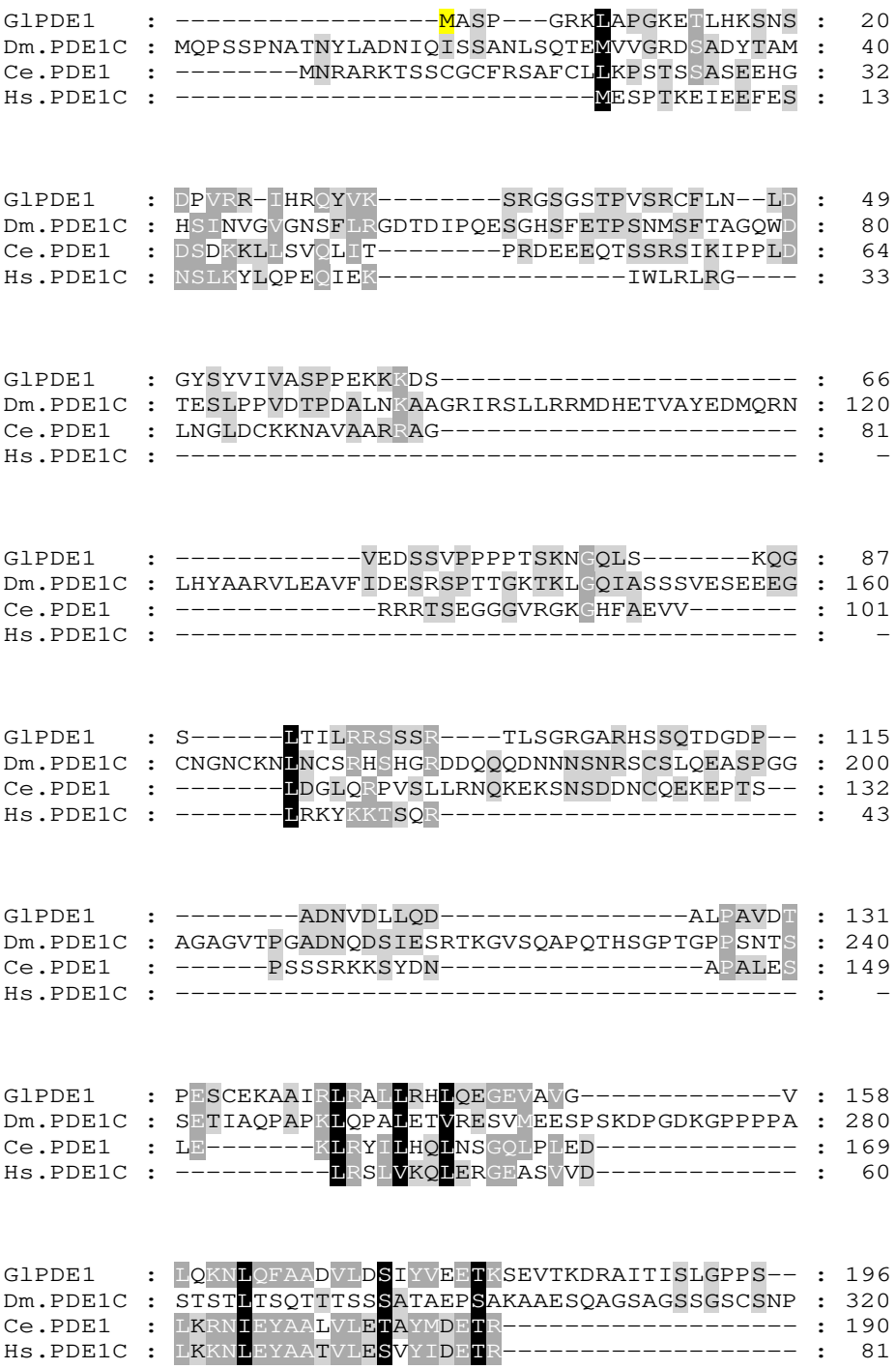
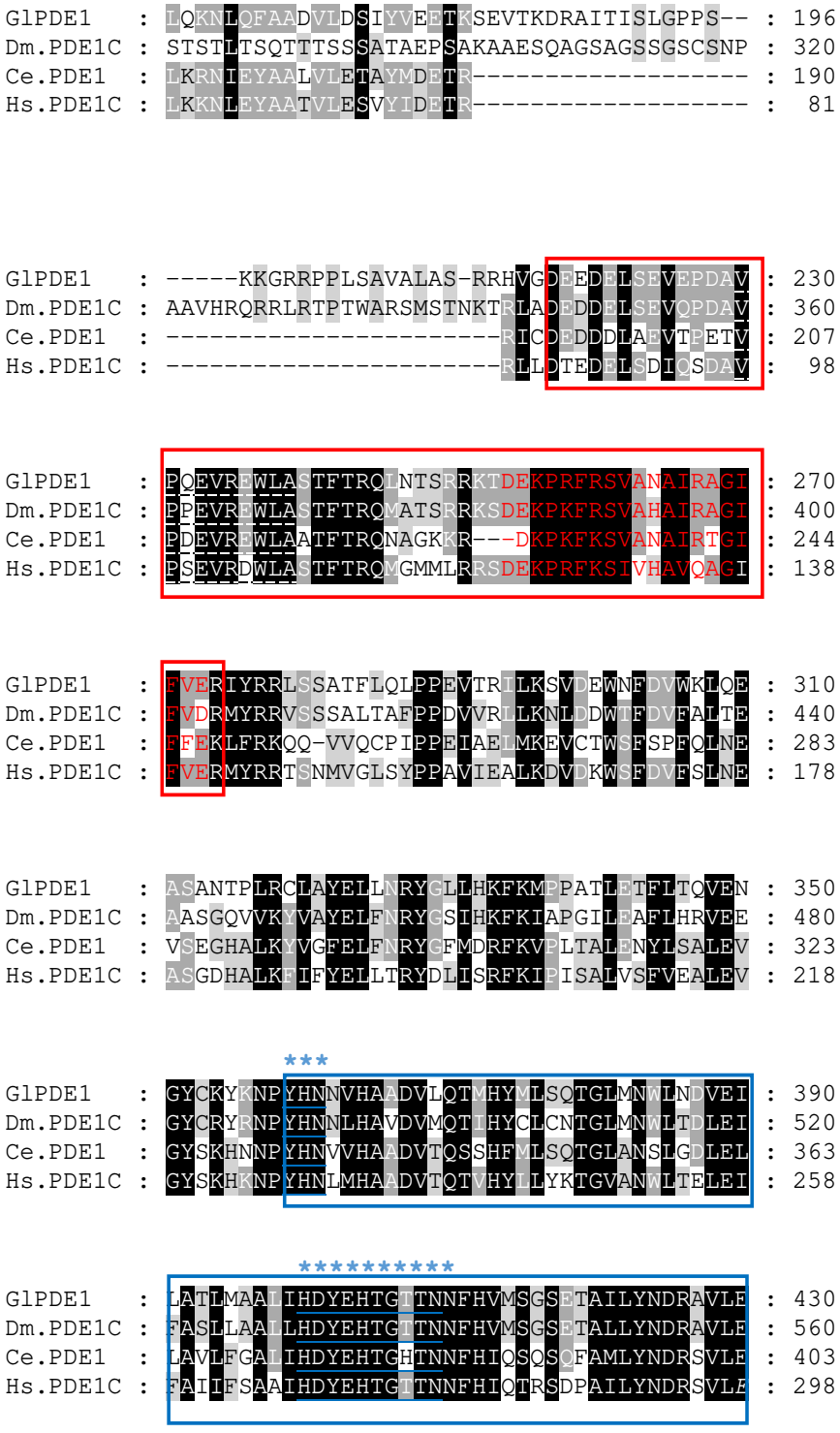
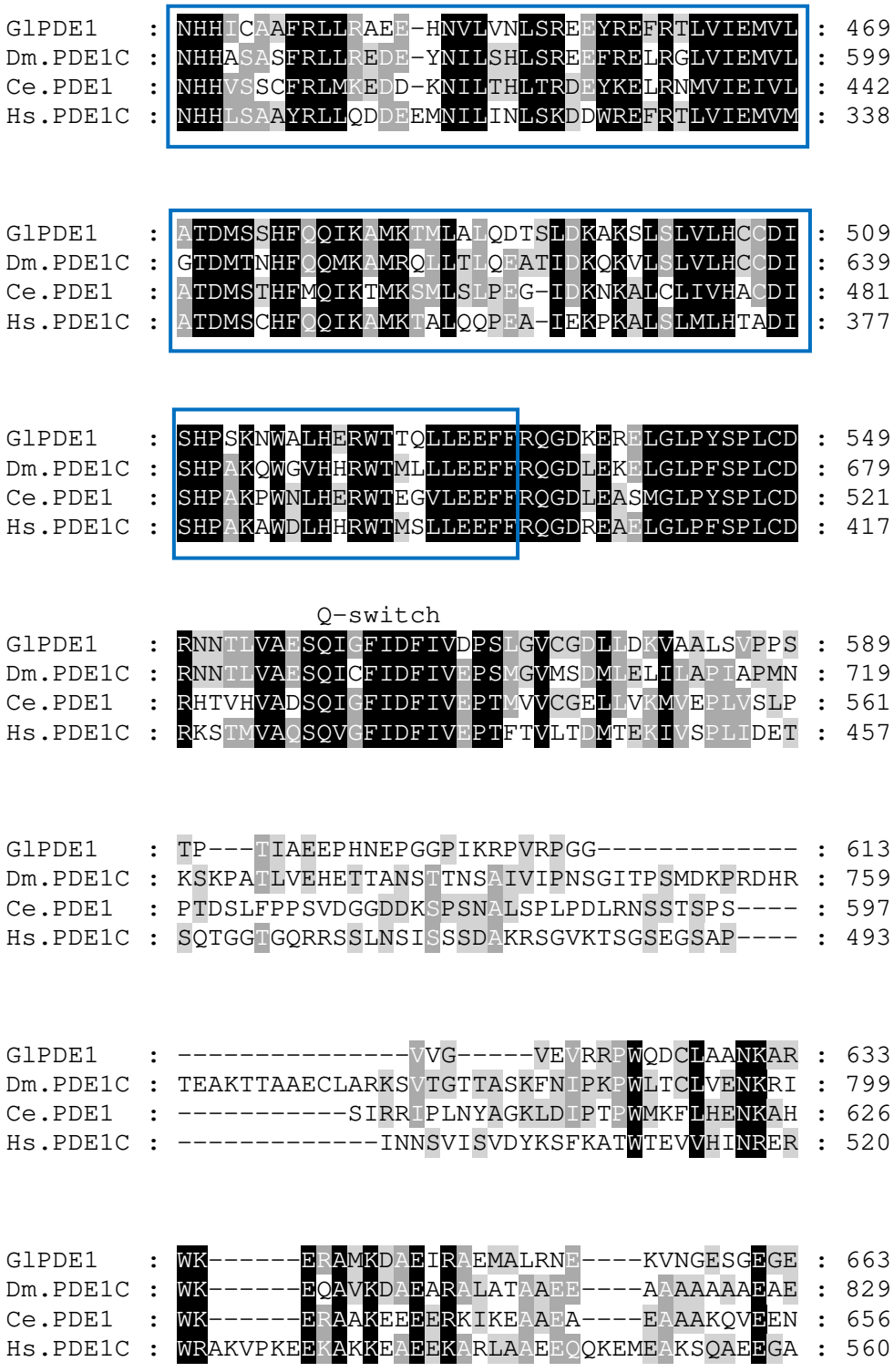


Figure.2.1.Nucleotide and amino acid sequence of cDNA encoding Gl-PDE1. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. Calcium/calmodulin regulatory domain is indicated by a red box and contains the PDEase_I_N found only in the N-terminus. Ca^{+2}/calmodulin binding site is indicated in bold red. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.


GlPDE1 : ----------MASP---GRKLAPGKETLHKSNS : 20
Dm.PDE1C : MQPSSPNATNYLADNIQISSANLSQTEMVVGRDSADYTAM : 40
Ce.PDE1 : --------MNRARKTSSCGCFRSAFCLLKPSTSSASEEHG : 32
Hs.PDE1C : ----------MESPTKEIEEFES : 13
GlPDE1 : DPVRR-IHRQYVK--------SRGSGSTPVSRCFLN--LD : 49
Dm.PDE1C : HSINVGVGNSFLRGDTDIPQESGHSFETPSNMSFTAGQWD : 80
Ce.PDE1 : DSDKKLLSVQLIT--------PRDEEEQTSSRSIKIPPLD : 64
Hs.PDE1C : NSLKYLQPEQIEK----------IWLRLRG---- : 33
GlPDE1 : GYSYVIVASPPEKKKDS---------- : 66
Dm.PDE1C : TESLPPVDTPDALNKAAGRIRSLLRRMDHETVAYEDMQRN : 120
Ce.PDE1 : LNGLDCKKNAVAARRAG---------- : 81
Hs.PDE1C : ---------- : -
GlPDE1 : ----------VEDSSVPPPPTSKNGQLS-------KQG : 87
Dm.PDE1C : LHYAARVLEAVFIDESRSPTTGKTKLGQIASSSVESEEEG : 160
Ce.PDE1 : ----------RRRTSEGGGVRGKGHFAEVV------- : 101
Hs.PDE1C : ---------- : -
GlPDE1 : S------LTILRRSSSR----TLSGRGARHSSQTDGDP-- : 115
Dm.PDE1C : CNGNCKNLNCSRHSHGRDDQQQDNNNSNRSCSLQEASPGG : 200
Ce.PDE1 : -------LDGLQRPVSLLRNQKEKSNSDDNCQEKEPTS-- : 132
Hs.PDE1C : -------LRKYKKTSQR---------- : 43
GlPDE1 : --------ADNVDLLQD----------ALPAVDT : 131
Dm.PDE1C : AGAGVTPGADNQDSIESRTKGVSQAPQTHSGPTGPPSNTS : 240
Ce.PDE1 : ------PSSSRKKSYDN----------APALES : 149
Hs.PDE1C : ---------- : -
GlPDE1 : PESCEKAAIRLRALLRHLQEGEVAVG----------V : 158
Dm.PDE1C : SETIAQPAPKLQPALETVRESVMEESPSKDPGDKGPPPPA : 280
Ce.PDE1 : LE-------KLRYILHQLNSGQLPILED---------- : 169
Hs.PDE1C : ----------LRSLVKQLERGEASVVD---------- : 60
GlPDE1 : LQKNLQFAADVLDSIYVEETKSEVTKDRAITISLGPPS-- : 196
Dm.PDE1C : STSTLTSQTTTSSSATAEPSAKAAESQAGSAGSSGSCSNP : 320
Ce.PDE1 : LKRNIEYAALVLETAYMDETR---------- : 190
Hs.PDE1C : LKKNLEYAATVLESVYIDETR---------- : 81


GlPDE1 : LQKNLQFAADVLDSIYVEETKSEVTKDRAITISLGPPS-- : 196
Dm.PDE1C : STSTLTSQTTTSSSATAEPSAKAAESQAGSAGSSGSCSNP : 320
Ce.PDE1 : LKRNIEYAALVLETAYMDETR------------------- : 190
Hs.PDE1C : LKKNLEYAATVLESVYIDETR------------------- : 81
GlPDE1 : -----KKGRRPPLSAVALAS-RRHVGDEEDELSEVEPDAV : 230
Dm.PDE1C : AAVHRQRRLRTPTWARSMSTNKTRLADEDDELSEVQPDAV : 360
Ce.PDE1 : ------------------------RICDEDDDLAEVTPETV : 207
Hs.PDE1C : ------------------------RLLDTEDELSDIQSDAV : 98
GlPDE1 : PQEVREWLASTFTRQLNTSRRKTDEKPRFRSVANAIRAGI : 270
Dm.PDE1C : PPEVREWLASTFTRQMATSRRKSDEKPKFRSVAHAIRAGI : 400
Ce.PDE1 : PDEVREWLAATFTRQNAGKKR---DKPKFKSVANAIRTGI : 244
Hs.PDE1C : PSEVRDWLASTFTRQMGMMLRRSDEKPRFKSIVHAVQAGI : 138
GlPDE1 : FVERIYRRLSSATFLQLPPEVTRILKSVDEWNFDVWKLQE : 310
Dm.PDE1C : FVDRMYRRVSSSALTAFPPDVVRLLKNLDDWTFDVFALTE : 440
Ce.PDE1 : FFEKLFRKQQ-VVQCPIPPEIAELMKEVCTWSFSPFQLNE : 283
Hs.PDE1C : FVERMYRRTSNMVGLSYPPAVIEALKDVDKWSFDVFSLNE : 178
GlPDE1 : ASANTPLRCLAYELLNRYGLLHKFKMPPATLETFLTQVEN : 350
Dm.PDE1C : AASGQVVKYVAYELFNRYGSIHKFKIAPGILEAFLHRVEE : 480
Ce.PDE1 : VSEGHALKYVGFELFNRYGFMDRFKVPLTALENYLSALEV : 323
Hs.PDE1C : ASGDHALKEIFYELLTRYDLISRFKIPISALVSFVEALEV : 218
***
GlPDE1 : GYCKYKNPYHNNVHAADVLQTMHYMLSQTGLMNWLNDVEI : 390
Dm.PDE1C : GYCRYRNPYHNNLHAVDVMQTIHYCLCNTGLMNWLTDLEI : 520
Ce.PDE1 : GYSKHNNPYHNVVHAADVTQSSHFMLSQTGLANSLGDLEL : 363
Hs.PDE1C : GYSKHKNPYHNLMHAADVTQTVHYLLYKTGVANWLTELEI : 258
****
GlPDE1 : LATLMAALIHDYEHTGTTNNFHVMSGSETAILYNDRAVLE : 430
Dm.PDE1C : FASLLAALLHDYEHTGTTNNFHVMSGSETALLYNDRAVLE : 560
Ce.PDE1 : LAVLFGALIHDYEHTGHTNNFHIQSQSQFAMLYNDRSVLE : 403
Hs.PDE1C : FAIIFSAAIHDYEHTGTTNNFHIQTRSDPAILYNDRSVLA : 298


GlPDE1 : NHHICAAFRLLRAEE-HNVLVNLSREEYREFRTLVIEMVL : 469
Dm.PDE1C : NHHASASFRLLREDE-YNILSHLSREEFRELRGLVIEMVL : 599
Ce.PDE1 : NHHVSSCFRLMKEDD-KNILTHLTRDEYKELRNMVIEIVL : 442
Hs.PDE1C : NHHLSAAYRLLQDDEEMNILINLSKDDWREFRTLVIEMVM : 338
GlPDE1 : ATDMSSHFQQIKAMKTMLALQDTSLDKAKSLSLVLHCCDI : 509
Dm.PDE1C : GTDMTNHFQQMKAMRQLLTLQEATIDKQKVLSLVLHCCDI : 639
Ce.PDE1 : ATDMSTHFMQIKTMKSMLSLPEG-IDKNKALCLIVHACDI : 481
Hs.PDE1C : ATDMSCHFQQIKAMKTALQQPEA-IEKPKALSLMLHTADI : 377
GlPDE1 : SHPSKNWALHERWTTQLLEEFFRQGDKERELGLPYSPLCD : 549
Dm.PDE1C : SHPAKQWGVHHRWTMLLLEEFFRQGDLEKELGLPFSPLCD : 679
Ce.PDE1 : SHPAKPWNLHERWTEGVLEEFFRQGDLEASMGLPYSPLCD : 521
Hs.PDE1C : SHPAKAWDLHHRWTMSLLEEFFRQGDREAELGLPFSPLCD : 417
Q-switch
GlPDE1 : RNNTLVAESQIGFIDFIVDPSLGVCGDLLDKVAALSVPPS : 589
Dm.PDE1C : RNNTLVAESQICFIDFIVEPSMGVMSDMLELILAPIAPMN : 719
Ce.PDE1 : RHTVHVADSQIGFIDFIVEPTMVVCGELLVKMVEPLVSLP : 561
Hs.PDE1C : RKSTMVAQSQVGFIDFIVEPTFTVLTDMTEKIVSPLIDET : 457
GlPDE1 : TP---TIAEEPHNEPGGPIKRPVRPGG---------- : 613
Dm.PDE1C : KSKPATLVEHETTANSTTNSAIVIPNSGITPSMDKPRDHR : 759
Ce.PDE1 : PTDSLFPPSVDGGDDKSPSNALSPLPDLRNSSTSPS---- : 597
Hs.PDE1C : SQTGGTGQRRSSLNSISSSDAKRSGVKTSGSEGSAP---- : 493
GlPDE1 : ----------VVG-----VEVRRPWQDCLAANKAR : 633
Dm.PDE1C : TEAKTTAAECLARKSVTGTTASKFNIPKPWLTCLVENKRI : 799
Ce.PDE1 : ----------SIRRIPLNYAGKLDIPTPWMKFLHENKAH : 626
Hs.PDE1C : ----------INNSVISVDYKSFKATWTEVVHINRER : 520
GlPDE1 : WK------ERAMKDAEIRAEMALRNE----KVNGESGEGE : 663
Dm.PDE1C : WK------EQAVKDAEARALATAAEE----AAAAAAEAE : 829
Ce.PDE1 : WK------ERAAKEEEERKIKEAAEA----EAAAKQVEEN : 656
Hs.PDE1C : WRAKVPKEEKAKKEAEEKARLAAEEQQKEMEAKSQAEEGA : 560

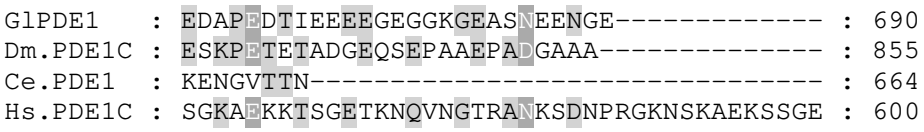
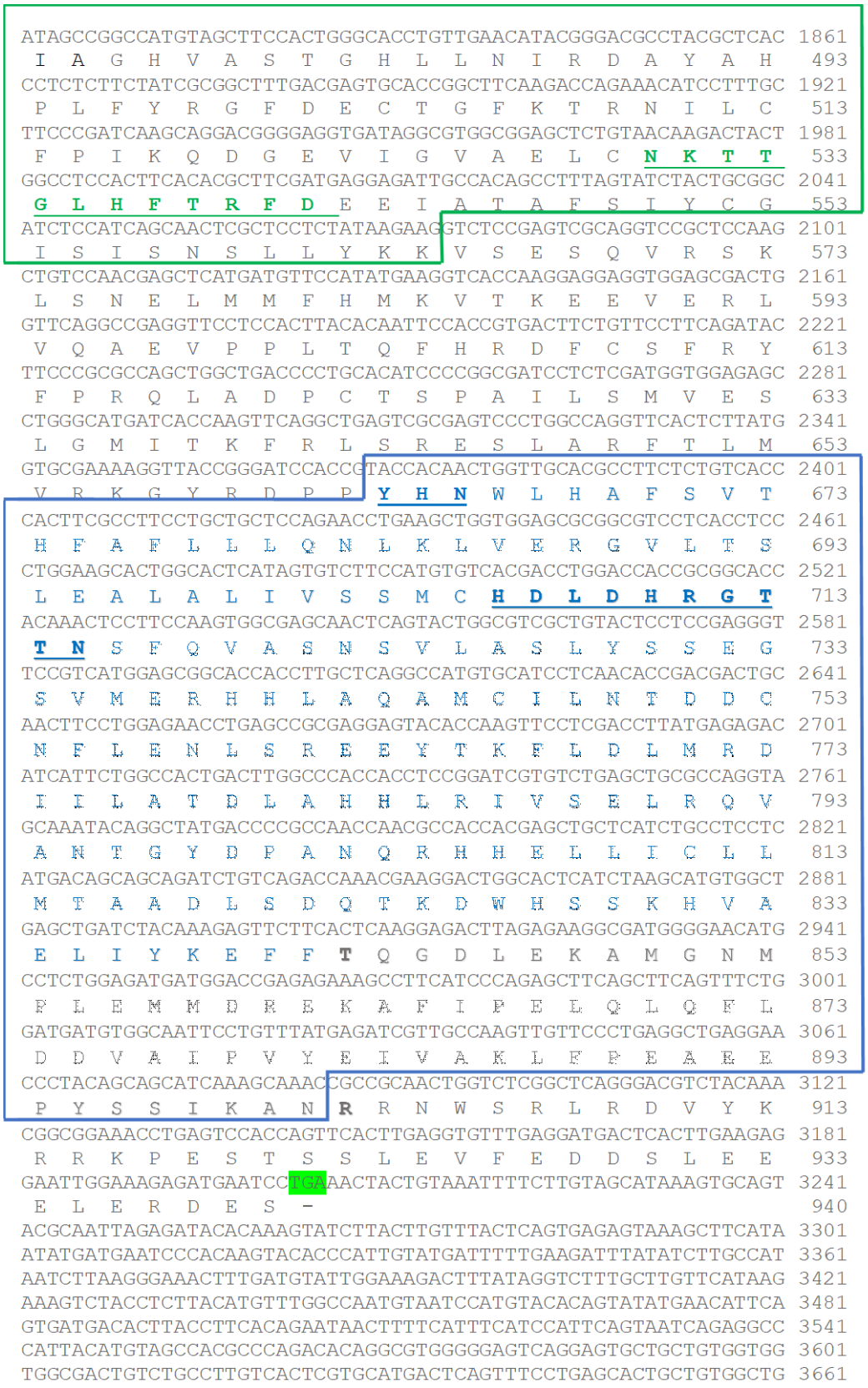


Figure 2.2. Multiple alignment of deduced amino acid sequences of PDE1 proteins in one crustacean species, one insect species, one nematode species and one mammalian species. Abbreviations: Gl: *G lateralis*; Dm:, *D melanogaster*; Ce: *C elegans*; Hs: *H sapiens*. Black shading indicates that amino acid residues that are identical or similar in all sequences; gray shading indicates identical or similar amino acids in most of the sequences. Dashes indicate gaps introduced to optimize the alignment and the boxes indicate highly conserved domains. Blue asterisks indicate the signature sequences in the catalytic domain. The colors of the boxes correspond to the colors of the domains in Fig. 2. 1.

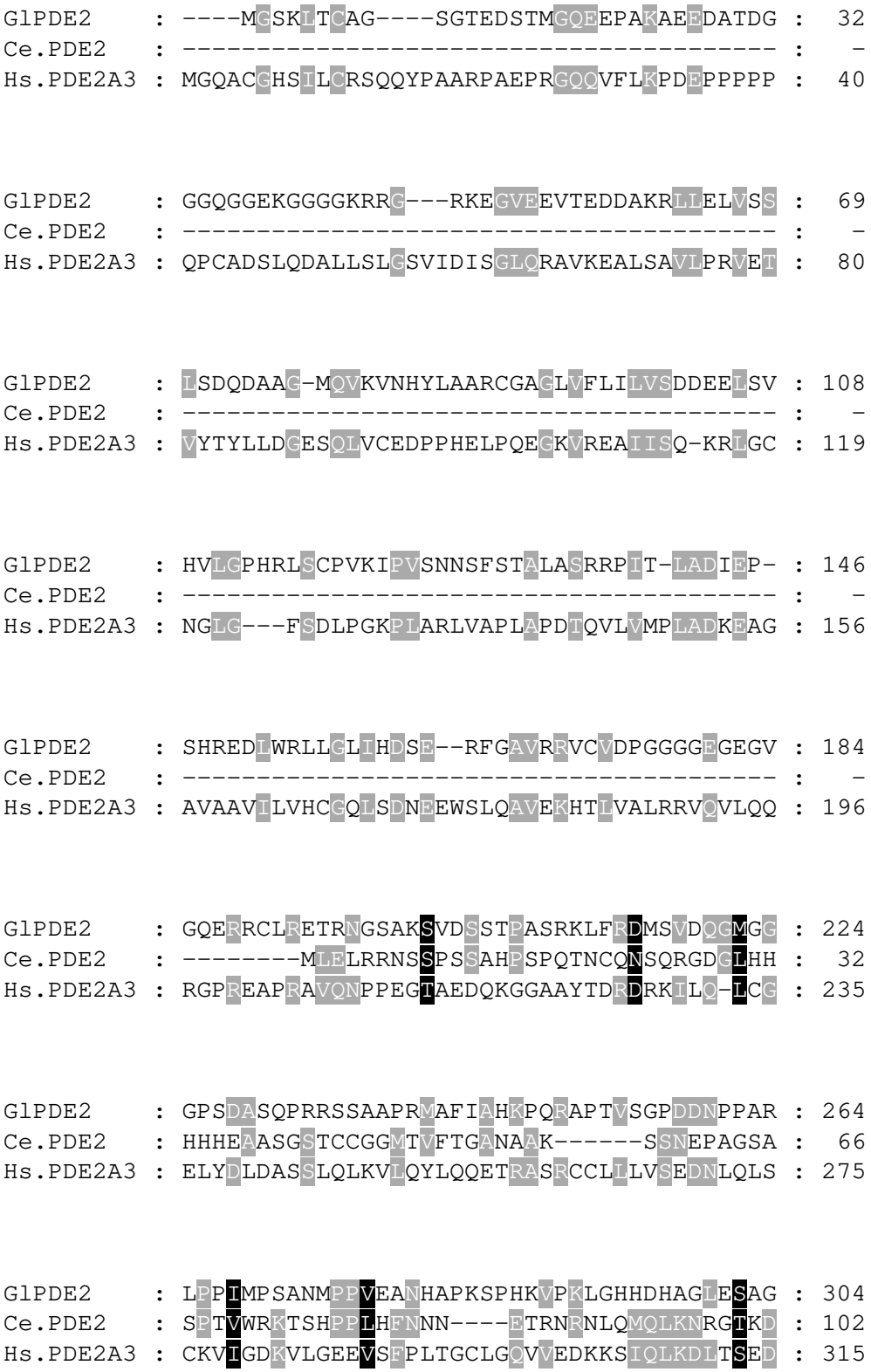
GLPDE2 4729 nucleotides, structure: sequence T

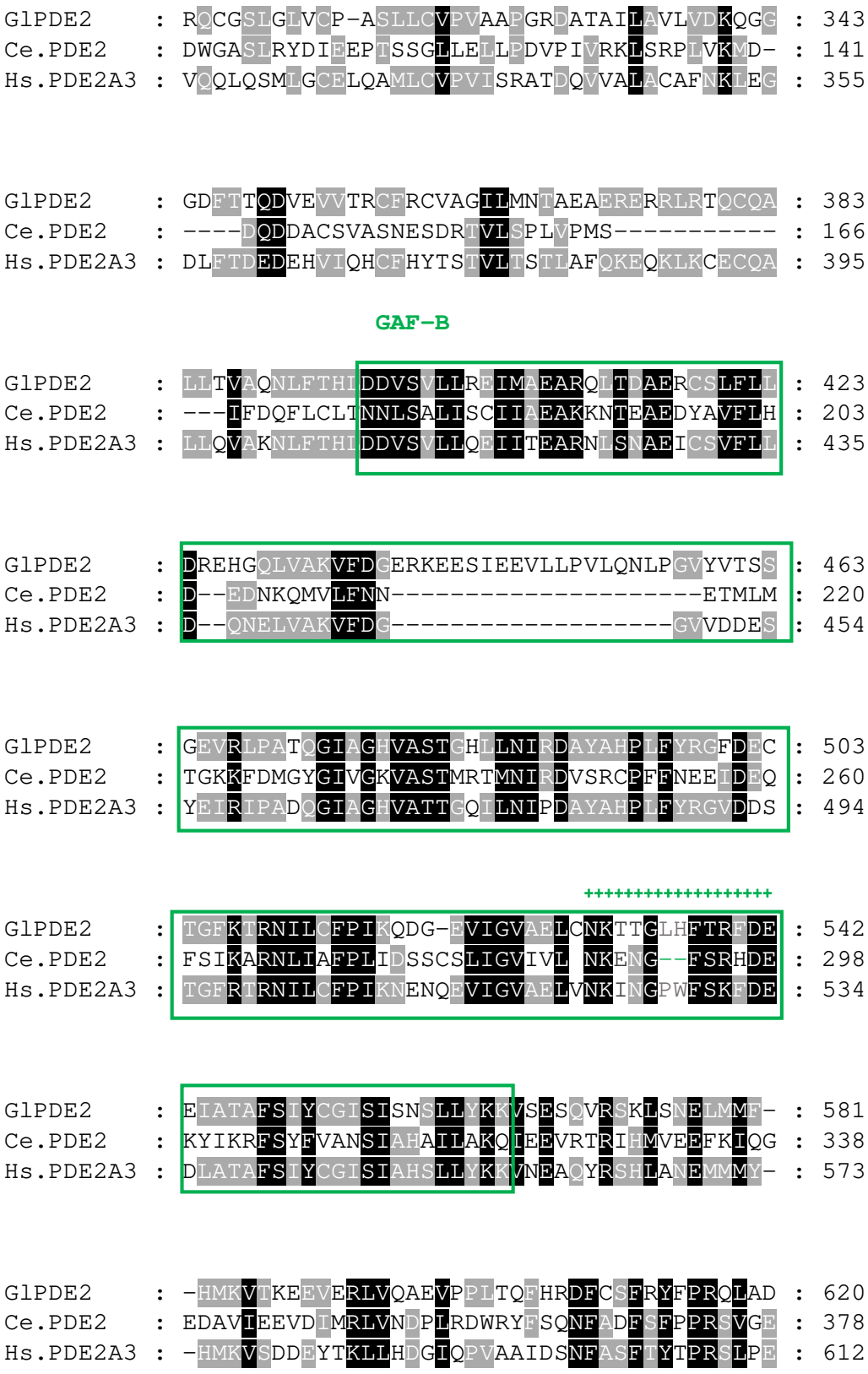
```
CACGTTTATAGTGTTGCCAGTTACCTGTGGTGGTGTGGGGCTGAGCAGTCGTGTAAAACT   61
AACCTCCGCTACTTGTTTTAAAAGAGGACGTGAACTTGTACTTCCTGTCTGGTTTTAGTG  121
ACGCGACTTCAAGAACAAGGATTGAACTTCTGTCCTGAACCTGTGCGTGCGAGTGTGCGT  181
GAAGTGAGAGAGGTGGAGGGTGAGAGGGAGGGAGAGGCGTCGACCGAACACTTAGTTACT  241
CGTGATGTGTGTCTCGGCGGCGAGGCCATGCTGTGGAGGAAGGTCGACTAGATGGCCTTT  301
ACCACTGAGCTCATGAAGAGGTGACCTAGGATGGGGTCACTCCCTCCTGCCCGAGACGCC  361
TGACGTGGCTTGACCCGAGGCATGGGGTCCAAGTTGACCTGCGCCGGCAGTGGCACGGAG  421
                      M  G  S  K  L  T  C  A  G  S  G  T  E   13
GACTCCACTATGGGGCAAGAGGAGCCAGCAAAGGCGGAGGAAGATGCCACTGATGGAGGA  481
 D  S  T  M  G  Q  E  E  P  A  K  A  E  E  D  A  T  D  G  G   33
GGACAAGGAGGTGAAAAAGGAGGAGGGGGGAAGAGAAGAGGAAGGAAAGAGGGAGTAGAG  541
 G  Q  G  G  E  K  G  G  G  G  K  R  R  G  R  K  E  G  V  E   53
GAGGTGACGGAAGACGACGCAAAAAGACTCCTGGAACTTGTCTCTTCGTTGAGCGATCAG  601
 E  V  T  E  D  D  A  K  R  L  L  E  L  V  S  S  L  S  D  Q   73
GACGCCGCGGGGATGCAAGTAAAAGTGAACCATTATCTGGCGGCGCGCTGTGGGGCGGGT  661
 D  A  A  G  M  Q  V  K  V  N  H  Y  L  A  A  R  C  G  A  G   93
CTCGTCTTCCTCATCCTCGTGAGTGACGATGAGGAGCTCTCCGTACACGTCCTGGGCCCT  721
 L  V  F  L  I  L  V  S  D  D  E  E  L  S  V  H  V  L  G  P  113
CACCGCCTCTCCTGCCCCGTCAAAATACCTGTCAGCAACAACAGCTTCAGCACGGCCCTC  781
 H  R  L  S  C  P  V  K  I  P  V  S  N  N  S  F  S  T  A  L  133
GCCTCGCGTCGGCCCATCACGCTGGCGGACATCGAGCCGTCGCACCGCGAGGACCTGTGG  841
 A  S  R  R  P  I  T  L  A  D  I  E  P  S  H  R  E  D  L  W  153
CGCCTGCTGGGCCTCATCCACGACAGCGAGCGGTTCGGGGCGGTGCGGCGCGTGTGTGTG  901
 R  L  L  G  L  I  H  D  S  E  R  F  G  A  V  R  R  V  C  V  173
GACCCTGGCGGCGGCGGTGAGGGCGAGGGCGTGGGCCAGGAGCGTCGCTGCCTGCGGGAG  961
 D  P  G  G  G  G  E  G  E  G  V  G  Q  E  R  R  C  L  R  E  193
ACACGCAATGGCTCGGCGAAGAGTGTGGACAGCTCCACCCCTGCCTCCCGCAAGCTGTTC 1021
 T  R  N  G  S  A  K  S  V  D  S  S  T  P  A  S  R  K  L  F  213
AGGGACATGTCTGTGGACCAGGGCATGGGCGGCGGCCCCAGCGATGCGTCGCAGCCCAGG 1081
 R  D  M  S  V  D  Q  G  M  G  G  G  P  S  D  A  S  Q  P  R  233
CGCTCCTCGGCGGCGCCCCGCATGGCCTTCATCGCGCACAAGCCCCAGCGGGCACCCACA 1141
 R  S  S  A  A  P  R  M  A  F  I  A  H  K  P  Q  R  A  P  T  253
GTCTCTGGCCCTGACGACAACCCCCCCGCCAGGCTGCCCCCCATCATGCCCTCTGCCAAC 1201
 V  S  G  P  D  D  N  P  P  A  R  L  P  P  I  M  P  S  A  N  273
ATGCCCCCAGTCGAGGCCAACCACGCCCCCAAGAGCCCCCACAAGGTGCCTAAGCTGGGG 1261
 M  P  P  V  E  A  N  H  A  P  K  S  P  H  K  V  P  K  L  G  293
CACCACGACCACGCGGGACTCGAGTCAGCGGGGCGGCAGTGCGGGTCGCTGGGCCTCGTG 1321
 H  H  D  H  A  G  L  E  S  A  G  R  Q  C  G  S  L  G  L  V  313
TGTCCCGCCTCGCTGCTGTGCGTGCCCGTGGCGGCGCCAGGCCGCGACGCCACCGCCATC 1381
 C  P  A  S  L  L  C  V  P  V  A  A  P  G  R  D  A  T  A  I  333
CTGGCCGTGCTGGTGGACAAACAGGGCGGCGGGGACTTTACTACGCAGGACGTAGAGGTC 1441
 L  A  V  L  V  D  K  Q  G  G  G  D  F  T  T  Q  D  V  E  V  353
GTAACGCGCTGCTTCAGGTGTGTGGCGGGCATCCTGATGAACACGGCGGAGGCGGAGCGG 1501
 V  T  R  C  F  R  C  V  A  G  I  L  M  N  T  A  E  A  E  R  373
GAGCGGCGGCTCAGGACCCAGTGCCAGGCCCTCCTGACGGTGGCACAGAACCTCTTCACT 1561
 E  R  R  L  R  T  Q  C  Q  A  L  L  T  V  A  Q  N  L  F  T  393
CACCTCGATGACGTGTCGGTGCTGCTGCGTGAGATCATGGCTGAGGCGCGGCAGCTGACG 1621
 H  L  D  D  V  S  V  L  L  R  E  I  M  A  E  A  R  Q  L  T  413
GACGCCGAACGGTGTTCTCTGTTCCTGCTGGACCGAGAACACGGGCAGCTGGTGGCCAAG 1681
 D  A  E  R  C  S  L  F  L  L  D  R  E  H  G  Q  L  V  A  K  433
GTGTTTGACGGGGAGCGGAAGGAGGAGTCCATCGAAGAAGTTCTCCTGCCAGTCTTGCAG 1741
 V  F  D  G  E  R  K  E  E  S  I  E  E  V  L  L  P  V  L  Q  453
AATTTACCTGGCGTCTACGTCACGAGCTCAGGTGAGGTGCGTCTGCCTGCCACTCAAGGG 1801
 N  L  P  G  V  Y  V  T  S  S  G  E  V  R  L  P  A  T  Q  G  473
```


ATAGCCGGCCATGTAGCTTCCACTGGGCACCTGTTGAACATACGGGACGCCTACGCTCAC 1861
I A G H V A S T G H L L N I R D A Y A H 493
CCTCTCTTCTATCGCGGCTTTGACGAGTGCACCGGCTTCAAGACCAGAAACATCCTTTGC 1921
P L F Y R G F D E C T G F K T R N I L C 513
TTCCCGATCAAGCAGGACGGGGAGGTGATAGGCGTGGCGGAGCTCTGTAACAAGACTACT 1981
F P I K Q D G E V I G V A E L C N K T T 533
GGCCTCCACTTCACACGCTTCGATGAGGAGATTGCCACAGCCTTTAGTATCTACTGCGGC 2041
G L H F T R F D E E I A T A F S I Y C G 553
ATCTCCATCAGCAACTCGCTCCTCTATAAGAAGGTCTCCGAGTCGCAGGTCCGCTCCAAG 2101
I S I S N S L L Y K K V S E S Q V R S K 573
CTGTCCAACGAGCTCATGATGTTCCATATGAAGGTCACCAAGGAGGAGGTGGAGCGACTG 2161
L S N E L M M F H M K V T K E E V E R L 593
GTTCAGGCCGAGGTTCCTCCACTTACACAATTCCACCGTGACTTCTGTTCCTTCAGATAC 2221
V Q A E V P P L T Q F H R D F C S F R Y 613
TTCCCGCGCCAGCTGGCTGACCCCTGCACATCCCCGGCGATCCTCTCGATGGTGGAGAGC 2281
F P R Q L A D P C T S P A I L S M V E S 633
CTGGGCATGATCACCAAGTTCAGGCTGAGTCGCGAGTCCCTGGCCAGGTTCACTCTTATG 2341
L G M I T K F R L S R E S L A R F T L M 653
GTGCGAAAAGGTTACCGGGATCCACCGTACCACAACTGGTTGCACGCCTTCTCTGTCACC 2401
V R K G Y R D P P Y H N W L H A F S V T 673
CACTTCGCCTTCCTGCTGCTCCAGAACCTGAAGCTGGTGGAGCGCGGCGTCCTCACCTCC 2461
H F A F L L L Q N L K L V E R G V L T S 693
CTGGAAGCACTGGCACTCATAGTGTCTTCCATGTGTCACGACCTGGACCACCGCGGCACC 2521
L E A L A L I V S S M C H D L D H R G T 713
ACAAACTCCTTCCAAGTGGCGAGCAACTCAGTACTGGCGTCGCTGTACTCCTCCGAGGGT 2581
T N S F Q V A S N S V L A S L Y S S E G 733
TCCGTCATGGAGCGGCACCACCTTGCTCAGGCCATGTGCATCCTCAACACCGACGACTGC 2641
S V M E R H H L A Q A M C I L N T D D C 753
AACTTCCTGGAGAACCTGAGCCGCGAGGAGTACACCAAGTTCCTCGACCTTATGAGAGAC 2701
N F L E N L S R E E Y T K F L D L M R D 773
ATCATTCTGGCCACTGACTTGGCCCACCACCTCCGGATCGTGTCTGAGCTGCGCCAGGTA 2761
I I L A T D L A H H L R I V S E L R Q V 793
GCAAATACAGGCTATGACCCCGCCAACCAACGCCACCACGAGCTGCTCATCTGCCTCCTC 2821
A N T G Y D P A N Q R H H E L L I C L L 813
ATGACAGCAGCAGATCTGTCAGACCAAACGAAGGACTGGCACTCATCTAAGCATGTGGCT 2881
M T A A D L S D Q T K D W H S S K H V A 833
GAGCTGATCTACAAAGAGTTCTTCACTCAAGGAGACTTAGAGAAGGCGATGGGGAACATG 2941
E L I Y K E F F T Q G D L E K A M G N M 853
CCTCTGGAGATGATGGACCGAGAGAAAGCCTTCATCCCAGAGCTTCAGCTTCAGTTTCTG 3001
P L E M M D R E K A F I P E L Q L Q F L 873
GATGATGTGGCAATTCCTGTTTATGAGATCGTTGCCAAGTTGTTCCCTGAGGCTGAGGAA 3061
D D V A I P V Y E I V A K L F P E A E E 893
CCCTACAGCAGCATCAAAGCAAACCGCCGCAACTGGTCTCGGCTCAGGGACGTCTACAAA 3121
P Y S S I K A N R R N W S R L R D V Y K 913
CGGCGGAAACCTGAGTCCACCAGTTCACTTGAGGTGTTTGAGGATGACTCACTTGAAGAG 3181
R R K P E S T S S L E V F E D D S L E E 933
GAATTGGAAAGAGATGAATCCTGAAACTACTGTAAATTTTCTTGTAGCATAAAGTGCAGT 3241
E L E R D E S - 940
ACGCAATTAGAGATACACAAAGTATCTTACTTGTTTACTCAGTGAGAGTAAAGCTTCATA 3301
ATATGATGAATCCCACAAGTACACCCATTGTATGATTTTTGAAGATTTATATCTTGCCAT 3361
AATCTTAAGGGAAACTTTGATGTATTGGAAAGACTTTATAGGTCTTTGCTTGTTCATAAG 3421
AAAGTCTACCTCTTACATGTTTGGCCAATGTAATCCATGTACACAGTATATGAACATTCA 3481
GTGATGACACTTACCTTCACAGAATAACTTTTCATTTCATCCATTCAGTAATCAGAGGCC 3541
CATTACATGTAGCCACGCCCAGACACAGGCGTGGGGGAGTCAGGAGTGCTGCTGTGGTGG 3601
TGGCGACTGTCTGCCTTGTCACTCGTGCATGACTCAGTTTCCTGAGCACTGCTGTGGCTG 3661

```
ACTCAGACTCTCCCTCACCGCAACTCTCCACATGCTGCACGCACTTACACAGACATTTTG 3721
GGACCCCGATGATGCTTACTCATCCATTAACTAAGAGTGTATATTTCCCTTTAGGAATAT 3781
CAGCCACTGTTTGCAGAACTTTAACATTTGTTGGTGGATCATCTGATGTGATTTCAATAT 3841
TCCTCTCATACATCAGTGTTATCTCTCTCTGCATTGATAGTCAGTCAACTGCTTGAAATA 3901
TTAAAGTACTTAGCCTCTGGTTCTTATATGCAAGCTGATTTGTACATTAAAGGCACCTTG 3961
TCTTACACATTATTCATATATGTATTTCTCTTTTCATACATCATCTTATATACCTACACA 4021
AACTATTACATAGTGTACACACTTTATACCTCGGGTAATTTTTCTGAAGTTATCAGTCAG 4081
GATATGTTAAGTCAAGCATCATACAAAGACAACTGCAACATTTTGTTTAGATAACTTATA 4141
TTTTGAAAATAACTCTGAGGATATTGTAACTTACCTCCTTAAGCCAAAGTTAACCCTCTA 4201
GAAGGCCAACACACTGTAAACTATGGTAGTGGTTGTCAAATGTATGTGATATGAAGCTAG 4261
TCAGACACTTTTATAAAACAGTGGTGCCTCTATTGTAGGTGTGGCTAGCCTGAGTTTTAT 4321
ACCATGAATGCTTTTAGTTTATATCATACCAATGAAGTGTGAAACCTGTATCACCTTACA 4381
CATGTAAATGAGAGTTTAGGTGAGTGATGTTGTGGCCTACATAGAGATCCTCTAAATTGC 4441
ACACCATAAGAAGAAATGTGTATGACTGAGGTCTTCAGGAATGAGACCAGTAATAACCTC 4501
GGACATTGTAGTGGTCAGAAGTAGTTGTATACATAAGGAAAGAGGAAAAGTTTAGAATAT 4561
ATAGTATAAAAGTGTTCCTTGAAGGGTGGTGTGAATGATGTCTGCTGTCACCTGTACAAC 4621
TGTATGATTAGACAGGCTGTATTGAGTCTGCTTGTTATTGGTCTTATACAACTATTTTTC 4681
TTTGTCTCATAATATTTTGTATTTCCATATGTGTGTGTGTGTGTGTGTT            4729
```

Figure 2.3. Nucleotide and amino acid sequence of cDNA encoding Gl-PDE2. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. The GAF-B regulatory domain is indicated by a green box and contains the (NKxxFDxxE) signature sequence found in all mammalian GAF domains; the sequence is underlined and in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.


GlPDE2 : ----MGSKLTCAG----SGTEDSTMGQEEPAKAEEDATDG : 32
Ce.PDE2 : ---------- : -
Hs.PDE2A3 : MGQACGHSILCRSQQYPAARPAEPRGQQVFLKPDEPPPPP : 40
GlPDE2 : GGQGGEKGGGGKRRG---RKEGVEEVTEDDAKRLIELVSS : 69
Ce.PDE2 : ---------- : -
Hs.PDE2A3 : QPCADSLQDALLSLGSVIDISGLQRAVKEALSAVIPRVET : 80
GlPDE2 : LSDQDAAG-MQVKVNHYLAARCGAGLVFLILVSDDEELSV : 108
Ce.PDE2 : ---------- : -
Hs.PDE2A3 : VYTYLLDGESQLVCEDPPHELPQEGKVREAIISQ-KRLGC : 119
GlPDE2 : HVLGPHRLSCPVKIPVSNNSFSTALASRRPIT-LADIEP- : 146
Ce.PDE2 : ---------- : -
Hs.PDE2A3 : NGLG---FSDLPGKPLARLVAPLAPDIQVLVMPLADKEAG : 156
GlPDE2 : SHREDLWRLLGLIHDSE--RFGAVRRVCVDPGGGGEGEGV : 184
Ce.PDE2 : ---------- : -
Hs.PDE2A3 : AVAAVILVHCGQLSDNPEWSLQAVEKHTLVALRRVQVLQQ : 196
GlPDE2 : GQERRCLRETRNGSAKSVDSSTPASRKLFRDMSVDQGMGG : 224
Ce.PDE2 : --------MLELRRNSSPSSAHPSPQTNCQNSQRGDGLHH : 32
Hs.PDE2A3 : RGPREAPRAVQNPPEGTAEDQKGGAAYTDRDRKILQ-LCG : 235
GlPDE2 : GPSDASQPRRSSAAPRMAFIAHKPQRAPTVSGPDDNPPAR : 264
Ce.PDE2 : HHHEAASGSTCCGGMTVFTGANAAK------SSNEPAGSA : 66
Hs.PDE2A3 : ELYDLDASSLQLKVLQYLQQETRASRCCLILVSEDNLQLS : 275
GlPDE2 : LPPIMPSANMPPVEANHAPKSPHKVPKLGHHDHAGLESAG : 304
Ce.PDE2 : SPTVWRKTSHPPLHFNNN----ETRNRNLQMQLKNRGTKD : 102
Hs.PDE2A3 : CKVIGDKVLGEEVSFPLTGCLGQVVEDKKSIQLKDLTSED : 315


GlPDE2 : RQCGSIGLVCP-ASLLCVPVAAPGRDATAILAVLVDKQGG : 343
Ce.PDE2 : DWGASIRYDIEEPTSSGLLELLPDVPIVRKLSRPLVKMD- : 141
Hs.PDE2A3 : VQQLQSMLGCELQAMLCVPVISRATDQVVALACAFNKLEG : 355
GlPDE2 : GDFTTQDVEVVTRCFRCVAGILMNTAEAERERRLRTQCQA : 383
Ce.PDE2 : ----DQDDACSVASNESDRTVLSPLVPMS---------- : 166
Hs.PDE2A3 : DLFTDEDEHVIQHCFHYTSTVLISTLAFQKEQKLKCECQA : 395
GAF-B
GlPDE2 : LLTVAQNLFTHIDDVSVLLRFIMAEARQLTDAERCSLFLL : 423
Ce.PDE2 : ---IFDQFLCLTNNLSALISCIIAEAKKNTEAEDYAVFLH : 203
Hs.PDE2A3 : LLQVAKNLFTHIDDVSVLLQFIITEARNLSNAEICSVFLL : 435
GlPDE2 : DREHGQLVAKVFDGERKEESIEEVLLPVLQNLPGVYVTSS : 463
Ce.PDE2 : D--EDNKQMVLFNN----------ETMLM : 220
Hs.PDE2A3 : D--QNELVAKVFDG----------GVVDDES : 454
GlPDE2 : GEVRLPATQGIAGHVASTGHLLNIRDAYAHPLFYRGFDEC : 503
Ce.PDE2 : TGKKFDMGYGIVGKVASTMRTMNIRDVSRCPFFNEEIDEQ : 260
Hs.PDE2A3 : YEIRIPADQGIAGHVATTGQILNIPDAYAHPLFYRGVDDS : 494
++++++++++++++++++
GlPDE2 : TGFKTRNILCFPIKQDG-EVIGVAELCNKTTGLHFTREDE : 542
Ce.PDE2 : FSIKARNLIAFPLIDSSCSLIGVIVL NKENG--FSRHDE : 298
Hs.PDE2A3 : TGFRTRNILCFPIKNENQEVIGVAELVNKINGPWFSKFDE : 534
GlPDE2 : EIATAFSIYCGISISNSLLYKKVSESQVRSKLSNELMMF- : 581
Ce.PDE2 : KYIKRFSYFVANSIAHAILAKQIEEVRTRIHMVEEFKIQG : 338
Hs.PDE2A3 : DLATAFSIYCGISIAHSLLYKKVNEAQYRSHLANEMMMY- : 573
GlPDE2 : -HMKVTKEEVERLVQAEVPPLTQFHRDFCSFRYFPRQLAD : 620
Ce.PDE2 : EDAVIEEVDIMRLVNDPLRDWRYFSQNFADFSFPPRSVGE : 378
Hs.PDE2A3 : -HMKVSDDEYTKLLHDGIQPVAAIDSNFASFTYTPRSLPE : 612

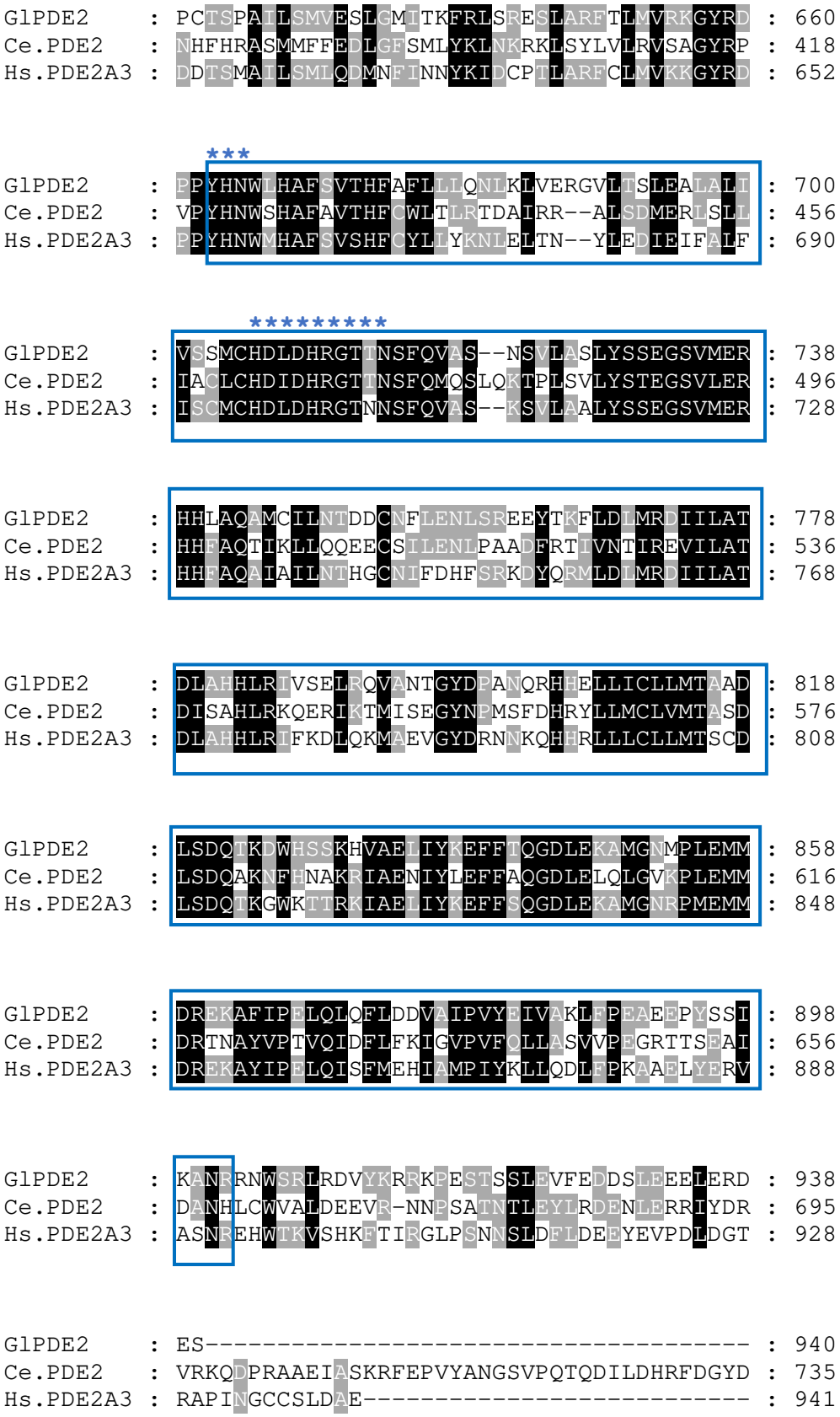
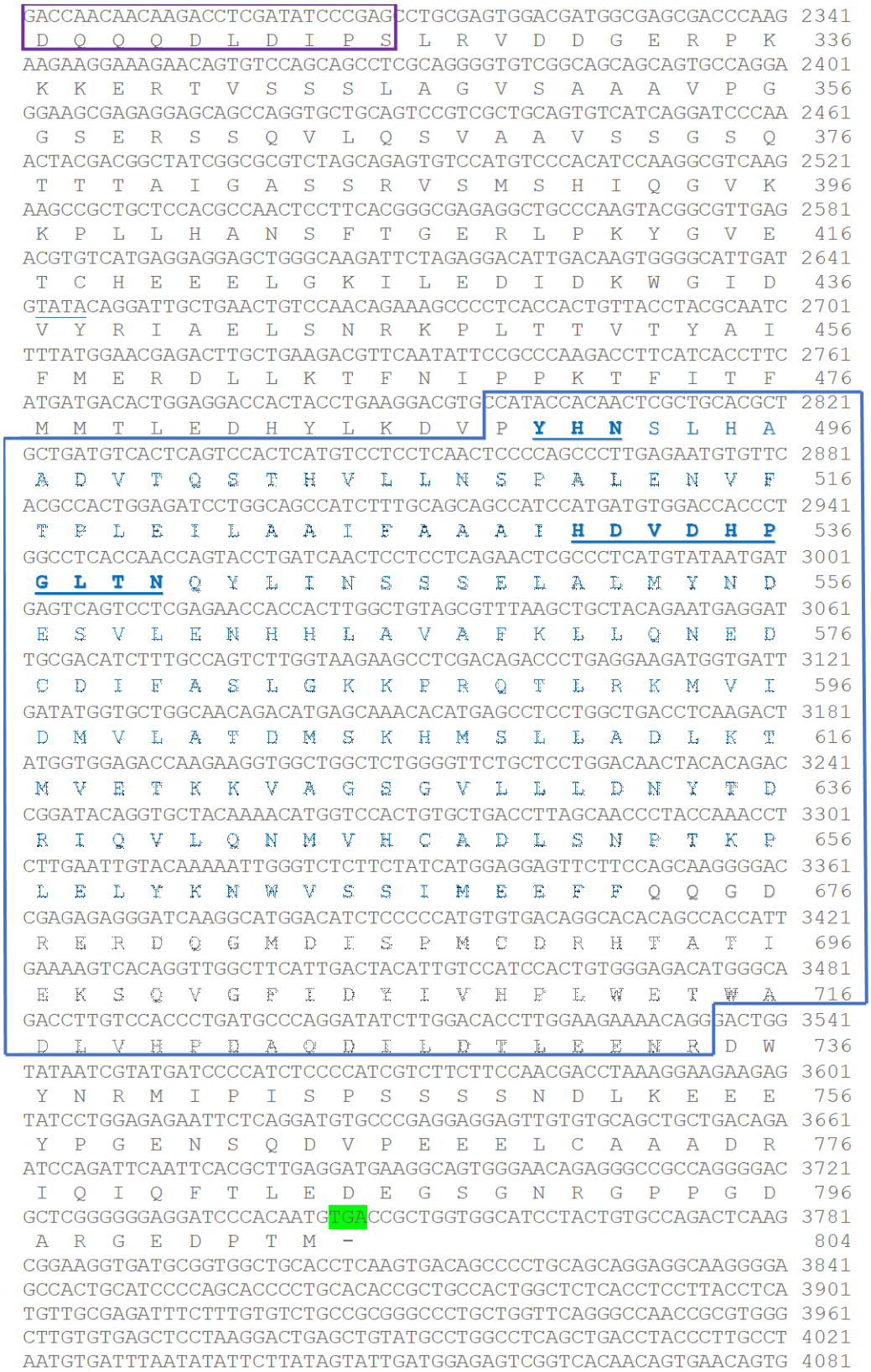

GlPDE2 : PCTSPAILSMVESLGMITKFRLSRESLARFTLMVRKGYRD : 660
Ce.PDE2 : NHFHRASMMFFEDLGFSMLYKLNKRKLSYLVLRVSAGYRP : 418
Hs.PDE2A3 : DDTSMAILSMLQDMNFINNYKIDCPTLARFCLMVKKGYRD : 652
***
GlPDE2 : PPYHNWLHAFSVTHFAFLLLQNLKLVERGVLTSLEALALI : 700
Ce.PDE2 : VPYHNWSHAFAVTHFCWLTLRTDAIRR--ALSDMERLSLL : 456
Hs.PDE2A3 : PPYHNWMHAFSVSHFCYLLYKNLELTN--YLEDIEIFALF : 690
****
GlPDE2 : VSSMCHDLDHRGTTNSFQVAS--NSVLASLYSSEGSVMER : 738
Ce.PDE2 : IACLCHDIDHRGTTNSFQMQSLQKTPLSVLYSTEGSVLER : 496
Hs.PDE2A3 : ISCMCHDLDHRGTNNSFQVAS--KSVLAALYSSEGSVMER : 728
GlPDE2 : HHLAQAMCILNTDDCNFLENLSREEYTKFLDLMRDIILAT : 778
Ce.PDE2 : HHFAQTIKLLQQEECSILENIPAADFRTIVNTIREVILAT : 536
Hs.PDE2A3 : HHFAQAIAILNTHGCNIFDHFSRKDYQRMLDLMRDIILAT : 768
GlPDE2 : DLAHHLRIVSELRQVANTGYDPANQRHHELLICLLMTAAD : 818
Ce.PDE2 : DISAHLRKQERIKTMISEGYNPMSFDHRYLLMCLVMTASD : 576
Hs.PDE2A3 : DLAHHLRIFKDLQKMAEVGYDRNNKQHHRLLLCLLMTSCD : 808
GlPDE2 : LSDQTKDWHSSKHVAELIYKEFFTQGDLEKAMGNMPLEMM : 858
Ce.PDE2 : LSDQAKNFHNAKRIAENIYLEFFAQGDLELQLGVKPLEMM : 616
Hs.PDE2A3 : LSDQTKGWKTTRKIAELIYKEFFSQGDLEKAMGNRPMEMM : 848
GlPDE2 : DREKAFIPELQLQFLDDVAIPVYFIVAKLFPEAEEPYSSI : 898
Ce.PDE2 : DRTNAYVPTVQIDFLFKIGVPVFQLLASVVPEGRTTSEAI : 656
Hs.PDE2A3 : DREKAYIPELQISFMEHIAMPIYKLLQDLFPKAAELYERV : 888
GlPDE2 : KANRRNWSRLRDVYKRRKPESTSSLEVFEDDSLEEELERD : 938
Ce.PDE2 : DANHLCWVALDEEVR-NNPSATNTLEYLRDENLERRIYDR : 695
Hs.PDE2A3 : ASNREHWTKVSHKFTIRGLPSNNSLDFLDEEYEVPDLDGT : 928
GlPDE2 : ES---------- : 940
Ce.PDE2 : VRKQDPRAAEIASKRFEPVYANGSVPQTQDILDHRFDGYD : 735
Hs.PDE2A3 : RAPINGCCSLDAE---------- : 941

Figure 2.4. Multiple alignment of deduced amino acid sequences of PDE2 proteins in one crustacean species, one nematode species and one mammal species. Abbreviations: Gl: *G lateralis*; Ce: *C elegans*; Hs: *H sapiens*. Black shading indicates that amino acid residues that are identical or similar in all sequences; gray shading indicates identical or similar amino acids in most of the sequences. Dashes indicate gaps introduced to optimize the alignment and the boxes indicate highly conserved domains. Blue asterisks indicate the signature sequences in the catalytic domain. Green plus signs indicate the motif sequence in the GAF domain. The colors of the boxes correspond to the colors of the domains in Fig. 2. 3.

GlPDE4: 6130 nucleotides, structure: C sequence

```
GTGGCGTGCGGGTGTGGCGTGGAGTCCGCGCTGGCTGTGGCTGTGAGTGCTCCGGTGCAC    61
AAGTGTGGTGGCGGCCCCGTCGACCACACGCTTCTCCCCGCGCGGCGTTCACGGCTGTGA   121
GGTAGGAGAGTCCTACGTGTCCGCACCCCGCGCTGTGTCCCCCGGGTGTCCTGGCGTGAC   181
GGTGTCGCTCCTGTGCCGCTCCTGCTTGTCCGGATGAATGGCAGCGGACAGGTGGAGGCT   241
CCCGGGAAGTGAGGTGACCCGCTGTCCTGCCGCCGTTCAGCATCACGGTGTAGTTGGCGT   301
CCTGCTGCCGCCACGCCAGGCTCGCCGCCGCCTCTCTCCCGCCGGCGGACCGGACGGTGC   361
GCACTGAGAGGCACGTCCATGCGGGGTGACGGTGAGGGGGTGGTTGGGGAGCAGCAGCCA   421
CCTGTGCTCACCTGTGCTCGCCTGGCCTCCTCTTGCTGACTGCATCACGCTCATGACGGG   481
CTCGTGTGCTCCTCAGCCCGCCCCGCCAGTCCTCGTGTGACGCTCCCCGGTGTTCAGACC   541
ATCGTAGTCACAGTAGTGCGTCGTAGCCTCACCGTAGCCCCGAGAGACAGTTCATCGTTT   601
AGAGCTAGTGTGAGCTTTGTTCGTGAGTGTAGTGTGTGCAGTACCTACTTGAGGCTCATT   661
CCTCTGTAGAAAAATGTTAGCCAGCGAATGTGTGCGAGTGTGTCTGTCTGTGTGGCGTGG   721
CCTGGCTAGACTTCCTGTCGTGTAGGGCAGTAGTCGTGATATGTGTTGTAGAAAAGAAAG   781
TCTGTAGAAAAACTAGCAGCCAAGCGCGTGGGTAGCGAGTCGCACGGAGCCTGAGTAGCG   841
GCTTGAGGCTGTGCCGAGGCCAACACCGCTTCTAGTAATCGTCGGTACAGTCGTTGCCAA   901
GCGTAGGTCATCGTTATAGTTGAGCCTCGCGTATACCTGAGTCCTTGTAGTGCCTCTCAG   961
CGTAGGTGTAGCCCAGGGAGTCTAGAATCCTTGGTGTAGCCGTGAGTTAGTGTGGAGGTC  1021
GCCGCTGTAGACCTGCCACGACCTGCTTCGCTGTGTAGGGCGTTCAGCGTCGCTGTAGTG  1081
AGGCAGTAGGCCTCGTGAGCCTCTCGTCGTCGCTGTCCTCGTGTCGTGTCGTTTAGCAAG  1141
TCTTGGTCAGCTTCCCGCCGCCTCACCGCCTCGTAGCCACGCCAGGGCAGCGGCCAGCAG  1201
TGCGTCGCCCCGCCGCTTGTGTCTGTGTGTGTGGCGGTGCAGCGCTGGTGATGCTGGGCT  1261
GGAGGATCGGACACAGGCCCCCCCGCCGCCGCTGCCCGCCGCCCCCCTCCCTTTAGTACC  1321
CTCACCGCCGCCATGCTGCAAGTTCCTGAGATCAGAGCCCCCGACGAGGACGAGGACGAT  1381
              M   L   Q   V   P   E   I   R   A   P   D   E   D   E   D   D    16
GATACCCCCACGCCCTCGGGACTGCGCCTCCAAGTCCCTGAATTCTCCCTCCTAGCGCCA  1441
 D   T   P   T   P   S   G   L   R   L   Q   V   P   E   F   S   L   L   A   P    36
CCTGACGACCCTCCTGATGGGGGCGGAGGCTGCGTCCACCTGCAGGTGCCTGAGATGGGA  1501
 P   D   D   P   P   D   G   G   G   G   C   V   H   L   Q   V   P   E   M   G    56
AGCTTCGTGCCCCCGGGAAGCAACATAGGCAGCTCAGCGACGCGCCTCGAGCCCCCCAAA  1561
 S   F   V   P   P   G   S   N   I   G   S   S   A   T   R   L   E   P   P   K    76
ACCCTACACCTGTCCCTGCCGCCCTTCCCCCAGCCAGGCGTGTCCAGCTTCCTGCAGGTG  1621
 T   L   H   L   S   L   P   P   F   P   Q   P   G   V   S   S   F   L   Q   V    96
CCTGGAGGCTACACGGGGCCCAGGAGGAGACACTCTTGGATCTGCAGTTTCGACGTTGAA  1681
 P   G   G   Y   T   G   P   R   R   R   H   S   W   I   C   S   F   D   V   E   116
AATGGCACCTCGCCCGGCCGAAGCCCCCTGGACGGGACCTCCCCCTCGGCGGGTCTCGTC  1741
 N   G   T   S   P   G   R   S   P   L   D   G   T   S   P   S   A   G   L   V   136
CTGCAGAACTTTCCCCAGCGGAGGGAAAGTTTCCTCTACAGGAGTGATTCAGACTTCGAG  1801
 L   Q   N   F   P   Q   R   R   E   S   F   L   Y   R   S   D   S   D   F   E   156
ATGTCGCCCAAAAGCATGTCTCGAAACTCCTCCATCGCCTCGGAAGGGTCACGAGTTATG  1861
 M   S   P   K   S   M   S   R   N   S   S   I   A   S   E   G   S   R   V   M   176
TTGGCCACCTTGAGCGAGTCTCGTGGGGCTAATGACGACAAGCCGATACATGGAGAGGAC  1921
 L   A   T   L   S   E   S   R   G   A   N   D   D   K   P   I   H   G   E   D   196
CTCATCGTGACCCCCTTCGCACAGATCCTGGCGTCGCTCCGCTCTGTGAGGAACAACTAC  1981
 L   I   V   T   P   F   A   Q   I   L   A   S   L   R   S   V   R   N   N   Y   216
ATCAACCTCACCAATGTTTCCACCTCCAAGTCTCGCCGGTCCAGCGCTGCGGCGGGCTGC  2041
 I   N   L   T   N   V   S   T   S   K   S   R   R   S   S   A   A   A   G   C   236
TCCACGCCTCAGCCCAAAAACTTTACCTATGGTGATGAGACGTACACCAAGGTGGCGCTG  2101
 S   T   P   Q   P   K   N   F   T   Y   G   D   E   T   Y   T   K   V   A   L   256
GAGACCCTGGAGGAGCTGGACTGGTGCCTCGACCAGCTGGAGACCATCCAGACCCATCGC  2161
 E   T   L   E   E   L   D   W   C   L   D   Q   L   E   T   I   Q   T   H   R   276
TCCGTCTCTGACATGGCCTCCTCCAAGTTCAAGAGGATGCTCAACAAGGAACTCAGCCAC  2221
 S   V   S   D   M   A   S   S   K   F   K   R   M   L   N   K   E   L   S   H   296
TTCTCGGAATCCAGCAAGTCGGGCAACCAGATTTCCGAATACATTTGCACAACCTTCCTC  2281
 F   S   E   S   S   K   S   G   N   Q   I   S   E   Y   I   C   T   T   F   L   316
```


GACCAACAACAAGACCTCGATATCCCGAGCCTGCGAGTGGACGATGGCGAGCGACCCAAG 2341
D Q Q Q D L D I P S L R V D D G E R P K 336
AAGAAGGAAAGAACAGTGTCCAGCAGCCTCGCAGGGGTGTCGGCAGCAGCAGTGCCAGGA 2401
K K E R T V S S S L A G V S A A A V P G 356
GGAAGCGAGAGGAGCAGCCAGGTGCTGCAGTCCGTCGCTGCAGTGTCATCAGGATCCCAA 2461
G S E R S S Q V L Q S V A A V S S G S Q 376
ACTACGACGGCTATCGGCGCGTCTAGCAGAGTGTCCATGTCCCACATCCAAGGCGTCAAG 2521
T T T A I G A S S R V S M S H I Q G V K 396
AAGCCGCTGCTCCACGCCAACTCCTTCACGGGCGAGAGGCTGCCCAAGTACGGCGTTGAG 2581
K P L L H A N S F T G E R L P K Y G V E 416
ACGTGTCATGAGGAGGAGCTGGGCAAGATTCTAGAGGACATTGACAAGTGGGGCATTGAT 2641
T C H E E E L G K I L E D I D K W G I D 436
GTATACAGGATTGCTGAACTGTCCAACAGAAAGCCCCTCACCACTGTTACCTACGCAATC 2701
V Y R I A E L S N R K P L T T V T Y A I 456
TTTATGGAACGAGACTTGCTGAAGACGTTCAATATTCCGCCCAAGACCTTCATCACCTTC 2761
F M E R D L L K T F N I P P K T F I T F 476
ATGATGACACTGGAGGACCACTACCTGAAGGACGTGCCATACCACAACTCGCTGCACGCT 2821
M M T L E D H Y L K D V P Y H N S L H A 496
GCTGATGTCACTCAGTCCACTCATGTCCTCCTCAACTCCCCAGCCCTTGAGAATGTGTTC 2881
A D V T Q S T H V L L N S P A L E N V F 516
ACGCCACTGGAGATCCTGGCAGCCATCTTTGCAGCAGCCATCCATGATGTGGACCACCCT 2941
T P L E I L A A I F A A A I H D V D H P 536
GGCCTCACCAACCAGTACCTGATCAACTCCTCCTCAGAACTCGCCCTCATGTATAATGAT 3001
G L T N Q Y L I N S S S E L A L M Y N D 556
GAGTCAGTCCTCGAGAACCACCACTTGGCTGTAGCGTTTAAGCTGCTACAGAATGAGGAT 3061
E S V L E N H H L A V A F K L L Q N E D 576
TGCGACATCTTTGCCAGTCTTGGTAAGAAGCCTCGACAGACCCTGAGGAAGATGGTGATT 3121
C D I F A S L G K K P R Q T L R K M V I 596
GATATGGTGCTGGCAACAGACATGAGCAAACACATGAGCCTCCTGGCTGACCTCAAGACT 3181
D M V L A T D M S K H M S L L A D L K T 616
ATGGTGGAGACCAAGAAGGTGGCTGGCTCTGGGGTTCTGCTCCTGGACAACTACACAGAC 3241
M V E T K K V A G S G V L L L D N Y T D 636
CGGATACAGGTGCTACAAAACATGGTCCACTGTGCTGACCTTAGCAACCCTACCAAACCT 3301
R I Q V L Q N M V H C A D L S N P T K P 656
CTTGAATTGTACAAAAATTGGGTCTCTTCTATCATGGAGGAGTTCTTCCAGCAAGGGGAC 3361
L E L Y K N W V S S I M E E F F Q Q G D 676
CGAGAGAGGGATCAAGGCATGGACATCTCCCCCATGTGTGACAGGCACACAGCCACCATT 3421
R E R D Q G M D I S P M C D R H T A T I 696
GAAAAGTCACAGGTTGGCTTCATTGACTACATTGTCCATCCACTGTGGGAGACATGGGCA 3481
E K S Q V G F I D Y I V H P L W E T W A 716
GACCTTGTCCACCCTGATGCCCAGGATATCTTGGACACCTTGGAAGAAAACAGGGACTGG 3541
D L V H P D A Q D I L D T L E E N R D W 736
TATAATCGTATGATCCCCATCTCCCCATCGTCTTCTTCCAACGACCTAAAGGAAGAAGAG 3601
Y N R M I P I S P S S S S N D L K E E E 756
TATCCTGGAGAGAATTCTCAGGATGTGCCCGAGGAGGAGTTGTGTGCAGCTGCTGACAGA 3661
Y P G E N S Q D V P E E E L C A A A D R 776
ATCCAGATTCAATTCACGCTTGAGGATGAAGGCAGTGGGAACAGAGGGCCGCCAGGGGAC 3721
I Q I Q F T L E D E G S G N R G P P G D 796
GCTCGGGGGGAGGATCCCACAATGTGACCGCTGGTGGCATCCTACTGTGCCAGACTCAAG 3781
A R G E D P T M - 804
CGGAAGGTGATGCGGTGGCTGCACCTCAAGTGACAGCCCCTGCAGCAGGAGGCAAGGGGA 3841
GCCACTGCATCCCCAGCACCCCTGCACACCGCTGCCACTGGCTCTCACCTCCTTACCTCA 3901
TGTTGCGAGATTTCTTTGTGTCTGCCGCGGGCCCTGCTGGTTCAGGGCCAACCGCGTGGG 3961
CTTGTGTGAGCTCCTAAGGACTGAGCTGTATGCCTGGCCTCAGCTGACCTACCCTTGCCT 4021
AATGTGATTTAATATATTCTTATAGTATTGATGGAGAGTCGGTCACAACAGTGAACAGTG 4081

```
CCAAATTGTTCCTTTTTAAAAGAAATATGGATCCTGTGGTGGTAGTTTGTTATATGAGAA 4141
AAGAACTCTTGTGGGGAGTTGTCTGTTATGTGAGGATTCTGTTCCTGAGTGGTCAGTCTT 4201
TTAAAAATACCAGTCCTATGAAGGCACGATGTGCAATGCCTCAAGAGAGCAGTTGAGGGA 4261
GTTAATTTTTCATTTTTTGTATGGTTTGAGGACAAAGGCTTCTGTGTGGTACATATCAGA 4321
TAGCATCAGTAACCGGCACATGAGTGGTGCTTTCATTGTGTGAGTCTGGGGCATTATAAT 4381
GTGAGCTCTTTCGTAATACAGGTGCCTGACAGGGTTCGCTGCTGCCGACACTAAAAGTAG 4441
ACTTGCTGTCGGAGAAGCCAAAGATAATTTATTGCTTCAGCCCACAGTGGACTTGGAGCC 4501
ACCACTGCTACCACACATTTCAGTTGCTGTGCAGATTGAGGGATGCAAGTTTGTGCAGCA 4561
GCTGAGGACATAGATGGTGGGTCTTGTCAGGCAGAAGAAAAAACATGTCCCTCACTTAAA 4621
ACTGATGTAAGAGTTTAGATAATATTTATTCTCTTTTTTCAATATTTATTTTAAGTAGTG 4681
TGAAAAAATTATTTGTCACCATTAGATCTTTTTTTTATTATTATCCCTTACTGTGCAAGG 4741
TTCATAAGTAGTTCGTTTTAAAAATTTTTTTTGCAATGTCACTTTGAGGTTTTCATCTTT 4801
TAGACAGCTTTTTTGGCGCTACCTTAGATGTTCGATGAACTTCACTGATGGGTTTTCACC 4861
TACTTTTGAACTAGGTAGCCATGAAAGAGGTTCAGATTTTAGCACTTCAACTTCTATCCA 4921
CCTGTTAAACAACACAGGCCTTTTTTTCTGTTTCCTCCTTTTCTTTTCAAGTAACCTAAA 4981
ATCAGCAAAAGTGTGCACCAACCATTTTCATATAAATCTTTCATCTCACAATCCCACAGC 5041
CTTGAGAGAATGTGCCACTCATCTCATCATGAATTGTGATATTGTTAAAGTCAGAGTTTG 5101
GTGAACTTTGGTCCATCAATGTAGAATATGAAGAAAGAGCTTGCAGACTTTGTATTTGCT 5161
TCAGTGTGTAGGCAGCATACTTGTTTGTGTTCATCAGAATATGTCTGCTATTTTCAGAAC 5221
TAATTACATTACTGAATACTTCCAGTATGTATATTTATTTCATATATTTGAGCTATAATT 5281
AATATAACATACACTATCAAGTAACAAAACTGGCCTGAGACACTCATACTGTTGGAATTG 5341
CCAACTTGTGAAGTGAAGCAAATCTGAGCCTCTAGTGTAGAATACTCCCTTGTGATGAAG 5401
AGAGCATAGTGTGCGTCATGTTCCATCCCACACCGCCCAGTGTCGCTCACCAGCCACACT 5461
CCCGACTCCTTCGGCTCGGCACTGGCAACACCAGCAGCCCTTGATTCCCTTCTACAGTCC 5521
CTTTCTTATCCTCCCCTCCCTTTATCCCTCCCTTCCTCCCTCCCTCCTTGTAGGAAGATC 5581
ATGGTGAAGGGGGAACTCTGATAAATATGCACCACACAGATAACCACTTCAATAGCCATG 5641
ATGTTTGTGTCCACTCGTTGCAGCCATTTCAGAGATCAGTGTTGCTCCTATCTTTATAAT 5701
GTAGTGTACTGTGCACTCAAGCCCTGGCATTGGCACTGTGCCTGGCACATGTGTCCCCTC 5761
ACAGTGGTGCTCCCACGGGCACTGTGTATGGAGCTTCACTTGCCATCAGGATAAAGTCAG 5821
CTCAAGAGGAGAAAAAAGTGGGACAATATATCTGCAGATGCTTTCCCACAGACCTGAGGA 5881
CAGACTCACCCTTCACTACATGCTAATTATTCAGATATATTTATTTTTTATAGTAGAAGC 5941
ATTAATCCCACCTTTATGACAGGGTGACTGTAGTTGTCTAATACCATGTAAATGTTAATT 6001
TTTCTCATAATTTATTGAATGGGTCTCCTATACATGTGTATTATTTTTAAAATATTTACA 6061
TGTATATAGAGGTGCAAGTAAACATTATCAGGTGTATAATAAGATGTATGAGTATTTAAG 6121
CAGTATTGC                                                     6130
```

Figure 2.5. Nucleotide and amino acid sequence of cDNA encoding Gl-PDE4. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue. Two Upstream Conserved Regions; UCR1 and UCR2, are found in purple boxes.

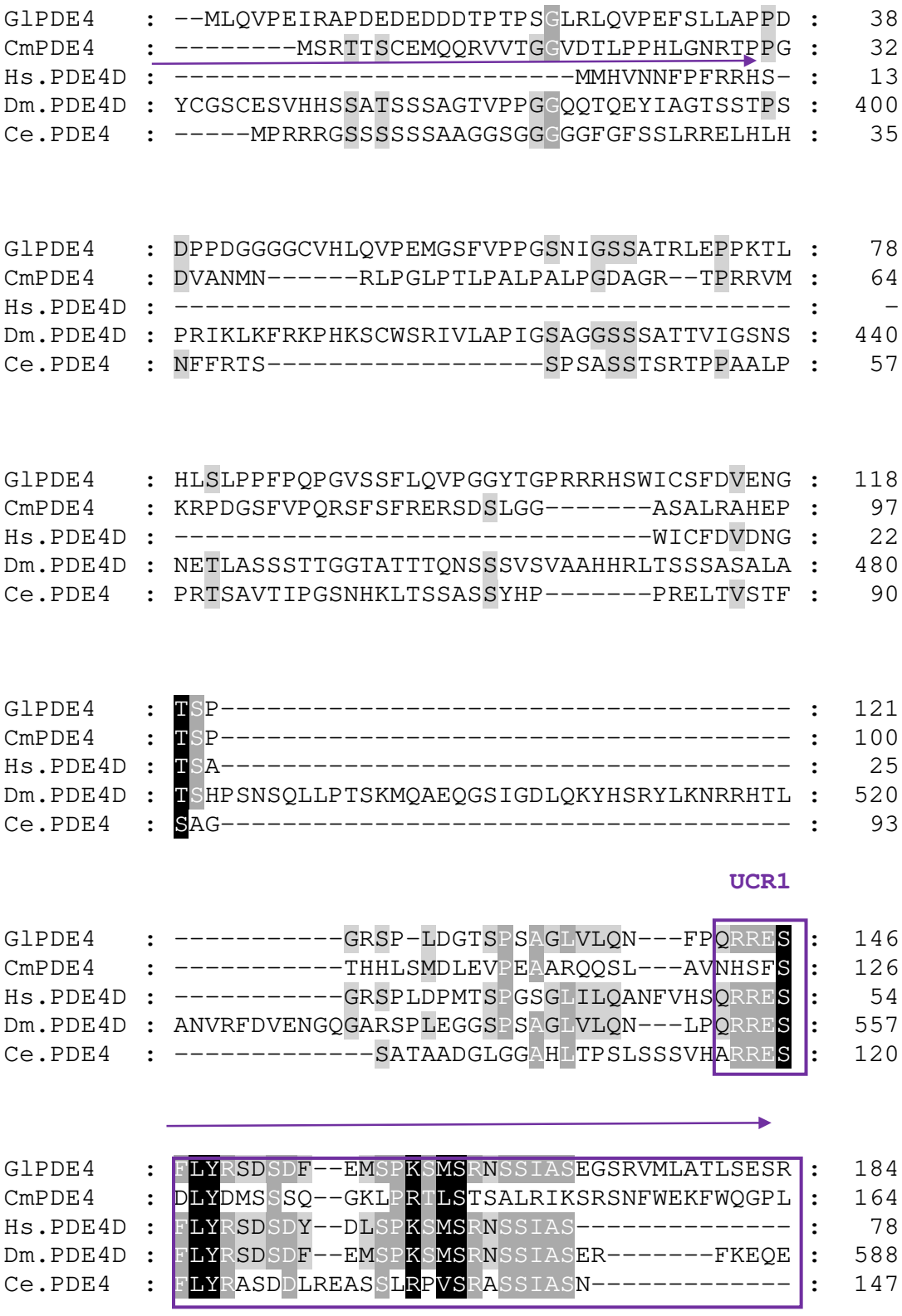
CmPDE4 6122 nucleuotides

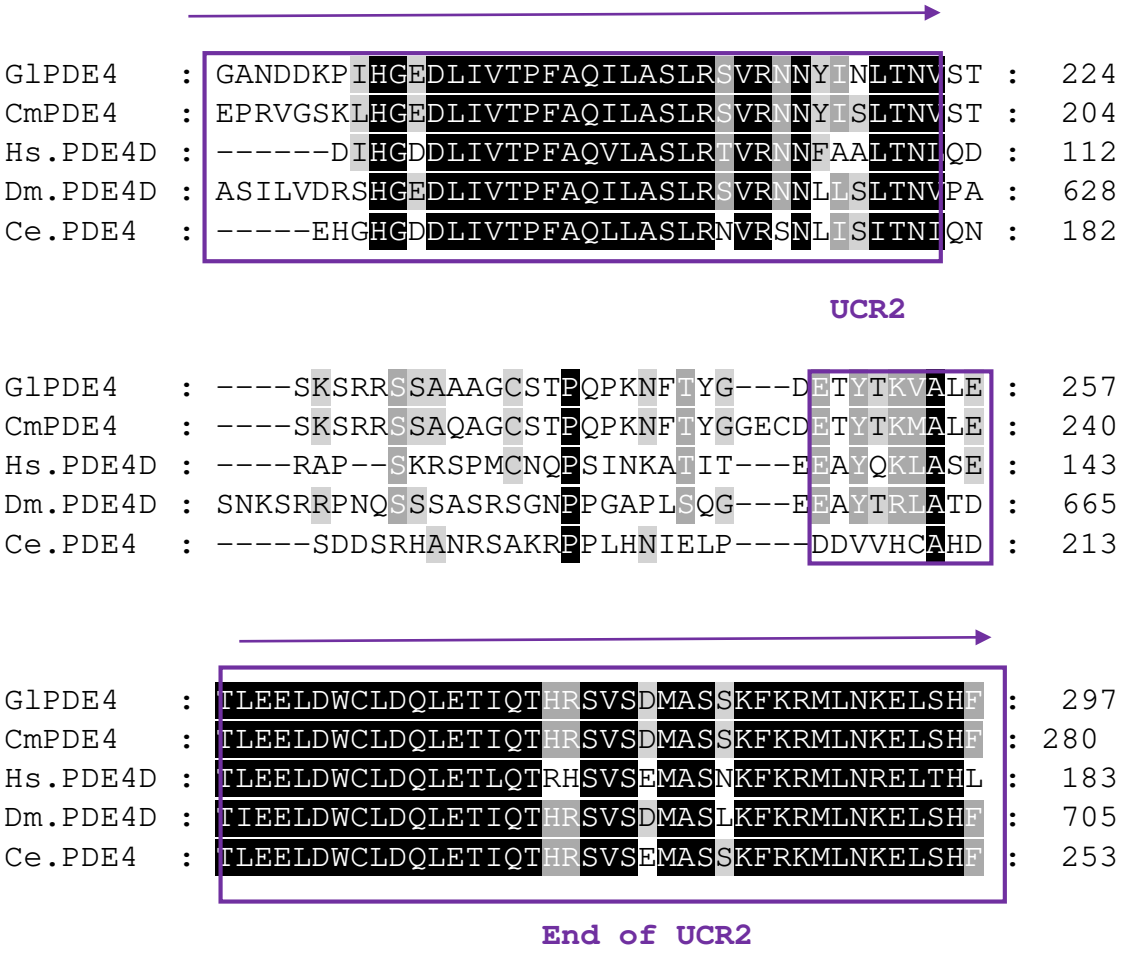
```
   1 TCAACAACAACAAAAGCAGCAGTGGCAGCGGCAGTCTCTGCTATGCGTTTATTTTTAGTC
  61 ACTCGTCACCGGTACGCGTTATCGCTTCCCTCGTCATCCTTGTTCCCTCCCTCTCCCTCT
 121 GTCTCTTCCTTCCTTCCTCCCTTCCTTCCTGGCATTCCTCTCTACCTCATTCCTTCCTTT
 181 CCTTCCTCTTCCTCCCTCCCCGCCACAGTGACCGGACGCGAGTGGCAGCATCACGTGAGC
 241 TGTGTTTCGCGTCGTGATCGATTACCGGGTGGAGTAACAAGGTCGAGGAACCTAACCCGC
 301 CACACTGCACCACACTCAGTCTGACCCTAAAGCGAGAGGAACATGACTGACTGACCGACT
 361 GACTGATGACTGTCTTGGCGAGGAGAGACTGTTCTGACAATTCTGAAACCGAATAACGTC
 421 GAGCGTGAGAATAAGAGTTCTAGGTTAGGAGGAATGGCGGAAGTGTGGCGTTTTTGTTGT
 481 GTTGGAGGAATACATTAGGTGAGCCGTGAGGGCTGAGATGGGTTGCCTGGCGTTACGCTG
 541 TGGCCACCATCCCTCCACCGCCGCCCCCGCGCATCTCTGTCGCGACATCGCCTCCTCAGT
 601 CGGGTGTGAGAACCCAAAGAGTCCGGGTCTGCGTTTTACGCGTATCAAGGTCGTGGTGGT
 661 GGTGGTGGTGGCTGTAGCGTCGTGCGAGGAGGACACGTGGCACAGGAAGTCCGGCTGAAG
 721 GCTGTGTGCGCCGAGGTAGCAGCGACCATCCTGCCCCACGCGGCCAGTGCGGCGCCGAGA
 781 ATAGTGTTTGCGTGATAGTGAGAGGCTGTGACGTGCACGGGTACTGGTGGTGCCAGGAGA
 841 GAGCCAGCACAGCCGCCGGCCCTCACAACAACCACAACCACCGGCCGAGCCCAACACGCC
 901 GTCAGTGGCCTTCCCGCACGCCCTCACAGAGCAGTCCCTTATTGATCCGAGACTACCATT
 961 GTCGGCAGCGTGATGACCGCCACGACCACCGACCAAGGGCCACGACCACCGCCGGGATGG
1021 GTTGTATCTAAGTGAAAGAGAGGAACACCGACTCGTGCCGTTGAAGGCACGTTCGTCCTG
1081 CCCTGCGAGGTGCGCAACCCGTCACACGTGGCTGCGCCGAGTGTTGTGGTGTCATCTACG
1141 TGCACCTCCCAACTCCACCTCTCCTGCTACTATTCCTCCTTCTCCTCCTCCTCCTCCTCC
1201 TCCTTGTCGCCCGGCCGTGGGTGTGGCCGGCTGCCAAGCGTGTGGGGTGGCCCTGTTGGC
1261 CGGGTAGCAGCCCTCCAACCGTGGCGCAGCGACTGGTCATCCTGGAGGAGCCCCCGGGGT
   4                                                   M  S  R  T
1321 CCCCAGGGTCCCCAGGGGTGTCCAGGACGGTTACCTCGTCCTCCTGGAAGATGTCCCGCA
  24    T  S  C  E  M  Q  Q  R  V  V  T  G  G  V  D  T  L  P  P  H
1381 CCACCTCCTGTGAAATGCAGCAGCGGGTGGTAACGGGCGGAGTGGACACGCTTCCGCCCC
  44   L  G  N  R  T  P  P  G  D  V  A  N  M  N  R  L  P  G  L  P
1441 ACCTGGGGAACAGGACGCCACCTGGGGACGTCGCTAACATGAATCGCCTCCCAGGTCTTC
  64   T  L  P  A  L  P  A  L  P  G  D  A  G  R  T  P  R  R  V  M
1501 CTACCCTCCCAGCACTTCCAGCCCTCCCGGGGGACGCAGGGCGGACGCCACGCCGCGTCA
  84   K  R  P  D  G  S  F  V  P  Q  R  S  F  S  F  R  E  R  S  D
1561 TGAAACGCCCAGACGGAAGTTTCGTTCCGCAGCGGAGCTTCTCTTTCAGGGAACGTTCAG
 104   S  L  G  G  A  S  A  L  R  A  H  E  P  T  S  P  T  H  H  L
1621 ATTCTTTGGGCGGAGCGAGTGCCTTGAGGGCCCACGAGCCCACCTCGCCCACCCACCACC
 124   S  M  D  L  E  V  P  E  A  A  R  Q  Q  S  L  A  V  N  H  S
1681 TCTCCATGGACTTGGAGGTGCCCGAGGCGGCGCGCCAACAGTCCCTGGCAGTGAATCACT
 144   F  S  D  L  Y  D  M  S  S  S  Q  G  K  L  P  R  T  L  S  T
1741 CCTTCTCCGACCTTTACGACATGTCGTCCAGCCAGGGCAAGCTGCCGCGTACACTCTCCA
 164   S  A  L  R  I  K  S  R  S  N  F  W  E  K  F  W  Q  G  P  L
1801 CTTCGGCGCTCCGTATCAAGAGTCGCTCCAACTTCTGGGAAAAGTTTTGGCAGGGTCCGT
 184   E  P  R  V  G  S  K  L  H  G  E  D  L  I  V  T  P  F  A  Q
1861 TGGAGCCGCGAGTGGGGAGCAAGCTACACGGAGAGGACCTGATCGTGACCCCCTTCGCGC
 204   I  L  A  S  L  R  S  V  R  N  N  Y  I  S  L  T  N  V  S  T
1921 AGATCTTGGCGTCGCTCAGATCGGTTCGCAACAACTACATCAGCCTCACCAATGTGTCCA
 224   S  K  S  R  R  S  S  A  Q  A  G  C  S  T  P  Q  P  K  N  F
1981 CCTCGAAGTCTCGGAGGTCCAGTGCACAAGCTGGCTGCTCCACACCTCAACCCAAAAACT
 244   T  Y  G  G  E  C  D  E  T  Y  T  K  M  A  L  E  T  L  E  E
2041 TTACCTATGGAGGTGAGTGCGATGAGACCTACACAAAGATGGCGCTGGAGACCTTGGAGG
 264   L  D  W  C  L  D  Q  L  E  T  I  Q  T  H  R  S  V  S  D  M
2101 AGCTGGACTGGTGCTTGGATCAGCTGGAGACCATTCAGACCCACCGCTCCGTGTCCGATA
 284   A  S  S  K  F  K  R  M  L  N  K  E  L  S  H  F  S  E  S  S
```

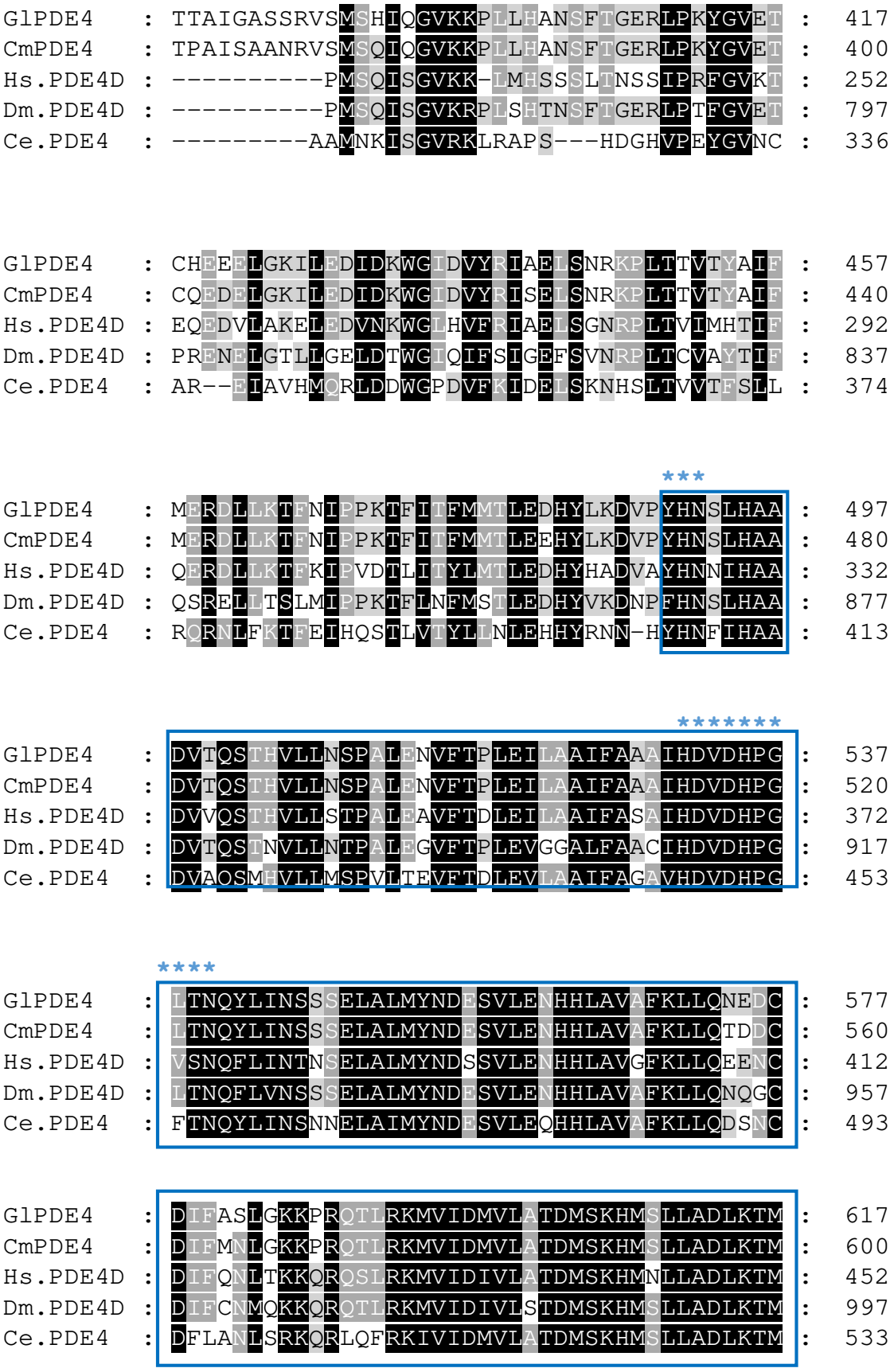
```
2281 ACCTTGACATCCCGAGTCTGCGAGTGGACGATGGCGAACGGCCTAAGAAGAAGGACAGAA
 344   H  S  G  T  F  A  G  V  S  S  A  A  I  S  G  G  G  E  R  S
2341 CCCATTCCGGCACCTTTGCGGGAGTGTCGTCAGCAGCAATATCAGGAGGCGGAGAGAGGA
 364   A  Q  T  L  Q  S  I  A  S  A  S  A  A  V  Q  G  T  P  A  I
2401 GTGCCCAAACGCTGCAGTCCATTGCCTCAGCCTCGGCAGCAGTACAAGGCACGCCAGCAA
 384   S  A  A  N  R  V  S  M  S  Q  I  Q  G  V  K  K  P  L  L  H
2461 TCAGCGCGGCCAACAGGGTGTCCATGTCCCAGATCCAGGGCGTCAAGAAACCACTACTCC
 404   A  N  S  F  T  G  E  R  L  P  K  Y  G  V  E  T  C  Q  E  D
2521 ACGCGAACTCCTTCACGGGCGAGAGACTGCCGAAGTATGGTGTGGAGACCTGTCAAGAAG
 424   E  L  G  K  I  L  E  D  I  D  K  W  G  I  D  V  Y  R  I  S
2581 ACGAGCTGGGCAAGATATTGGAGGACATTGATAAGTGGGGCATTGATGTGTACAGGATCT
 444   E  L  S  N  R  K  P  L  T  T  V  T  Y  A  I  F  M  E  R  D
2641 CTGAACTGTCCAACAGGAAGCCACTTACCACCGTTACCTACGCAATCTTCATGGAAAGAG
 464   L  L  K  T  F  N  I  P  P  K  T  F  I  T  F  M  M  T  L  E
2701 ACCTGCTGAAGACGTTCAATATTCCGCCCAAGACCTTCATCACCTTCATGATGACACTGG
 484   E  H  Y  L  K  D  V  P  Y  H  N  S  L  H  A  A  D  V  T  Q
2761 AGGAGCACTACCTGAAGGATGTGCCCTACCACAACTCATTGCACGCTGCTGACGTCACAC
 504   S  T  H  V  L  L  N  S  P  A  L  E  N  V  F  T  P  L  E  I
2821 AGTCCACCCATGTCCTCCTTAACTCCCCAGCCCTTGAGAATGTGTTCACGCCTCTGGAAA
 524   L  A  A  I  F  A  A  A  I  H  D  V  D  H  P  G  L  T  N  Q
2881 TCCTGGCAGCCATCTTTGCAGCGGCCATCCATGACGTGGACCACCCAGGCCTCACCAACC
 544   Y  L  I  N  S  S  S  E  L  A  L  M  Y  N  D  E  S  V  L  E
2941 AGTACCTGATCAACTCCTCCTCAGAACTTGCCCTCATGTATAATGATGAGTCGGTCCTGG
 564   N  H  H  L  A  V  A  F  K  L  L  Q  T  D  D  C  D  I  F  M
3001 AGAACCACCACCTGGCCGTGGCATTCAAGCTGCTCCAGACTGACGACTGTGACATCTTCA
 584   N  L  G  K  K  P  R  Q  T  L  R  K  M  V  I  D  M  V  L  A
3061 TGAACCTTGGTAAGAAGCCCCGGCAGACCCTGAGAAAGATGGTGATTGACATGGTGCTGG
 604   T  D  M  S  K  H  M  S  L  L  A  D  L  K  T  M  V  E  T  K
3121 CAACAGACATGAGCAAACACATGAGCCTTCTAGCTGACCTCAAGACCATGGTGGAGACTA
 624   K  V  A  G  S  G  V  L  L  L  D  N  Y  T  D  R  I  Q  V  L
3181 AAAAGGTGGCTGGCTCTGGAGTGTTGCTTCTTGACAACTACACAGACAGGATACAGGTGC
 644   Q  N  M  V  H  C  A  D  L  S  N  P  T  K  P  L  E  M  Y  K
3241 TACAAAACATGGTCCACTGTGCTGATCTGAGCAACCCCACCAAGCCTCTTGAGATGTACA
 664   N  W  V  S  S  I  M  E  E  F  F  Q  Q  G  D  R  E  R  D  Q
3301 AAAACTGGGTCTCTTCTATCATGGAGGAGTTCTTCCAGCAAGGTGACCGAGAGAGGGATC
 684   G  M  D  I  S  P  M  C  D  R  H  T  A  T  I  E  K  S  Q  V
3361 AGGGAATGGACATCTCTCCAATGTGTGACAGACATACAGCCACCATCGAAAAGTCACAGG
 704   G  F  I  D  Y  I  V  H  P  L  W  E  T  W  A  D  L  V  H  P
3421 TTGGCTTCATTGACTACATTGTCCATCCACTGTGGGAGACATGGGCAGATCTGGTCCATC
 724   D  A  Q  D  I  L  D  T  L  E  E  N  R  D  W  Y  N  R  M  I
3481 CTGATGCCCAGGACATCTTGGACACCTTGGAGGAGAACAGAGATTGGTACAACCGCATGA
 744   P  I  S  P  S  S  S  S  N  D  L  K  E  E  D  Y  P  G  E  N
3541 TTCCCATCTCCCCATCTTCCTCATCTAATGACCTGAAGGAGGAGGATTATCCTGGAGAGA
 764   S  Q  D  A  P  E  E  E  L  C  A  A  A  D  R  I  Q  I  Q  F
3601 ATTCTCAGGATGCACCCGAGGAGGAGCTTTGTGCAGCTGCTGACAGGATCCAGATTCAGT
 784   T  L  E  D  E  G  S  G  N  R  G  P  P  G  D  A  R  G  E  D
3661 TTACACTTGAGGACGAAGGCAGTGGGAATAGAGGGCCGCCAGGTGACGCTCGGGGGGAGG
 787   P  T  M
3721 ATCCCACAATGTGACCGCTGGTGGCGTCCTACTGTGCCAGACTAAAGCGGAAGGTGATGC
3781 GGTGGCTGCACCTCAAGTGACGGCCCCTGCAGCAGGAGGCAAGGGAGGCTGCTGCATCCC
3841 CAGTCCTCCTGCACACCCCTGCTTCTGGCCCTCACCTCCTTACCTCATGTTGCGAGATTT
3901 ATTTGTGTCTGCCGCGGGCCCTGCTGGTTCGGGGCCGACCGCGTGGGCTTGTGTGAGCTC
3961 CCATTGACTGAGCTATATGCCTGGCCTCAGCCAACCTACCCTTGCCTAATGTGATTTAAT
```

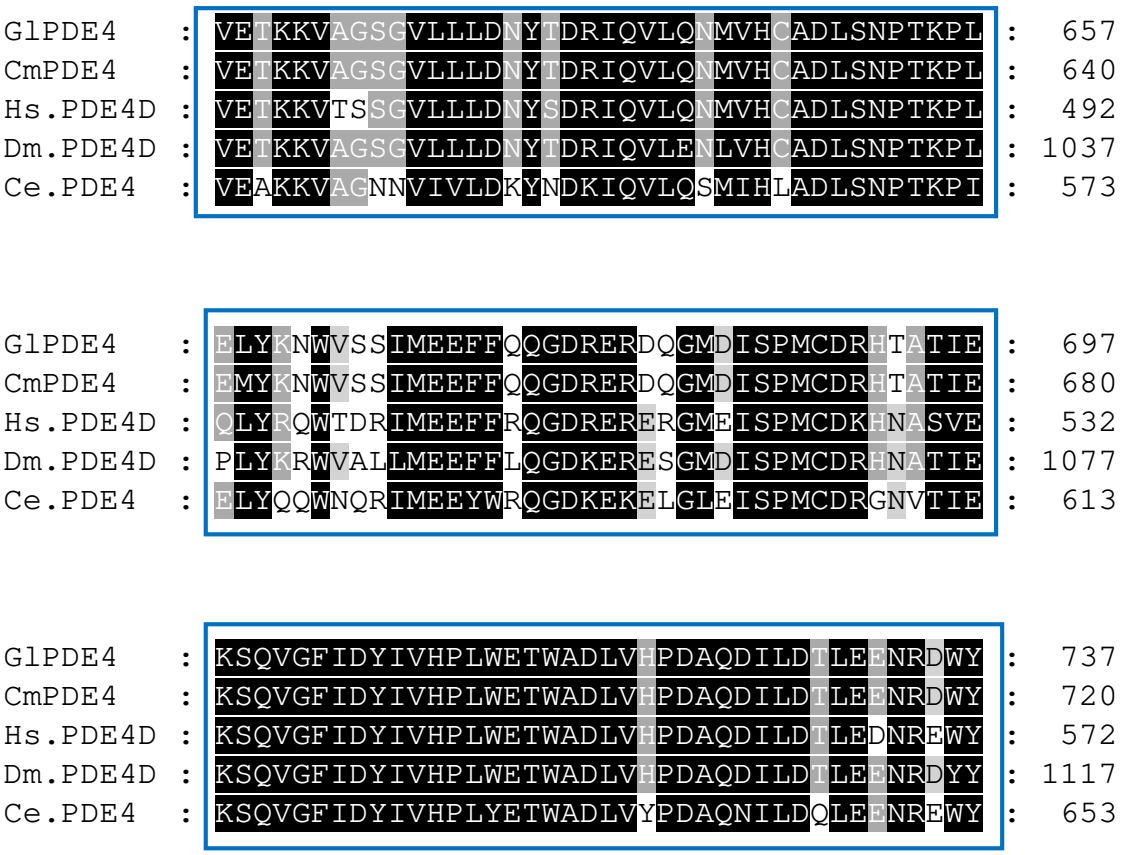
```
4021 ATATTCTTATAGTATTGATGGAGAGCCGGTCACAACAGTGAACAGTGCCAAATCGTTCCT
4081 TTTAAAAAGAAATGTGGATCCTGTGGTGGTGGTTTGTTACATGAGAAAAAAGAACATTCG
4141 TAGGGAGTTGTCCATCATGCGAGGACTCTGTTCCTGGGTGGTTATTCCTTTAAAATACCA
4201 ATCCCATGAAGGCAACAATGTGTCATGCCTCAAGAGCACAATTGTGGGGAGTTGATTATT
4261 CTTTCTTTCTTTCTTTCTTTTTTTTTCTAACGTTTTGAGGACTAAGGCTTCTGTGTGGTA
4321 CATATCAGATAGCAGCATCAGTAAGCGGCTCGAGAGTAGTGCTTTCACTGTGCAAGTCTG
4381 GGGCATTATTGCGTGAGCTCTTTCGTAACACAGGTGCCTGACGGGATTAGCCGCTGCCAA
4441 CACTCAAAGTAAACTAGCTGTCGGAGAAGCCAAAGATAATTTATTGCTTCAACCCCAGTG
4501 GATTAAGAGCCACTACTGTGACTGCACATTGCAGCTGCTGCACAGCTCGAGGGATTTAAT
4561 TTTGTACAGCAGCTGAGAACATTGATGGTGGGTCCTGTCAGGTAAATAAAACAAAAAAAT
4621 AAAAATAAAAAAATAAAAAAAAAAGTCCCTCACTTGAAACTGATGTAAGAGTTTAGATGAT
4681 ATTATTGATTCCCTTTTTCATTACTTATTTTAAGTAGTGTGGAAAAATAATTTGCCACCA
4741 TTAGATTTTTTTTTCATTGCTCCTTACAGTGCAAAGTTCATGAGTAGTTCGTTTTTAATA
4801 TTTTTGCAATGTCACTTTGAGATTTTATTCTTTTGACAGCTTTTTTGGCGCTACCTTAGA
4861 TGTTCAATGAACTTGACTGATGGGTTTTCACCTCCTTTTGAACTAGGTAGCCATGAAGAA
4921 GAGGTTCAGATTTTAGCACTTCATTCACTATCCGCAGTAATCAACGCAGGCACTTTGTAA
4981 ACCTTCTCTCATTCTCTAGAAAGCCTTTATGCTACCATGTATTTTCCTGTCTTAAATTCT
5041 TCAACTTATAAACAATGTAGCATTGACCTCCTCATGAAATGTGTTGTTTTCAAGGTAGAT
5101 GCCTATAAAAGCATCATTCAGTCCAACTGTCCATCAACCTGAAATGTGCAATTTAGAAAG
5161 AACCTATAAACTTTTACAATTGTTTCAGTCTGTAGGACACATTACTTATTCTATGTCATT
5221 AGACAAAATATTTTTGCTCTTTTGATAAAGTAAATTACTGTCAGTCAGTAATATTTCATT
5281 CACTGTGTATTGAGTCAGTAATTTACATTATTAAGTCACCAAATTGACCTGTGATACTCA
5341 AACCATTGGACTTGCCTGAAATGAAGCAAATCTGAGCCTCTAATGTAGAATACTCCCTTG
5401 TGATGAAGAGAGCCTAATGTGTGTCACGTTCCATCCCACGCCGCCCAGTGTCACTCACCA
5461 GCCACTCTCCCAATTCCCCCTCGGCTCGGCACTGGTGACACCGGCAGCCCTCGGATCCTT
5521 CCTCCCGCCCCCTCCCTTTGTTCTTCCCTCCCTCCTTGTAGGCAGATAATGGCAATGGGG
5581 ACACTGATAAATATGCACCACACAGATAACCACTTCCATAGCCATGATGTTTGTGTCCAC
5641 TCATTGCAGTCATTTCAGAGATCAGTGTTGCTCCTGTCTTTATAATGTACGTAGTGTACT
5701 GTACACTCCAGCCTTGGTGTTGGCACTGTGCAAGGCACAAGTGTTCCCTCACAGTGGTAC
5761 TCCCACAGGCACTGTGAGTGGAGCCTAACTTGCCATCAGAAAAATAAGACAGCTCAAGAG
5821 GAGAAAAGTGGGACAAATTTTCTCTGCAGATGTTTCCCCACAGACCTGAGGAAAGACTCG
5881 CCCTTCACTAAATGCTAATTATTCAGATATATTTATTTTTTATAGTAGAAGCATTAATCC
5941 CACCCTTATGACAGGGTGACTGTATGTAGTTGTCTAATACCATGTAAATGTTAATTTTTC
6001 TCATAATTTATTGAATGGGTCTCCTATACATGTGTATTATTTTTAAAGTATTTACATGTA
6061 TATAGAGGTGCAAGTAAATATTATCAGGTGTATAATAAGATGTATGAGTATTTAAGCAGT
6121 AT
```

Figure 2.6. Nucleotide and amino acid sequence of cDNA encoding Cm-PDE4. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue. One Upstream Conserved Region; UCR2, is found in a purple box.


GlPDE4 : --MLQVPEIRAPDEDEDDDTPTPSCLRLQVPEFSLLAPPD : 38
CmPDE4 : --------MSRTTSCEMQQRVVTGGVDTLPPHLGNRTPPG : 32
Hs.PDE4D : -------------------------MMHVNNFPFRRHS- : 13
Dm.PDE4D : YCGSCESVHHSSATSSSAGTVPPGGQQTQEYIAGTSSTPS : 400
Ce.PDE4 : -----MPRRRGSSSSSSAAGGSGGCGGFGFSSLRRELHLH : 35
GlPDE4 : DPPDGGGGCVHLQVPEMGSFVPPGSNIGSSATRLEPPKTL : 78
CmPDE4 : DVANMN------RLPGLPTLPALPALPGDAGR--TPRRVM : 64
Hs.PDE4D : -- : -
Dm.PDE4D : PRIKLKFRKPHKSCWSRIVLAPIGSAGGSSSATTVIGSNS : 440
Ce.PDE4 : NFFRTS------------------SPSASSTSRTPPAALP : 57
GlPDE4 : HLSLPPFPQPGVSSFLQVPGGYTGPRRRHSWICSFDVENG : 118
CmPDE4 : KRPDGSFVPQRSFSFRERSDSLGG-------ASALRAHEP : 97
Hs.PDE4D : ------------------------------WICFDVDNG : 22
Dm.PDE4D : NETLASSSTTGGTATTTQNSSSVSVAAHHRLTSSSASALA : 480
Ce.PDE4 : PRTSAVTIPGSNHKLTSSASSYHP-------PRELTVSTF : 90
GlPDE4 : TSP------------------------------------ : 121
CmPDE4 : TSP------------------------------------ : 100
Hs.PDE4D : TSA------------------------------------ : 25
Dm.PDE4D : TSHPSNSQLLPTSKMQAEQGSIGDLQKYHSRYLKNRRHTL : 520
Ce.PDE4 : SAG------------------------------------ : 93
UCR1
GlPDE4 : -----------GRSP-LDGTSPSAGLVLQN---FPQRRES : 146
CmPDE4 : -----------THHLSMDLEVPEAARQQSL---AVNHSFS : 126
Hs.PDE4D : -----------GRSPLDPMTSPGSGLILQANFVHSQRRES : 54
Dm.PDE4D : ANVRFDVENGQGARSPLEGGSPSAGLVLQN---LPQRRES : 557
Ce.PDE4 : ------------SATAADGLGGAHLTPSLSSSVHARRES : 120
GlPDE4 : FLYRSDSDF--EMSPKSMSRNSSIASEGSRVMLATLSESR : 184
CmPDE4 : DLYDMSSSQ--GKLPRTLSTSALRIKSRSNFWEKFWQGPL : 164
Hs.PDE4D : FLYRSDSDY--DLSPKSMSRNSSIAS-------------- : 78
Dm.PDE4D : FLYRSDSDF--EMSPKSMSRNSSIASER-------FKEQE : 588
Ce.PDE4 : FLYRASDDLREASSLRPVSRASSIASN------------ : 147


GlPDE4 : GANDDKPIHGEDLIVTPFAQILASLRSVRNNYINLTNVST : 224
CmPDE4 : EPRVGSKLHGEDLIVTPFAQILASLRSVRNNYISLTNVST : 204
Hs.PDE4D : ------DIHGDDLIVTPFAQVLASLRTVRNNFAALTNIQD : 112
Dm.PDE4D : ASILVDRSHGEDLIVTPFAQILASLRSVRNNLLSLTNVPA : 628
Ce.PDE4 : -----EHGHGDDLIVTPFAQLLASLRNVRSNLISITNIQN : 182
UCR2
GlPDE4 : ----SKSRRSSAAAGCSTPQPKNFTYG---DETYTKVALE : 257
CmPDE4 : ----SKSRRSSAQAGCSTPQPKNFIYGGECDETYTKMALE : 240
Hs.PDE4D : ----RAP--SKRSPMCNQPSINKATIT---EEAYQKLASE : 143
Dm.PDE4D : SNKSRRPNQSSSASRSGNPPGAPLSQG---EEAYTRLATD : 665
Ce.PDE4 : -----SDDSRHANRSAKRPPLHNIELP----DDVVHCAHD : 213
GlPDE4 : TLEELDWCLDQLETIQTHRSVSDMASSKFKRMLNKELSHF : 297
CmPDE4 : TLEELDWCLDQLETIQTHRSVSDMASSKFKRMLNKELSHF : 280
Hs.PDE4D : TLEELDWCLDQLETLQTRHSVSEMASNKFKRMLNRELTHL : 183
Dm.PDE4D : TIEELDWCLDQLETIQTHRSVSDMASLKFKRMLNKELSHF : 705
Ce.PDE4 : TLEELDWCLDQLETIQTHRSVSEMASSKFRKMLNKELSHF : 253
End of UCR2
GlPDE4 : SESSKSGNQISEYICTTFLDQQQDLDIPSLRVDDGERPKK : 337
CmPDE4 : SESSKSGNQISEYICTTFLDQQQDLDIPSLRVDDGERPKK : 320
Hs.PDE4D : SEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKKR : 223
Dm.PDE4D : SESSRSGNQISEYICSTFLDKQQEFDLPSLRVEDNPELVA : 745
Ce.PDE4 : AESSKSGTQVSKFLITTYMDKEE--DEPSIEIEVPTEVQG : 291
GlPDE4 : KERTVSSSLAGVSAAAVPGGSERSSQVLQSVAAVSSGSQT : 377
CmPDE4 : KDRTHSGTFAGVSSAAISGGGERSAQTLQSIASASAAVQG : 360
Hs.PDE4D : ---------- : -
Dm.PDE4D : ANAAAGQQSAGQYARSRSPRGP---------- : 767
Ce.PDE4 : PSTSGPMTLSILKKAQT---------- : 308


GlPDE4 : TTAIGASSRVSMSHIQGVKKPLLHANSFTGERLPKYGVET : 417
CmPDE4 : TPAISAANRVSMSQIQGVKKPLLHANSFTGERLPKYGVET : 400
Hs.PDE4D : ----------PMSQISGVKK-LMHSSSLTNSSIPRFGVKT : 252
Dm.PDE4D : ----------PMSQISGVKRPLSHTNSFTGERLPTFGVET : 797
Ce.PDE4 : ---------AAMNKISGVRKLRAPS---HDGHVPEYGVNC : 336
GlPDE4 : CHEEELGKILEDIDKWGIDVYRIAELSNRKPLTTVTYAIF : 457
CmPDE4 : CQEDELGKILEDIDKWGIDVYRISELSNRKPLTTVTYAIF : 440
Hs.PDE4D : EQEDVLAKELEDVNKWGLHVFRIAELSGNRPLTVIMHTIF : 292
Dm.PDE4D : PRENELGTLLGELDTWGIQIFSIGEFSVNRPLTCVAYTIF : 837
Ce.PDE4 : AR--EIAVHMQRLDDWGPDVFKIDELSKNHSLTVVTFSLL : 374
***
GlPDE4 : MERDLLKTFNIPPKTFITFMMTLEDHYLKDVPYHNSLHAA : 497
CmPDE4 : MERDLLKTFNIPPKTFITFMMTLEEHYLKDVPYHNSLHAA : 480
Hs.PDE4D : QERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIHAA : 332
Dm.PDE4D : QSRELLTSLMIPPKTFLNFMSTLEDHYVKDNPFHNSLHAA : 877
Ce.PDE4 : RQRNLFKTFEIHQSTLVTYLLNLEHHYRNN-HYHNFIHAA : 413
****
GlPDE4 : DVTQSTHVLLNSPALENVFTPLEILAAIFAAAIHDVDHPG : 537
CmPDE4 : DVTQSTHVLLNSPALENVFTPLEILAAIFAAAIHDVDHPG : 520
Hs.PDE4D : DVVQSTHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPG : 372
Dm.PDE4D : DVTQSTNVLLNTPALEGVFTPLEVGGALFAACIHDVDHPG : 917
Ce.PDE4 : DVAQSMHVLLMSPVLTEVFTDLEVLAAIFAGAVHDVDHPG : 453
****
GlPDE4 : LTNQYLINSSSELALMYNDESVLENHHLAVAFKLLQNEDC : 577
CmPDE4 : LTNQYLINSSSELALMYNDESVLENHHLAVAFKLLQTDDC : 560
Hs.PDE4D : VSNQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEENC : 412
Dm.PDE4D : LTNQFLVNSSSELALMYNDESVLENHHLAVAFKLLQNQGC : 957
Ce.PDE4 : FTNQYLINSNNELAIMYNDESVLEQHHLAVAFKLLQDSNC : 493
GlPDE4 : DIFASLGKKPRQTLRKMVIDMVLATDMSKHMSLLADLKTM : 617
CmPDE4 : DIFMNLGKKPRQTLRKMVIDMVLATDMSKHMSLLADLKTM : 600
Hs.PDE4D : DIFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLLADLKTM : 452
Dm.PDE4D : DIFCNMQKKQRQTLRKMVIDIVLSTDMSKHMSLLADLKTM : 997
Ce.PDE4 : DFLANLSRKQRLQFRKIVIDMVLATDMSKHMSLLADLKTM : 533


GlPDE4 : VETKKVAGSGVLLLDNYTDRIQVLQNMVHCADLSNPTKPL : 657
CmPDE4 : VETKKVAGSGVLLLDNYTDRIQVLQNMVHCADLSNPTKPL : 640
Hs.PDE4D : VETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPL : 492
Dm.PDE4D : VETKKVAGSGVLLLDNYTDRIQVLENLVHCADLSNPTKPL : 1037
Ce.PDE4 : VEAKKVAGNNVIVLDKYNDKIQVLQSMIHLADLSNPTKPI : 573
GlPDE4 : ELYKNWVSSIMEEFFQQGDRERDQGMDISPMCDRHTATIE : 697
CmPDE4 : EMYKNWVSSIMEEFFQQGDRERDQGMDISPMCDRHTATIE : 680
Hs.PDE4D : QLYRQWTDRIMEEFFRQGDRERERGMEISPMCDKHNASVE : 532
Dm.PDE4D : PLYKRWVALLMEEFFLQGDKERESGMDISPMCDRHNATIE : 1077
Ce.PDE4 : ELYQQWNQRIMEEYWRQGDKEKELGLEISPMCDRGNVTIE : 613
GlPDE4 : KSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEENRDWY : 737
CmPDE4 : KSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEENRDWY : 720
Hs.PDE4D : KSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEDNREWY : 572
Dm.PDE4D : KSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEENRDYY : 1117
Ce.PDE4 : KSQVGFIDYIVHPLYETWADLVYPDAQNILDQLEENREWY : 653
GlPDE4 : NRMIPISPSSS--SNDLKE----------EEYPG : 759
CmPDE4 : NRMIPISPSSS--SNDLKE----------EDYPG : 742
Hs.PDE4D : QSTIPQSPSPA--PDDPEEGRQGQTEKFQFELTLEEDGES : 610
Dm.PDE4D : QSMIPPSPPPSGVDENPQEDRIR-------FQVTLEESDQ : 1150
Ce.PDE4 : QSRIPEEPDTAR---------- : 665
GlPDE4 : ENSQD----VPEEELCAAADRIQIQFTLEDEGSGNRGPPG : 795
CmPDE4 : ENSQD----APEEELCAAADRIQIQFTLEDEGSGNRGPPG : 778
Hs.PDE4D : DTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEE : 650
Dm.PDE4D : ENLAELE--EGDESGGESTTTGTTGTTAASALSGAGGGGG : 1188
Ce.PDE4 : ----------TVTEDDEHK------- : 674

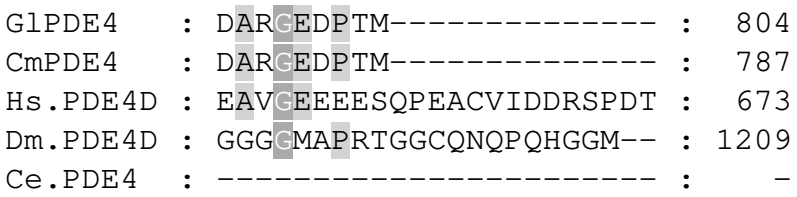
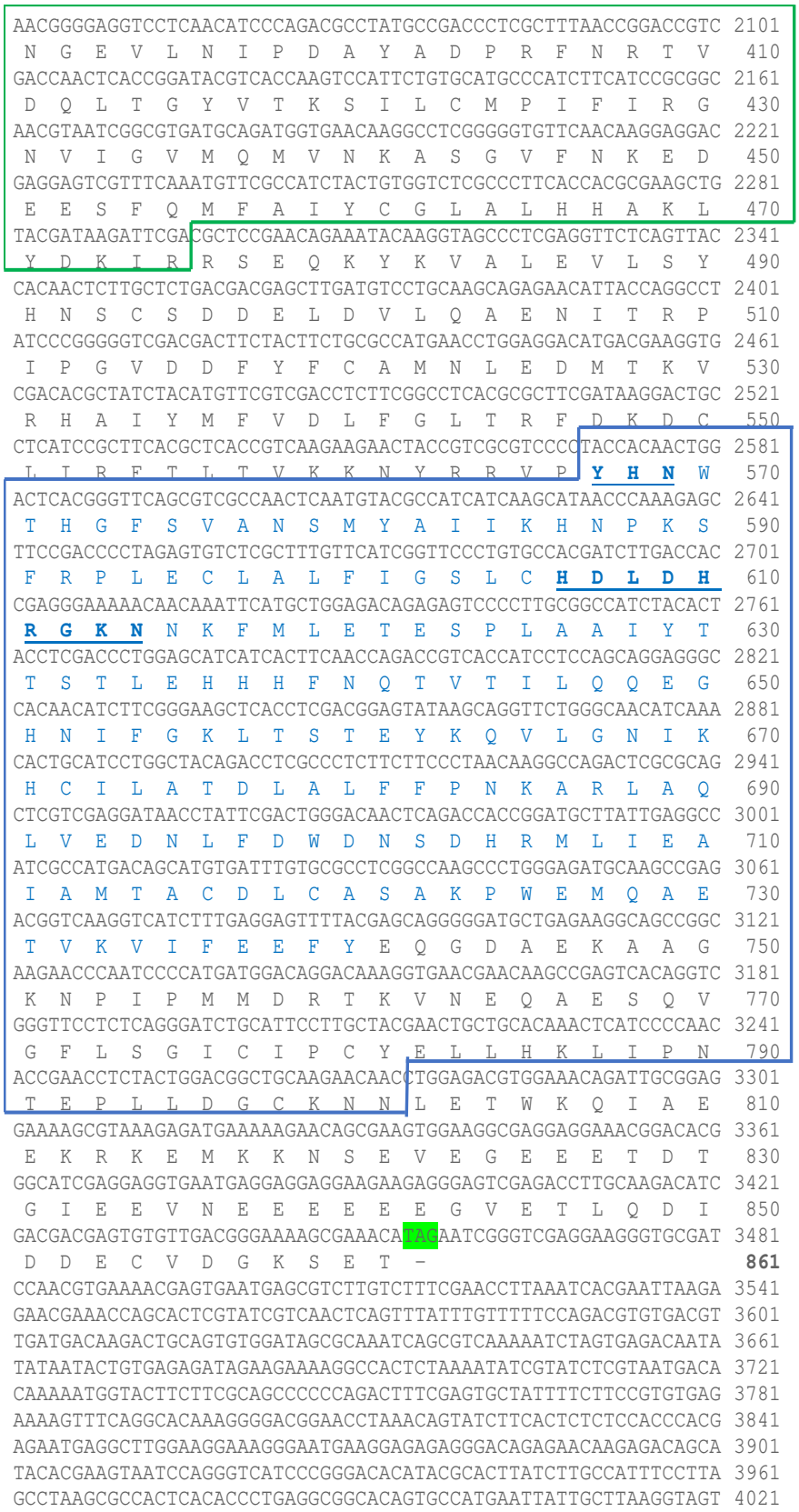


Figure 2.7. Multiple alignment of deduced amino acid sequences of PDE4 proteins in two crustacean species, one insect species, one nematode species and one mammal species. Abbreviations: Gl: *G lateralis*; Cm: *C maenas*; Dm: *D melanogaster*; Ce: *C elegans*; Hs: *H sapiens*. Black shading indicates that amino acid residues that are identical or similar in all sequences; gray shading indicates identical or similar amino acids in most of the sequences. Dashes indicate gaps introduced to optimize the alignment and the boxes indicate highly conserved domains. Blue asterisks indicate the signature sequences in the catalytic domain. The colors of the boxes correspond to the colors of the domains in Fig. 2. 5.

GlPDE5 6761 nucleotides, structure: G sequence

```
AGAGAGAGAGAGAGAGAGTGGGAGACGCTTTTAACCGGGACAGGAGGGAGAGAGACGC    61
TTTAAACCGGGACAACAATAAGTCGATTATGAACAAAGCCAGGGAGGGGACAGCGAGGAG  121
TCCTGAGGGAGACGTGTGGCCAGCGGAGACCTGCCTTTGTGGACCTGTGTGCCCTGGACT  181
GAACGCGTGCCCTCTAGACGTGTGTTCCTAAGTGTGACCTTGTGGGACGTGCCTCTGGGA  241
GACGTGCCCCTTGGAAACTGTACCCCAAGAGAAACGTGACTTCAGTGAAACGAGCCCAAA  301
GAACTGTTCTCTGAGGAGCCCACTTTTGTAGACCTACGGAAAAGGACTCGAAAAGGAATG  361
AGTTAGGAATAGTGGCCCTTTCCAAGACTTGCACAAAGTACTGTCCCCTGATATGCTCAC  421
ACTGACTGTGAAGAAGTACAAAAGAAGACTCGAAAAGGAAAACACTGAAGCCGTTTTCCT  481
TAAGAAGTAGACGAATCCCAAATCAAACGTACCTCAGGGGAAAAGTGTCCAAAGATCTGT  541
TTACCGATAAAACCGCATCTGAAAAAGTACAAACGAGAACTCGAAAACAAAGGCGTCGGA  601
ACAGTTTACCCAGCGAAAGGTACACGTGCCTGGGGAAACGTTCATCAAGATCTGTTCTTT  661
TACAATCCCACGCTTGAAGACGTATGTAAGAGGACGAAAAATTTAAGACGCTGAGATCGT  721
GTCCCGCAAAGAGAGACGTGCCTCAGGATCTGCTTGAAGACACAAAACGGAAGACGCTGA  781
AGTAGTTCCCCCCCCCTGAAAGAATAGAGGGACGGACCCTTGAAGGCAGCACGACCCCCA  841
GCCCTCTTCTCCCCTCCTGCAGCCGCCGCAATGAGCTGTCTGCTGGAGGAAGGGGGACAG  901
                              M  S  C  L  L  E  E  G  G  Q    10
GATGGGGTGGTGGGGGGTGGGGGTGTTGGTGTCAAGCGGGGACCCACGCCCATACGCCGC  961
 D  G  V  V  G  G  G  G  V  G  V  K  R  G  P  T  P  I  R  R    30
CGAAGATCAGGGGTCGGGGCGCCGGGCCTCGTTGTGAACGGGCGGAACAAGATGAACAGC 1021
 R  R  S  G  V  G  A  P  G  L  V  V  N  G  R  N  K  M  N  S    50
CACGACCTTCCGTTAACAGCCGAGGACATGACGGGGGAGGCGGTGGGGCAGTACCTGAAG 1081
 H  D  L  P  L  T  A  E  D  M  T  G  E  A  V  G  Q  Y  L  K    70
GCGCACCCTGAGTTCCTCGAGAGTTGGCTCATGGAACAGGTGGAGCTGGAGACGCTGGAG 1141
 A  H  P  E  F  L  E  S  W  L  M  E  Q  V  E  L  E  T  L  E    90
CGGTGGATGATACGCCGCACCCAACGCGACAAACAGAAGAGCTTGGAGAACGGCACGAAT 1201
 R  W  M  I  R  R  T  Q  R  D  K  Q  K  S  L  E  N  G  T  N   110
GGTAAAATCATCAGAAAAACAAGCCTATCCAGATGGAAGTTCTGCGTGCATGCTGACAAG 1261
 G  K  I  I  R  K  T  S  L  S  R  W  K  F  C  V  H  A  D  K   130
CGCAAGATGCTGCAGGAGCTCACATCGTCCCTCTACGTACGGCCCAACAAGCCACACGTC 1321
 R  K  M  L  Q  E  L  T  S  S  L  Y  V  R  P  N  K  P  H  V   150
CTCTGGGAGCTCACGCGCTGCATCTCCTCGGCCGTGAACGCAGACGGCTGTAACCTGTAC 1381
 L  W  E  L  T  R  C  I  S  S  A  V  N  A  D  G  C  N  L  Y   170
CTGGCTGACCTGGACACCAACACACTCATGCGATACGTGGAGAGCAAAGACGGGGAGGGG 1441
 L  A  D  L  D  T  N  T  L  M  R  Y  V  E  S  K  D  G  E  G   190
GACAGCTCGACCTGGAGCTGTCAAGTGGGCGGCGGGGCGTGGCTGTGTGGGTACGTGGCC 1501
 D  S  S  T  W  S  C  Q  V  G  G  G  A  W  L  C  G  Y  V  A   210
AGCTCGCGTCAGGCCGTCAGGGTGACCTGCCCCATCACTGACCCAAGGTTCCCTAAAGGA 1561
 S  S  R  Q  A  V  R  V  T  C  P  I  T  D  P  R  F  P  K  G   230
TGCCCCTTCGCTGAGGAGCAGGAAGTGCACCACCTTCTCGTGATGGCGGTGGTGCAGAGC 1621
 C  P  F  A  E  E  Q  E  V  H  H  L  L  V  M  A  V  V  Q  S   250
GACGGAGAGTTGGCGGCCGTTCTGGAGCTGTACAGGCGACGAGGGGGCGAGGCCTTCCAC 1681
 D  G  E  L  A  A  V  L  E  L  Y  R  R  R  G  G  E  A  F  H   270
ACGGAGGACGAGGAGATCGTCAACTCGTACCTGGTGTGGGGAGGCATCGCCCTGCACTAC 1741
 T  E  D  E  E  I  V  N  S  Y  L  V  W  G  G  I  A  L  H  Y   290
GCCGAGCTCTACCACAGCATGGTGAAGCAACGCACGCTCAATGAGTTCATCCTCTCCGTC 1801
 A  E  L  Y  H  S  M  V  K  Q  R  T  L  N  E  F  I  L  S  V   310
GTGAAGTCGATCTTCCAGGACATGGTTAGTATGGACACTCTCATAATGAAGGTGATGAAC 1861
 V  K  S  I  F  Q  D  M  V  S  M  D  T  L  I  M  K  V  M  N   330
TTTGCCCAGAAGCTTGTGAACGCCGATCGAGCTTCCCTCTTCCTCGTCGACTCCAAGAAC 1921
 F  A  Q  K  L  V  N  A  D  R  A  S  L  F  L  V  D  S  K  N   350
AAGCAACTCTACGCCCGCATCTTCGATATGGGCAGTGAATTCAGTGAAGACAATCCCCCA 1981
 K  Q  L  Y  A  R  I  F  D  M  G  S  E  F  S  E  D  N  P  P   370
CAGTCATTCAAGGAGATCAGGTTCGCCATTGGGAAAGGGATCGCCGGCATCGTGGCCCAG 2041
 Q  S  F  K  E  I  R  F  A  I  G  K  G  I  A  G  I  V  A  Q   390
```


AACGGGGAGGTCCTCAACATCCCAGACGCCTATGCCGACCCTCGCTTTAACCGGACCGTC 2101
N G E V L N I P D A Y A D P R F N R T V 410
GACCAACTCACCGGATACGTCACCAAGTCCATTCTGTGCATGCCCATCTTCATCCGCGGC 2161
D Q L T G Y V T K S I L C M P I F I R G 430
AACGTAATCGGCGTGATGCAGATGGTGAACAAGGCCTCGGGGGTGTTCAACAAGGAGGAC 2221
N V I G V M Q M V N K A S G V F N K E D 450
GAGGAGTCGTTTCAAATGTTCGCCATCTACTGTGGTCTCGCCCTTCACCACGCGAAGCTG 2281
E E S F Q M F A I Y C G L A L H H A K L 470
TACGATAAGATTCGACGCTCCGAACAGAAATACAAGGTAGCCCTCGAGGTTCTCAGTTAC 2341
Y D K I R R S E Q K Y K V A L E V L S Y 490
CACAACTCTTGCTCTGACGACGAGCTTGATGTCCTGCAAGCAGAGAACATTACCAGGCCT 2401
H N S C S D D E L D V L Q A E N I T R P 510
ATCCCGGGGGTCGACGACTTCTACTTCTGCGCCATGAACCTGGAGGACATGACGAAGGTG 2461
I P G V D D F Y F C A M N L E D M T K V 530
CGACACGCTATCTACATGTTCGTCGACCTCTTCGGCCTCACGCGCTTCGATAAGGACTGC 2521
R H A I Y M F V D L F G L T R F D K D C 550
CTCATCCGCTTCACGCTCACCGTCAAGAAGAACTACCGTCGCGTCCCCTACCACAACTGG 2581
L I R F T L T V K K N Y R R V P Y H N W 570
ACTCACGGGTTCAGCGTCGCCAACTCAATGTACGCCATCATCAAGCATAACCCAAAGAGC 2641
T H G F S V A N S M Y A I I K H N P K S 590
TTCCGACCCCTAGAGTGTCTCGCTTTGTTCATCGGTTCCCTGTGCCACGATCTTGACCAC 2701
F R P L E C L A L F I G S L C H D L D H 610
CGAGGGAAAAACAACAAATTCATGCTGGAGACAGAGAGTCCCCTTGCGGCCATCTACACT 2761
R G K N N K F M L E T E S P L A A I Y T 630
ACCTCGACCCTGGAGCATCATCACTTCAACCAGACCGTCACCATCCTCCAGCAGGAGGGC 2821
T S T L E H H H F N Q T V T I L Q Q E G 650
CACAACATCTTCGGGAAGCTCACCTCGACGGAGTATAAGCAGGTTCTGGGCAACATCAAA 2881
H N I F G K L T S T E Y K Q V L G N I K 670
CACTGCATCCTGGCTACAGACCTCGCCCTCTTCTTCCCTAACAAGGCCAGACTCGCGCAG 2941
H C I L A T D L A L F F P N K A R L A Q 690
CTCGTCGAGGATAACCTATTCGACTGGGACAACTCAGACCACCGGATGCTTATTGAGGCC 3001
L V E D N L F D W D N S D H R M L I E A 710
ATCGCCATGACAGCATGTGATTTGTGCGCCTCGGCCAAGCCCTGGGAGATGCAAGCCGAG 3061
I A M T A C D L C A S A K P W E M Q A E 730
ACGGTCAAGGTCATCTTTGAGGAGTTTTACGAGCAGGGGGATGCTGAGAAGGCAGCCGGC 3121
T V K V I F E E F Y E Q G D A E K A A G 750
AAGAACCCAATCCCCATGATGGACAGGACAAAGGTGAACGAACAAGCCGAGTCACAGGTC 3181
K N P I P M M D R T K V N E Q A E S Q V 770
GGGTTCCTCTCAGGGATCTGCATTCCTTGCTACGAACTGCTGCACAAACTCATCCCCAAC 3241
G F L S G I C I P C Y E L L H K L I P N 790
ACCGAACCTCTACTGGACGGCTGCAAGAACAACCTGGAGACGTGGAAACAGATTGCGGAG 3301
T E P L L D G C K N N L E T W K Q I A E 810
GAAAAGCGTAAAGAGATGAAAAAGAACAGCGAAGTGGAAGGCGAGGAGGAAACGGACACG 3361
E K R K E M K K N S E V E G E E E T D T 830
GGCATCGAGGAGGTGAATGAGGAGGAGGAAGAAGAGGGAGTCGAGACCTTGCAAGACATC 3421
G I E E V N E E E E E E G V E T L Q D I 850
GACGACGAGTGTGTTGACGGGAAAAGCGAAACATAGAATCGGGTCGAGGAAGGGTGCGAT 3481
D D E C V D G K S E T - 861
CCAACGTGAAAACGAGTGAATGAGCGTCTTGTCTTTCGAACCTTAAATCACGAATTAAGA 3541
GAACGAAACCAGCACTCGTATCGTCAACTCAGTTTATTTGTTTTTCCAGACGTGTGACGT 3601
TGATGACAAGACTGCAGTGTGGATAGCGCAAATCAGCGTCAAAAATCTAGTGAGACAATA 3661
TATAATACTGTGAGAGATAGAAGAAAAGGCCACTCTAAAATATCGTATCTCGTAATGACA 3721
CAAAAATGGTACTTCTTCGCAGCCCCCCAGACTTTCGAGTGCTATTTTCTTCCGTGTGAG 3781
AAAAGTTTCAGGCACAAAGGGGACGGAACCTAAACAGTATCTTCACTCTCTCCACCCACG 3841
AGAATGAGGCTTGGAAGGAAAGGGAATGAAGGAGAGAGGGACAGAGAACAAGAGACAGCA 3901
TACACGAAGTAATCCAGGGTCATCCCGGGACACATACGCACTTATCTTGCCATTTCCTTA 3961
GCCTAAGCGCCACTCACACCCTGAGGCGGCACAGTGCCATGAATTATTGCTTAAGGTAGT 4021

```
GTGCTGTGTAAGCCTGTCTGTTACCTGAAAACTGAGAAAAATGCTAAGGTATGGGTGTAT 4081
GTCTGTGTCTCTTTATGTGAATGTATGCATGTGTGTATGCGTGTGGATGTGTGCCAATGT 4141
ATCTTAAGCTTAATTTTTCTTTATGCCTGAAATGGCTTATCAGCGATTGTGAATAAATTA 4201
AACGAGGGACTAGAGCAAGGAAACTAAATATCAGAGGTGAAATTTTGGGGTTTAGTGTCC 4261
TGGAAAGCATCTATATCTTCTTTTGTTATGCTGTAAATGTTTTAATCTTACGAGTGAAAG 4321
AACAGAATAGTATTGTTTATTATTATGTTATGCCGTTGTTCCACTGGCTTTATTAATTTG 4381
TCAGGTTGTAGTAAGACCAGATTAATCATATTTATCGATGATGTAAAAACTTGTACCTTG 4441
GAAAAAAATCGATGACGAGAATGAGAAACAAACACGACATATTTGTGATTTATAATATTT 4501
ATCAAACAGGCTTTTCTTTGAAGTGAAGGACAACAGTATTTAATGAGTTTCGCAGTTTCT 4561
TTTCATCTGGTTATGTGTGAATATGTATATAGATGTGTGTGTATGCATGTAAGTAAGAGC 4621
AGAGCCTTCAAAAGTCTTGTGTATAAATGCAAGAGTGTGGGACTTTCAAAACACAAGTAC 4681
GTATGTGAAAGAGAACCAAAACTCCGAAAAAGCCTACGCGAGTGGATGGAAAAGTGAGAT 4741
TTTCAGAATATTCCGCTTCCGGATCACTCACATATAACTTCCTCCCACACACACACTCCC 4801
GTGCGATGTTTCCACTTCCACGACCCACCGTAACAAAACAAGCATGCGTGTGAAAGAACG 4861
AAAACTTCAAAGAGCCTACATGTGTGGATGAAAGAGTGTGAGATCTTCAGAATATTCCAC 4921
TCTCGTATCACTCACAAAATAACCCCGCTACGTTTCTACTCCCGCCGCCCACCCAAACAA 4981
AACACACAGGAACACATTCGCTCCAGTTCGCCGTTGCAACAAAAACAAGACTTGGTAACA 5041
ACAGGAATCTACTGAGCGCTGCCTTTCTTGGTATAATTTACAAACTTCCTATACAGTTCG 5101
TACCTCATTCTCGCGACCACAACTTCCACAGCCCCTCTCGATGTGTGTTTTTTACTCCCG 5161
TTGTGTACGCGATGTTGGAAACTGTGAAGAGGAAAATAATTATATCACTTCTGAAGGCCA 5221
CATAGCAGGGTTCAGTGATATAAAGTGTGGCTTCTGGGGACGGAAATCAGATTAATCTAA 5281
CACAGAGACTGTCAGGTTGTTTGTTCTGCCGACGTGAAAAGGAAAAACATTAGATTTTCT 5341
CGTAAGGTCACATAGCAGGGTTCAGTAATCAAACTGTGGCTTCGGGGGATGGAATACTGA 5401
CTTAACTAACCTGTAGACTGTCATGTTGTGTACTCAAAAGTGTTAGATTCCCTCTTGAGG 5461
CCACGTATCAGAATTCATTAATCTAAAATGTGACTTTGGGTGATGGAATAAAGACTAAGC 5521
CAACGCAGAGAGTGTCAGGCTGTATGTTCTGCCGAGAGGGAGAAAATCGAGGGCTGCCTG 5581
TCTTTCCGTGTGTCTGAAGAGCCTTACATACCTACCTACCACCGTCTTCACCGATGCCTT 5641
ATTACTCTGACCACTCATTAGCTCCCTCAACACTGGTCGCCCGCCTCACCCAAGGACCAG 5701
CAGACCCCTCTTTGATGATTGTCTGTACAGGATAAACCAATAATACGTACTACAATGCCT 5761
TTTCAGAGTGGTTATATGAAGAAACAAATTAAGAGTTAGAGCTACTTCCACGCATAAAAC 5821
ACGCCTCTTTCAGGTCTTATCTGGGGTCGTACGGTAGTGAGAGATATGTAGTGTATAATA 5881
TTCTTATAGCCTGTAGTTACTTCTGCCTTTTCCTGAGAGCTAAACTTCGGTGCGTCCACG 5941
CTAATATGGTGAATAAGCCCCGCCCACCCCAGTTCAGCAGCCATTAGTAAAATCCAGTCT 6001
TTCTATCAATCCGCCAGTCACATGCAGCCTGAGTCAGGAGGGGACATGGCCAACTAGTGG 6061
ATTGGCCAAAGGTATGCGTCTGAGGAGGGGAAGGGGAAGGGGTTTATCCGCCAAGCTAAA 6121
GCGGACGGAGTGTAGTGATCTGGCTGCCTCTTCTTGGCGCGCATCTTGATAGCCTAGCAT 6181
CCAAGTATCGTGTATCTGCCTTTCACGTGCACGATAGGAAAGAGTAACTGAGGCGAGACA 6241
CTATTTTCACACTAGAATGTTTGGAACCGTTAGGATTATTATGAATTAAGGATAATGCAC 6301
GTAGGAAGCTGGGGAGTATTACTTTTCTCAGGGCGAGAGGGAGGGAGGGAAGGAGCGGAA 6361
AAGGGAAAGGTAGAGATATTTAGAAGGAAGAAAGGGAAGGGAGGATGCCGTAAGGAAGAA 6421
TGAAAGATAAGATAAGCTGATCTCTTTCCCATCAGTTTTCATACCCGGTGTCTCTTGGGT 6481
TTTGCTTAGAGTGAGACGTTTCGAGACTGAACAGAGGAAAAGACGAATACTTGCCAACAA 6541
GACAAAAATACAAAGAGTTACAGTTAAGGTGAGAGATAGAGACGCTTGTTCTTGTTCATT 6601
ATTCTTGCCAGTCACAGACCTCACCGCACCGAGCGATCTGTGTCTCTCTTCTTAGTCCCG 6661
TTGCGGTTAGGTCCTCCAAATGCCTAACAAGCTGGTGAGTGAGTGTGCGTTGGGTTGATA 6721
CTTACCAAAGAATTCAGAGAGGTGTATTATGTATATAGAG                       6761
```

Figure 2.8. Nucleotide and amino acid sequence of cDNA encoding Gl-PDE5. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. Two GAF regulatory domains; GAF-A &GAF-B are indicated by green boxes and contains the (NKxxFDxxE) signature sequence found in all mammalian GAF domains, the sequence is underlined and in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.

CmPDE5 2996 nucleotides

```
   1 TGCACTCGTGCGCTATGAATGTCAACAAAGCCAGGGTAAGACAAGAGGAGCCCAGAAGGG
  61 AGACGTGACCAGCGGAGACGTACCCCTGTGAACGTGCCCTGGAAAACGTGCCCTTGAGAC
 121 GTGTTCTTGGACGTGTACCATTGAGGAACGTGCCTTAGGGAGACGTACCCTTTGGAAGAC
 181 GTGTATGCTATAGTAAACAAACGTGTGCTGAAAGAAAACTGTCCTTGAAATCCAGAGCAA
 241 TAAAAAACGTGATCTGGGAAACTTTCTACGAGGACCTCGAGACGGATACGCTCTTGTATA
 301 GGTGTTGAGGGATTAAGAAGCAGCGCCCTCTACCCTCCCCCCTTCATCCCCTACCGCAGT
   1   M  M  S  C  L  L  E  E  G  G  Q  E  G  V  V  G  G  G  G  V
 361 CATGATGAGCTGTCTGCTGGAGGAAGGGGGACAGGAGGGAGTGGTGGGTGGTGGAGGCGT
  21   G  V  K  R  A  S  V  P  L  R  R  R  R  T  G  I  G  A  P  G
 421 GGGTGTTAAGAGAGCGTCTGTACCCTTACGTCGACGCAGAACAGGGATCGGGGCGCCGGG
  41   L  T  V  N  G  R  S  N  M  N  S  H  D  L  S  L  T  A  E  D
 481 CCTCACTGTGAACGGGAGAAGTAATATGAACAGCCACGATCTCTCCCTAACAGCCGAGGA
  81  M  T  G  E  E  V  G  Q  Y  L  R  A  H  P  E  F  L  E  S  W
 541 CATGACGGGGGAGGAGGTGGGCCAATACCTTCGAGCACACCCAGAGTTCCTCGAGTCGTG
 101   L  M  E  Q  V  E  L  E  T  L  E  R  W  M  I  R  R  T  Q  R
 601 GCTCATGGAACAGGTGGAACTGGAGACGCTGGAGAGGTGGATGATACGACGCACTCAACG
 121   D  K  Q  K  S  L  E  N  D  T  N  G  K  I  I  R  K  T  S  L
 661 AGATAAACAGAAAAGCCTGGAGAACGATACGAATGGTAAAATCATCAGAAAAACGAGCTT
 141   S  R  W  K  F  C  V  H  A  D  K  R  K  M  L  Q  E  L  T  S
 721 ATCCAGATGGAAGTTTTGCGTGCACGCCGACAAACGCAAGATGCTGCAGGAGCTCACGTC
 161   S  L  Y  V  R  P  N  K  P  H  V  L  W  E  L  T  R  C  I  S
 781 GTCCCTCTACGTGAGACCCAACAAGCCACACGTTCTCTGGGAGCTCACCCGCTGCATCTC
 181   S  A  V  N  A  D  G  C  N  L  Y  L  A  D  L  D  T  N  M  L
 841 CTCAGCAGTGAACGCGGACGGATGCAACCTTTACCTCGCTGATCTCGACACTAACATGCT
 201   R  R  Y  V  E  S  K  D  G  E  G  D  S  T  G  W  S  C  P  M
 901 CAGACGTTACGTGGAGAGTAAAGACGGGGAAGGAGACAGCACAGGATGGAGCTGCCCCAT
 221   S  G  G  D  W  L  C  G  W  V  A  S  S  R  Q  A  L  R  A  T
 961 GAGCGGCGGGGACTGGCTGTGTGGATGGGTAGCCAGCTCTCGTCAGGCCCTTAGAGCCAC
 241   Y  P  I  T  D  P  R  F  P  K  G  S  P  F  A  E  E  Q  D  V
1021 CTATCCCATCACTGACCCGAGATTCCCCAAAGGATCCCCCTTCGCTGAGGAGCAGGACGT
 261   H  H  A  L  V  M  A  V  M  Q  S  D  G  E  L  A  A  V  L  E
1081 ACATCACGCTCTGGTCATGGCGGTGATGCAGAGCGACGGGGAGTTAGCAGCTGTGTTGGA
 281   L  Y  R  R  R  G  C  E  S  F  H  T  E  D  E  E  I  V  N  S
1141 GCTGTACAGGAGGCGAGGTTGCGAGTCGTTCCACACAGAGGATGAGGAGATCGTCAATTC
 301   Y  L  V  W  G  G  I  A  L  H  Y  A  E  L  Y  H  S  M  V  K
1201 CTACCTGGTGTGGGGTGGCATTGCGCTGCACTACGCCGAGCTCTACCACAGCATGGTGAA
 321   Q  R  T  L  N  E  F  I  L  S  V  V  K  S  I  F  Q  D  M  V
1261 GCAACGCACACTTAATGAGTTCATCCTCTCCGTTGTAAAATCGATCTTCCAGGACATGGT
 341   S  M  D  T  L  I  M  K  V  M  N  F  A  Q  R  L  V  N  A  D
1321 GAGCATGGACACACTCATTATGAAAGTGATGAATTTCGCCCAAAGACTTGTGAACGCCGA
 361   R  A  S  L  F  L  V  D  S  K  N  K  Q  L  Y  A  R  I  F  D
1381 CCGTGCTTCTCTCTTCCTCGTCGACTCCAAGAACAAACAACTTTATGCTCGTATATTCGA
 381   M  G  S  E  F  S  E  D  N  P  Q  Q  S  F  K  E  I  R  F  A
1441 CATGGGCAGCGAATTCAGTGAAGACAATCCCCAGCAGTCATTCAAGGAGATCAGGTTCGC
 401   I  G  T  G  I  A  G  I  V  A  Q  N  G  E  V  L  N  I  P  D
1501 CATAGGGACAGGTATCGCCGGCATCGTGGCTCAGAACGGAGAGGTGCTAAACATTCCAGA
 421   A  Y  A  D  P  R  F  N  R  T  V  D  Q  L  T  G  Y  V  T  K
1561 CGCTTACGCCGACCCTCGCTTTAACCGCACCGTGGACCAACTCACGGGCTACGTCACCAA
 441   S  I  L  C  M  P  I  F  I  R  G  N  V  I  G  V  M  Q  M  V
```

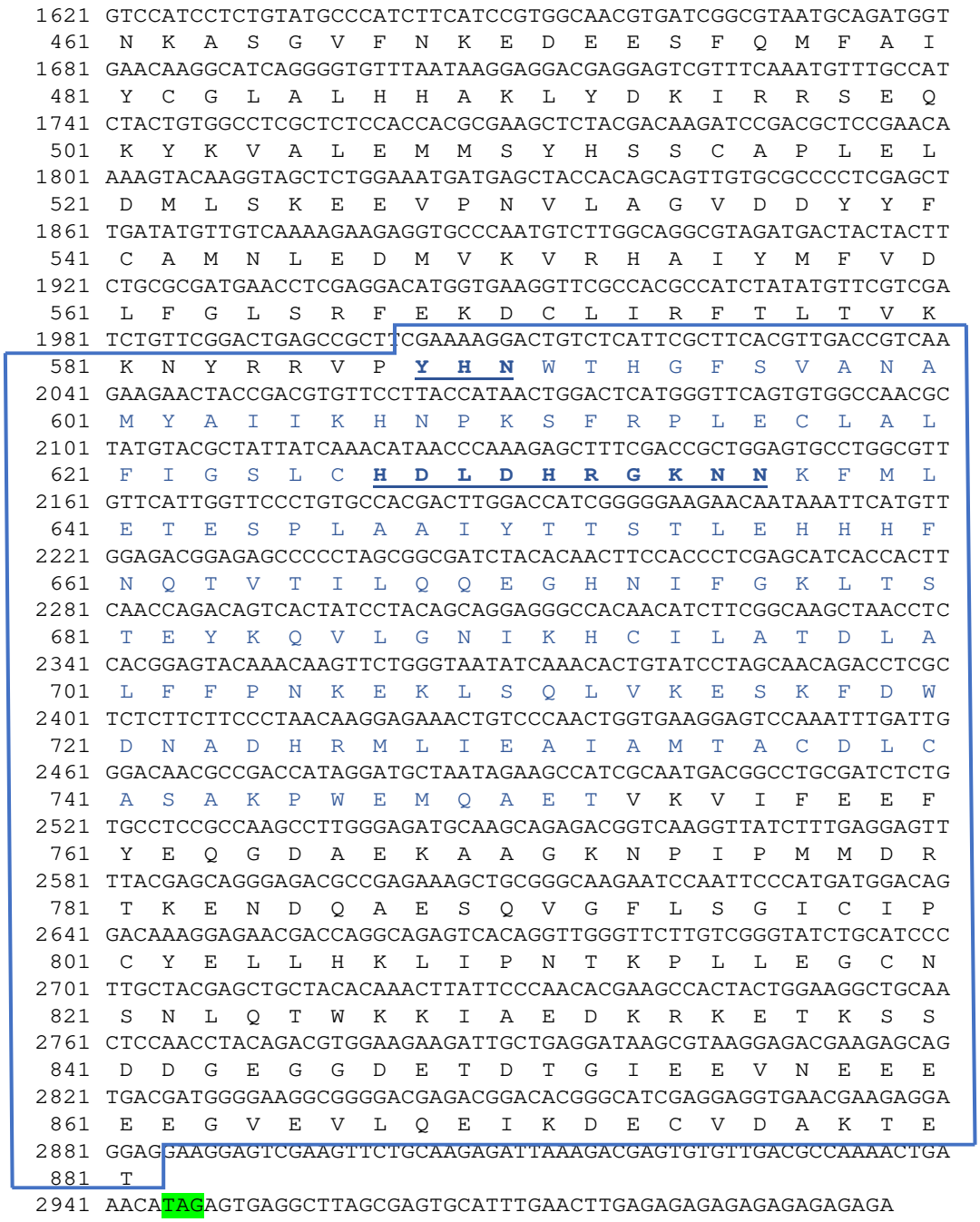
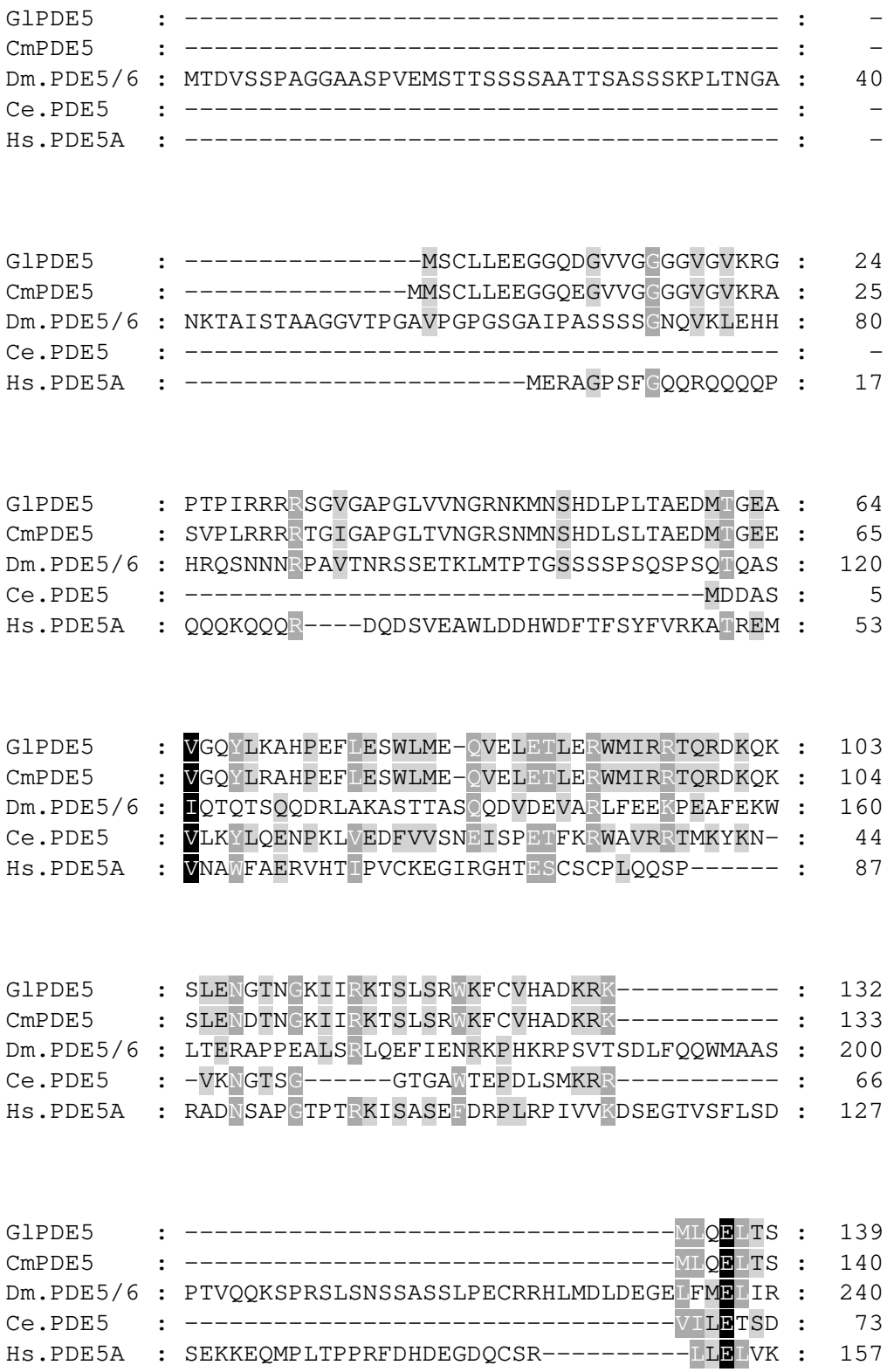
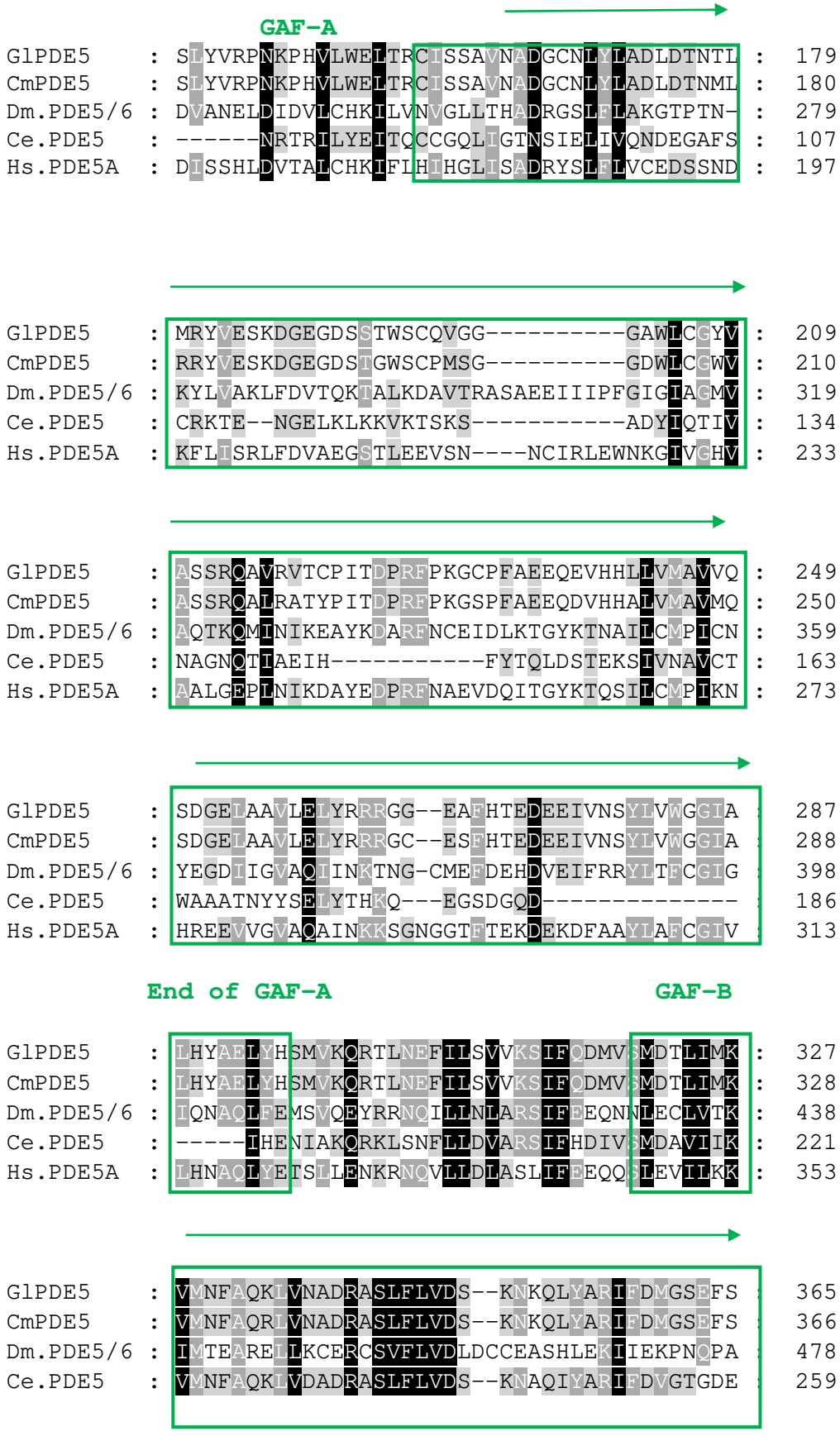
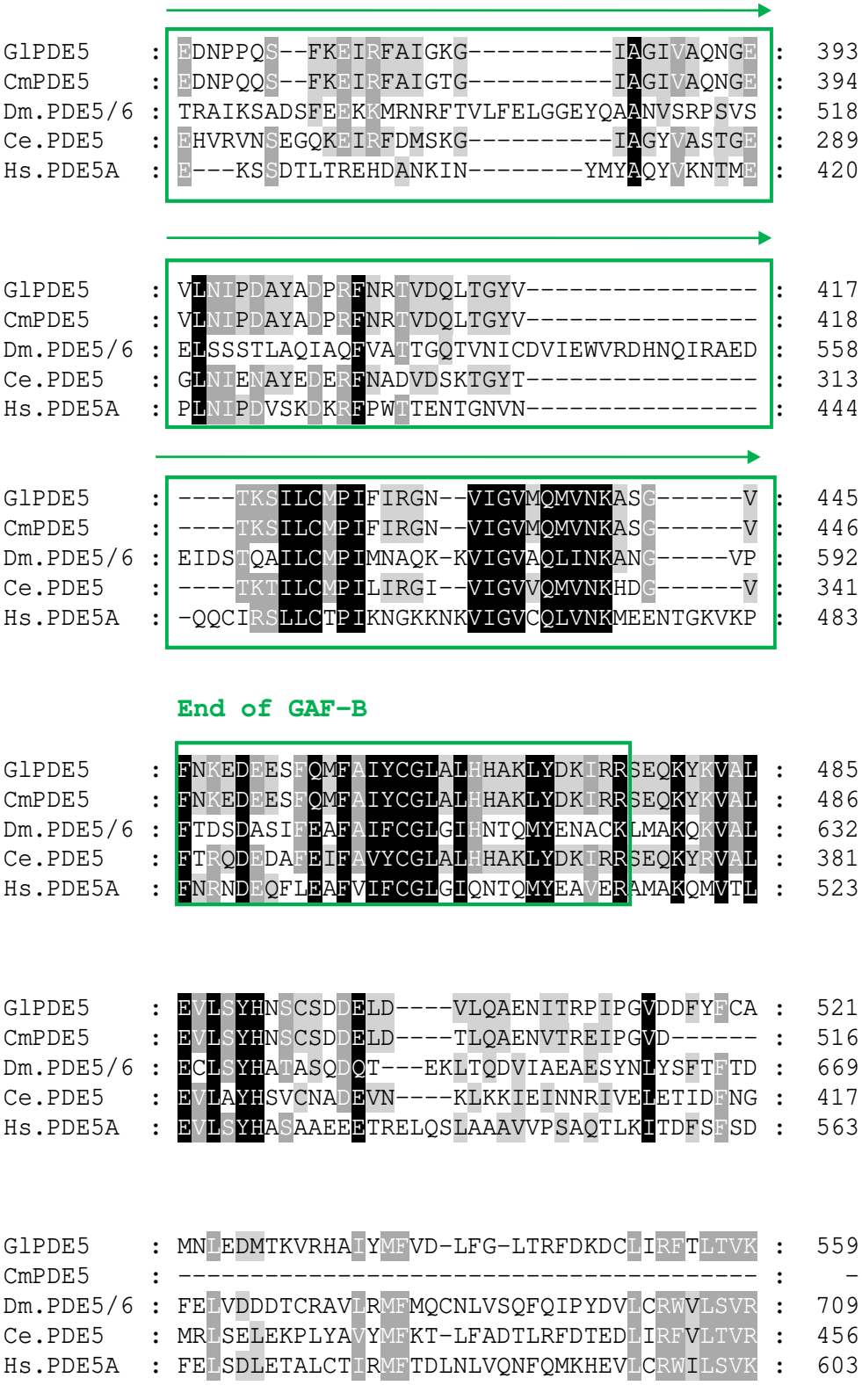
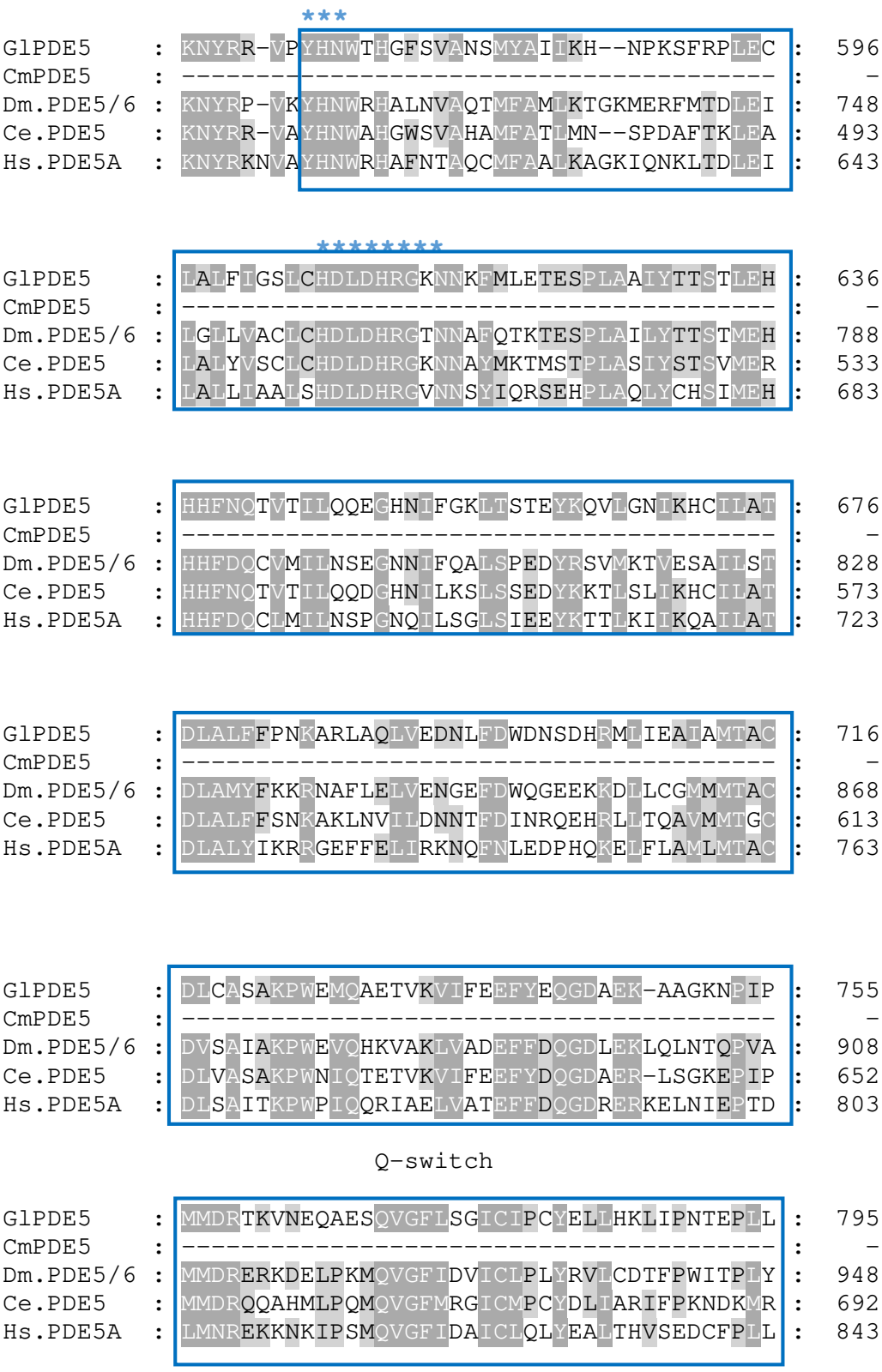


Figure 2.9. Nucleotide and amino acid sequence of cDNA encoding Cm-PDE5. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. Two GAF regulatory domains; GAF-A &GAF-B are indicated by green boxes and contains the (NKxxFDxxE) signature sequence found in all mammalian GAF domains, the sequence is underlined and in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.


GlPDE5 : ---------- : -
CmPDE5 : ---------- : -
Dm.PDE5/6 : MTDVSSPAGGAASPVEMSTTSSSSAATTSASSSKPLTNGA : 40
Ce.PDE5 : ---------- : -
Hs.PDE5A : ---------- : -
GlPDE5 : ----------MSCLLEEGGQDGVVGGGGVGVKRG : 24
CmPDE5 : ----------MMSCLLEEGGQEGVVGGGGVGVKRA : 25
Dm.PDE5/6 : NKTAISTAAGGVTPGAVPGPGSGAIPASSSSGNQVKLEHH : 80
Ce.PDE5 : ---------- : -
Hs.PDE5A : ----------MERAGPSFGQQRQQQQP : 17
GlPDE5 : PTPIRRRRSGVGAPGLVVNGRNKMNSHDLPLTAEDMTGEA : 64
CmPDE5 : SVPLRRRRTGIGAPGLTVNGRSNMNSHDLSLTAEDMTGEE : 65
Dm.PDE5/6 : HRQSNNNRPAVTNRSSETKLMTPTGSSSSPSQSPSQTQAS : 120
Ce.PDE5 : ----------MDDAS : 5
Hs.PDE5A : QQQKQQQR----DQDSVEAWLDDHWDFTFSYFVRKATREM : 53
GlPDE5 : VGQYLKAHPEFLESWLME-QVELETLERWMIRRTQRDKQK : 103
CmPDE5 : VGQYLRAHPEFLESWLME-QVELETLERWMIRRTQRDKQK : 104
Dm.PDE5/6 : IQTQTSQQDRLAKASTTASQQDVDEVARLFEEKPEAFEKW : 160
Ce.PDE5 : VLKYLQENPKLVEDFVVSNEISPETFKRWAVRRTMKYKN- : 44
Hs.PDE5A : VNAWFAERVHTIPVCKEGIRGHTESCSCPLQQSP------ : 87
GlPDE5 : SLENGTNGKIIRKTSLSRWKFCVHADKRK---------- : 132
CmPDE5 : SLENDTNGKIIRKTSLSRWKFCVHADKRK---------- : 133
Dm.PDE5/6 : LTERAPPEALSRLQEFIENRKPHKRPSVTSDLFQQWMAAS : 200
Ce.PDE5 : -VKNGTSG------GTGAWTEPDLSMKRR---------- : 66
Hs.PDE5A : RADNSAPGTPTRKISASEFDRPLRPIVVKDSEGTVSFLSD : 127
GlPDE5 : ----------MLQELTS : 139
CmPDE5 : ----------MLQELTS : 140
Dm.PDE5/6 : PTVQQKSPRSLSNSSASSLPECRRHLMDLDEGELFMELIR : 240
Ce.PDE5 : ----------VILETSD : 73
Hs.PDE5A : SEKKEQMPLTPPRFDHDEGDQCSR----------LLELVK : 157


GAF-A
GlPDE5 : SLYVRPNKPHVLWELTRCISSAVNADGCNLYLADLDTNTL : 179
CmPDE5 : SLYVRPNKPHVLWELTRCISSAVNADGCNLYLADLDTNML : 180
Dm.PDE5/6 : DVANELDIDVLCHKILVNVGLLTHADRGSLFLAKGTPTN- : 279
Ce.PDE5 : ------NRTRILYEITQCCGQLIGTNSIELIVQNDEGAFS : 107
Hs.PDE5A : DISSHLDVTALCHKIFLHIHGLISADRYSLFLVCEDSSND : 197
GlPDE5 : MRYVESKDGEGDSSTWSCQVGG----------GAWLCGYV : 209
CmPDE5 : RRYVESKDGEGDSTGWSCPMSG----------GDWLCGWV : 210
Dm.PDE5/6 : KYLVAKLFDVTQKTALKDAVTRASAEEIIIPFGIGIAGMV : 319
Ce.PDE5 : CRKTE--NGELKLKKVKTSKS----------ADYIQTIV : 134
Hs.PDE5A : KFLISRLFDVAEGSTLEEVSN----NCIRLEWNKGIVGHV : 233
GlPDE5 : ASSRQAVRVTCPITDPRFPKGCPFAEEQEVHHLLVMAVVQ : 249
CmPDE5 : ASSRQALRATYPITDPRFPKGSPFAEEQDVHHALVMAVMQ : 250
Dm.PDE5/6 : AQTKQMINIKEAYKDARFNCEIDLKTGYKTNAILCMPICN : 359
Ce.PDE5 : NAGNQTIAEIH----------FYTQLDSTEKSIVNAVCT : 163
Hs.PDE5A : AALGEPLNIKDAYEDPRFNAEVDQITGYKTQSILCMPIKN : 273
GlPDE5 : SDGELAAVLELYRRRGG--EAFHTEDEEIVNSYLVWGGIA : 287
CmPDE5 : SDGELAAVLELYRRRGC--ESFHTEDEEIVNSYLVWGGIA : 288
Dm.PDE5/6 : YEGDIIGVAQIINKTNG-CMEFDEHDVEIFRRYLTFCGIG : 398
Ce.PDE5 : WAAATNYYSELYTHKQ---EGSDGQD-------------- : 186
Hs.PDE5A : HREEVVGVAQAINKKSGNGGTFTEKDEKDFAAYLAFCGIV : 313
End of GAF-A
GAF-B
GlPDE5 : LHYAELYHSMVKQRTLNEFILSVVKSIFQDMVSMDTLIMK : 327
CmPDE5 : LHYAELYHSMVKQRTLNEFILSVVKSIFQDMVSMDTLIMK : 328
Dm.PDE5/6 : IQNAQLFEMSVQEYRRNQILLNLARSIFEEQNNLECLVTK : 438
Ce.PDE5 : -----IHENIAKQRKLSNFLLDVARSIFHDIVSMDAVIIK : 221
Hs.PDE5A : LHNAQLYETSLLENKRNQVLLDLASLIFEEQQSLEVILKK : 353
GlPDE5 : VMNFAQKLVNADRASLFLVDS--KNKQLYARIFDMGSEFS : 365
CmPDE5 : VMNFAQRLVNADRASLFLVDS--KNKQLYARIFDMGSEFS : 366
Dm.PDE5/6 : IMTEARELLKCERCSVFLVDLDCCEASHLEKIIEKPNQPA : 478
Ce.PDE5 : VMNFAQKLVDADRASLFLVDS--KNAQIYARIFDVGTGDE : 259


GlPDE5 : EDNPPQS--FKEIRFAIGKG----------IAGIVAQNGE : 393
CmPDE5 : EDNPQQS--FKEIRFAIGTG----------IAGIVAQNGE : 394
Dm.PDE5/6 : TRAIKSADSFEEKKMRNRFTVLFELGGEYQAANVSRPSVS : 518
Ce.PDE5 : EHVRVNSEGQKEIRFDMSKG----------IAGYVASTGE : 289
Hs.PDE5A : E---KSSDTLTREHDANKIN--------YMYAQYVKNTME : 420
GlPDE5 : VLNIPDAYADPRFNRTVDQLTGYV---------------- : 417
CmPDE5 : VLNIPDAYADPRFNRTVDQLTGYV---------------- : 418
Dm.PDE5/6 : ELSSSTLAQIAQFVATTGQTVNICDVIEWVRDHNQIRAED : 558
Ce.PDE5 : GLNIENAYEDERFNADVDSKTGYT---------------- : 313
Hs.PDE5A : PLNIPDVSKDKRFPWTTENTGNVN---------------- : 444
GlPDE5 : ----TKSILCMPIFIRGN--VIGVMQMVNKASG------V : 445
CmPDE5 : ----TKSILCMPIFIRGN--VIGVMQMVNKASG------V : 446
Dm.PDE5/6 : EIDSTQAILCMPIMNAQK-KVIGVAQLINKANG-----VP : 592
Ce.PDE5 : ----TKIILCMPILIRGI--VIGVVQMVNKHDG------V : 341
Hs.PDE5A : -QQCIRSLLCTPIKNGKKNKVIGVCQLVNKMEENTGKVKP : 483
End of GAF-B
GlPDE5 : FNKEDEESFQMFAIYCGLALHHAKLYDKIRRSEQKYKVAL : 485
CmPDE5 : FNKEDEESFQMFAIYCGLALHHAKLYDKIRRSEQKYKVAL : 486
Dm.PDE5/6 : FTDSDASIFEAFAIFCGLGIHNTQMYENACKLMAKQKVAL : 632
Ce.PDE5 : FTRQDEDAFEIFAVYCGLALHHAKLYDKIRRSEQKYRVAL : 381
Hs.PDE5A : FNRNDEQFLEAFVIFCGLGIQNTQMYEAVERAMAKQMVTL : 523
GlPDE5 : EVLSYHNSCSDDELD----VLQAENITRPIPGVDDFYFCA : 521
CmPDE5 : EVLSYHNSCSDDELD----TLQAENVTREIPGVD------ : 516
Dm.PDE5/6 : ECLSYHATASQDQT---EKLTQDVIAEAESYNLYSFTFTD : 669
Ce.PDE5 : EVLAYHSVCNADEVN----KLKKIEINNRIVELETIDFNG : 417
Hs.PDE5A : EVLSYHASAAEEETRELQSLAAAVVPSAQTLKITDFSFSD : 563
GlPDE5 : MNLEDMTKVRHAIYMFVD-LFG-LTRFDKDCLIRFTLTVK : 559
CmPDE5 : ---------- : -
Dm.PDE5/6 : FELVDDDTCRAVLRMFMQCNLVSQFQIPYDVLCRWVLSVR : 709
Ce.PDE5 : MRLSELEKPLYAVYMFKT-LFADTLRFDTEDLIRFVLTVR : 456
Hs.PDE5A : FELSDLETALCTIRMFTDLNLVQNFQMKHEVLCRWILSVK : 603


***
GlPDE5 : KNYRR-VPYHNWTHGFSVANSMYAIIKH--NPKSFRPLEC : 596
CmPDE5 : ---------- : -
Dm.PDE5/6 : KNYRP-VKYHNWRHALNVAQTMFAMLKTGKMERFMTDLEI : 748
Ce.PDE5 : KNYRR-VAYHNWAHGWSVAHAMFATLMN--SPDAFTKLEA : 493
Hs.PDE5A : KNYRKNVAYHNWRHAFNTAQCMFAALKAGKIQNKLTDLEI : 643
****
GlPDE5 : LALFIGSLCHDLDHRGKNNKFMLETESPLAAIYTTSTLEH : 636
CmPDE5 : ---------- : -
Dm.PDE5/6 : LGLLVACLCHDLDHRGTNNAFQTKTESPLAILYTTSTMEH : 788
Ce.PDE5 : LALYVSCLCHDLDHRGKNNAYMKTMSTPLASIYSTSVMER : 533
Hs.PDE5A : LALLIAALSHDLDHRGVNNSYIQRSEHPLAQLYCHSIMEH : 683
GlPDE5 : HHFNQTVTILQQEGHNIFGKLTSTEYKQVLGNIKHCILAT : 676
CmPDE5 : ---------- : -
Dm.PDE5/6 : HHFDQCVMILNSEGNNIFQALSPEDYRSVMKTVESAILST : 828
Ce.PDE5 : HHFNQTVTILQQDGHNILKSLSSEDYKKTLSLIKHCILAT : 573
Hs.PDE5A : HHFDQCLMILNSPGNQILSGLSIEEYKTTLKIIKQAILAT : 723
GlPDE5 : DLALFFPNKARLAQLVEDNLFDWDNSDHRMLIEAIAMTAC : 716
CmPDE5 : ---------- : -
Dm.PDE5/6 : DLAMYFKKRNAFLELVENGEFDWQGEEKKDLLCGMMMTAC : 868
Ce.PDE5 : DLALFFSNKAKLNVILDNNTFDINRQEHRLLTQAVMMTGC : 613
Hs.PDE5A : DLALYIKRRGEFFELIRKNQFNLEDPHQKELFLAMLMTAC : 763
GlPDE5 : DLCASAKPWEMQAETVKVIFEEFYEQGDAEK-AAGKNRIP : 755
CmPDE5 : ---------- : -
Dm.PDE5/6 : DVSAIAKPWEVQHKVAKLVADEFFDQGDLEKLQLNTQPVA : 908
Ce.PDE5 : DLVASAKPWNIQTETVKVIFEEFYDQGDAER-LSGKEPIP : 652
Hs.PDE5A : DLSAITKPWPIQQRIAELVATEFFDQGDRERKELNIEPTD : 803
Q-switch
GlPDE5 : MMDRTKVNEQAESQVGFLSGICIPCYELIHKLIPNTEPLL : 795
CmPDE5 : ---------- : -
Dm.PDE5/6 : MMDRERKDELPKMQVGFIDVICLPLYRVLCDTFPWITPLY : 948
Ce.PDE5 : MMDRQQAHMLPQMQVGFMRGICMPCYDLIARIFPKNDKMR : 692
Hs.PDE5A : LMNRERKKNKIPSMQVGFIDAICLQLYEALTHVSEDCFPLL : 843

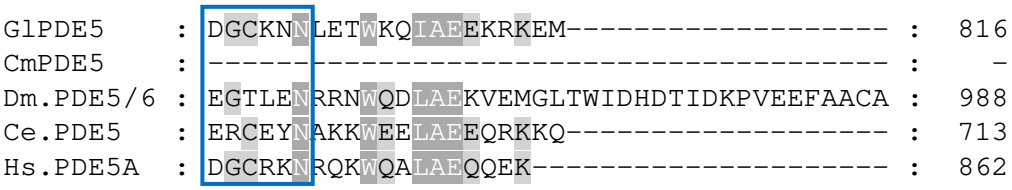


Figure 2.10. Multiple alignment of deduced amino acid sequences of PDE5 proteins in two crustacean species, one insect species, one nematode species and one mammal species. Abbreviations: Gl: *G lateralis*; Cm: *C maenas*; Dm: *D melanogaster*; Ce: *C elegans*; Hs: *H sapiens*. Black shading indicates that amino acid residues that are identical or similar in all sequences; gray shading indicates identical or similar amino acids in most of the sequences. Dashes indicate gaps introduced to optimize the alignment and the boxes indicate highly conserved domains. Blue asterisks indicate the signature sequences in the catalytic domain.The colors of the boxes correspond to the colors of the domains in Fig. 2. 7.

GlPDE7 7531 nucleotides, structure: TC

```
AGTCCATGTAAAGGGGTAGGATGCTGTTCACGCCGATCCCTGAGAGGCTTGTGTTGACGT   61
GCTGTGCTTCTGAGTGTTGAGCCTGTGTTTTGGTCTGGACAGTGTGCAGTGCTGGGCAGG  121
CACAGCGGGGCGGCAGTAGGCAACCGCTGCGCGTGGAGGGGACGCACACGCTGAGGTGAC  181
GAGTAGGAGGGTTTTTTCTGTGCTCCACGCGTCCAGCTTCCTTTCCCTCGTTTTGTTGTT  241
ACGATGGCGGTGGTGGTGACGCTGGTGCTGAGCGGCCTCTCCGGTAAAATTCAGTCCTTC  301
CCGTCGCTCTCTTGAGGATTAAACCCACCTGTACATCTCCTGGCCCTCACCTGTCTAGTG  361
AACGCTTCAAGCATCCTCTCCTCCACGTCTTACTGTCACGGTGGTGGCGGCGGTGGCGGG  421
GTCGAGGAGCCTCGCCAAAGACTCAATCCGCCACGTTGCTCCTGTGTGTGGACTTACCTC  481
TACAACTCCTGGCCGCCGCACCTGTCCCAGCATCCACCTCCCCACCGACTTGTAACACTC  541
GTGGCGTGAGCTGAGGAAGAGTCCCGGCAGTGTGTGGGGACTGAGGCGCGACAGACAGAC  601
TAGACAGATAGAGGCAGTGACTTAGTGTGTGTTGTATATGAAATGAACGAGAATTAGTGG  661
TGACATAAGTTAATCAAACGGAGCCCTTGTCATCAGCCGCTACAGCGAACAGCAAGGAGT  721
GACCAGAGGAGGGAGGGGGTCAGGCTGGACCAGACCTCCCAAAATGGCCTCATGGTGCGA  781
                                                    M  V  R     3
ATGTTAGGTGACGTGAAGGGCAATACAACCGTGGAAACGTCCCTCGCAGCCACCGTCGGG  841
 M  L  G  D  V  K  G  N  T  T  V  E  T  S  L  A  A  T  V  G    23
CCTTCTCGCTCCACCGATGCCCGCCTGGACGCCGCCAACACGGAGTCGCGAGCCAGGGCC  901
 P  S  R  S  T  D  A  R  L  D  A  A  N  T  E  S  R  A  R  A    43
ACTCTCACCATGAACTACAGCCTCGGGGACATGAACGTGGGCGAGGCGGAGCAGCGGCTG  961
 T  L  T  M  N  Y  S  L  G  D  M  N  V  G  E  A  E  Q  R  L    63
CTGGGCGGGGCGGGACGCGGGCCTTACTCCAGGTTCCTGACGCTCCACCGCCGCCGCCGC 1021
 L  G  G  A  G  R  G  P  Y  S  R  F  L  T  L  H  R  R  R  R    83
CGGAAGCTGCCCACACGCCGCCTTGACAAGCAACACCAGGCGCTGCTAGACGACCTGTAC 1081
 R  K  L  P  T  R  R  L  D  K  Q  H  Q  A  L  L  D  D  L  Y   103
AACGGCCTCACGCAGTACTTGTTGTCCCGCGTGGGCGTGTGGGGGTTCAATGCCTTCACC 1141
 N  G  L  T  Q  Y  L  L  S  R  V  G  V  W  G  F  N  A  F  T   123
CTAGACAGCGTGTGTGGGGGCCGGCCCGTCTCCGTGTTGTGCGTCTTCCTCCTCCACGAG 1201
 L  D  S  V  C  G  G  R  P  V  S  V  L  C  V  F  L  L  H  E   143
TATGGCCTCATCGAGCACTTCAAGTTCGACACCGTGACTGTGTGGAAGTGTTTCAGTCTG 1261
 Y  G  L  I  E  H  F  K  F  D  T  V  T  V  W  K  C  F  S  L   163
ATTGAGGATGGGTACCATGCCTCCAACCCCTACCACAATGCTATTCATGCAGCAGATGTG 1321
 I  E  D  G  Y  H  A  S  N  P  Y  H  N  A  I  H  A  A  D  V   183
ACCCAGGCAATGCACTGCTACCTGCAGGAGGAAAAGATCCACCAACACCTGACACCATTG 1381
 T  Q  A  M  H  C  Y  L  Q  E  E  K  I  H  Q  H  L  T  P  L   203
GAGATGTTGGCTGCTCTCATTGCTGCTGTCTGCCATGACCTGGACCACCCTGGGGTCAAC 1441
 E  M  L  A  A  L  I  A  A  V  C  H  D  L  D  H  P  G  V  N   223
CAGCCATTCCTCATTGCGACCGACAACCATCTGGCCGCACTATACAAAAACTTTAGTGTC 1501
 Q  P  F  L  I  A  T  D  N  H  L  A  A  L  Y  K  N  F  S  V   243
CTTGAGAACCATCACTGGCGGTGTGCAATGGGGTGCCTGTGGGAGTCTGGGCTTCTGGAC 1561
 L  E  N  H  H  W  R  C  A  M  G  C  L  W  E  S  G  L  L  D   263
TCATGGGACCCAGATGACGTGGCCACCCTCCAGGACATGCTGCGCTCCCTTATCTTGGCC 1621
 S  W  D  P  D  D  V  A  T  L  Q  D  M  L  R  S  L  I  L  A   283
ACAGACATCACACGCCAGCAGGAGTTCCTCACAAGATTTAAGCAATACCTGGACAGTGGC 1681
 T  D  I  T  R  Q  Q  E  F  L  T  R  F  K  Q  Y  L  D  S  G   303
ACCTTTGACATGGGGGAGCCTGAGCACCGCCACTTCTCTCTCCAAATTGCCTTGAAGTGT 1741
 T  F  D  M  G  E  P  E  H  R  H  F  S  L  Q  I  A  L  K  C   323
GCTGACATTTGCAACCCCTGTCGGCCCTGGGAGGTCTCACAGAAGTGGTCGTACAAGATC 1801
 A  D  I  C  N  P  C  R  P  W  E  V  S  Q  K  W  S  Y  K  I   343
TGTGATGAGTTTTATCGTCAAGGAGACTATGAACGCCAGCTGAACCTTCCTGTAACTCTG 1861
 C  D  E  F  Y  R  Q  G  D  Y  E  R  Q  L  N  L  P  V  T  L   363
CTGTGTGACAGCAGCAGGATGTCTGTGGCCAAAATACAAACAGATTTCATCAAGTATGTG 1921
 L  C  D  S  S  R  M  S  V  A  K  I  Q  T  D  F  I  K  Y  V   383
GTGTCACCTCTGTTTGATGTTTGGGACCGCTGGTTGGAAACAAGTTTATCAAACTCAATG 1981
```

[redacted] D R W L E T S L S N S M 403

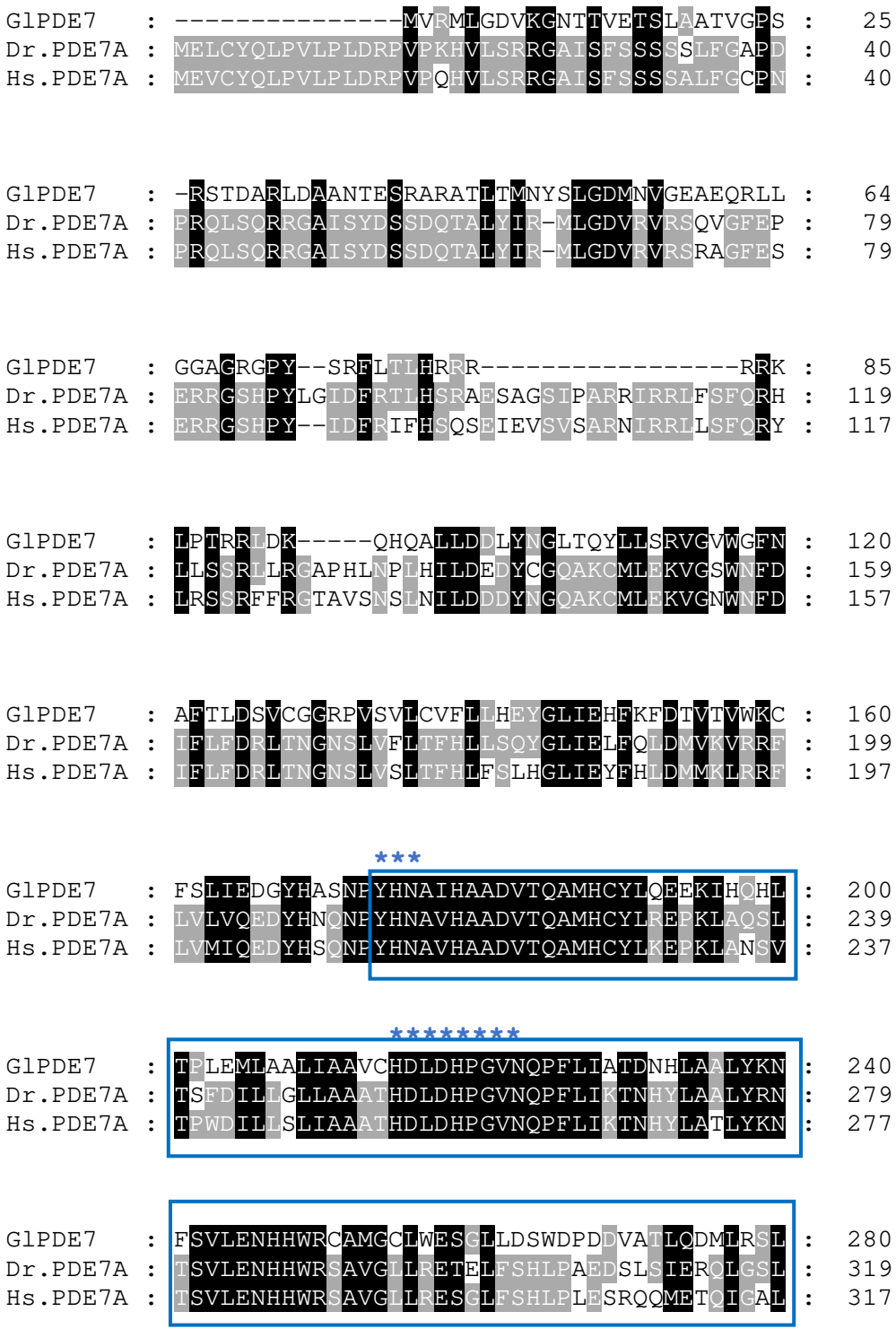
GTGACCAACATGAATATAAACCTGCAGAAATGGGAAGAGAAGCTGCTGAAGAAGCTGGCT 2041
V T N M N I N L Q K W E E K L L K K L A 423
GAGGATGCTGCTGCACAGCCAGTGGTTGTACCCGAAGACTCTTCCGAGAACCCAGTGGTG 2101
E D A A A Q P V V V P E D S S E N P V V 443
GAGAGTGCTGAGGAGGAACCCGAGGAGGTGGATGGCGGGACATCCCCCAGCAGCAGTGGA 2161
E S A E E E P E E V D G G T S P S S S G 463
GACAGTCACCAGTCAGTGCTTGGCTCTCTAGAGAATGTGTCACGATCACTTCAGCTTGGC 2221
D S H Q S V L G S L E N V S R S L Q L G 483
AGGAGACACTCTGTTCCTCTGAACTTGCCACGCTTACTTCCTCGCACAATTATAAGGAGG 2281
R R H S V P L N L P R L L P R T I I R R 503
GAGAGTCTGCCGGAAAATGGAAACCAGCCCTTAGTTGTAGAGACCGTGACCATGGGAGGG 2341
E S L P E N G N Q P L V V E T V T M G G 523
ATGTCTCTCACATCCCTTTCCTCTCGTCCAACTGACCTGAACTCCACACTGACACCTGAT 2401
M S L T S L S S R P T D L N S T L T P D 543
GCCCTTCTGCCCGAGCCTTCCATCACAACCATGGGTGGCGTGGGCATCAGGTTACCTCGT 2461
A L L P E P S I T T M G G V G I R L P R 563
CCAGCCACCAGACTCACTCGACGCAAGTCCCTCCCCCCCCTGTGGCTTCAGCAGACAAGG 2521
P A T R L T R R K S L P P L W L Q Q T R 583
GCAAGAACCTCACACATGCTGCAGCCCCTGTCACCCACTCCAAGTGAAGACGCTTCTTCT 2581
A R T S H M L Q P L S P T P S E D A S S 603
CACCAAACAACTCAGGAAAATTCCCCACAGGATACTGAGAATGCACCAAGGTTAGGTGCA 2641
H Q T T Q E N S P Q D T E N A P R L G A 623
GTGCATGGTGTTGGCTCAGGTCACTCAAGTGGGAATAACAGTAGTGGTTGTGGTGGAAGT 2701
V H G V G S G H S S G N N S S G C G G S 643
GGAGGTTGTGATAGAGTGAAAAGTGGTGGTGGTGAATGTTCAGACCCAGTGGAACCAATA 2761
G G C D R V K S G G G E C S D P V E P I 663
CATCATCCCATTAGGAGTGACACAGACCCATTGACTGCAGCTCAAGACGTGGGTGTTCAT 2821
H H P I R S D T D P L T A A Q D V G V H 683
GGTAGGAAGTGTGTTTCCAATCATCAAGGTGCTACAACATTCCTCCACGCTGACTGCCTC 2881
G R K C V S N H Q G A T T F L H A D C L 703
GACAACCACCCCTACAGACCATTCATTGGGAGGGGCTTGGTGCGCCGTGCTTCACTAGAC 2941
D N H P Y R P F I G R G L V R R A S L D 723
AGTACAAGTATTAGTAGTAAACGGGAGTTCTTGGATCGCCTGCTACACGAGAGCAGTAAA 3001
S T S I S S K R E F L D R L L H E S S K 743
TTCCCTCGTGTAGACCGAACTAATAGTGAGTTAGATAAAGAAAACTTAGTGCCAAGAGAA 3061
F P R V D R T N S E L D K E N L V P R E 763
GCACTAACATACCGAGAGCAGCAGCAGCAGCAGTCCTCATGGAAAACGCGTGCTTGG 3121
A L T Y R E Q Q Q Q Q Q S S W K T R A W 783
CGGAGTCTCAACTGTGATGACGAAAATGTGTGTGATCCACGTGAAAAGCTGATGAAGCCA 3181
R S L N C D D E N V C D P R E K L M K P 803
GGCATGTACAAGCTCAATCAGTCTCAGGGAAGTCTGTATGCTCATGGCCGCCGGGGGTCG 3241
G M Y K L N Q S Q G S L Y A H G R R G S 823
GCACCACTGCTGCGGCCAGAGGAGTTGTTGGGGGGTCTTCGAGGGGGAGCTGAGAGGCCA 3301
A P L L R P E E L L G G L R G G A E R P 843
GACTATAACTATCTCTCACTGCGGAGAGGCTCAGCACCATCACAAGCCAACCAAAAAGGC 3361
D Y N Y L S L R R G S A P S Q A N Q K G 863
TGTGATGTTGAAACTGGGTCTGAGGTGGGTGGCCGCCACACTCCCCTCACCTCAACGGAA 3421
C D V E T G S E V G G R H T P L T S T E 883
AATCTGCCCTTTAGCGAGTACGTCAGCTCACATTCCAGACTCAGGGGGCGGCGGCGAGGC 3481
N L P F S E Y V S S H S R L R G R R R G 903
TCCATACCCTTTGATCATCCTGGACTATGCCGGCAAGGGTCTGGTGGGGTGGAGGTGCTG 3541
S I P F D H P G L C R Q G S G G V E V L 923
CCTTCAAGGACGGGTGTGGGGGGTAACCTGGCGGTGTCTGGTGGAGGCAGCGGGGCAGCT 3601
P S R T G V G G N L A V S G G G S G A A 943
GGCTTCCCCTCGGTGTTGCCCCACTACTACCATGAGTATCACAGGGGGTCTGGAGGCCTA 3661

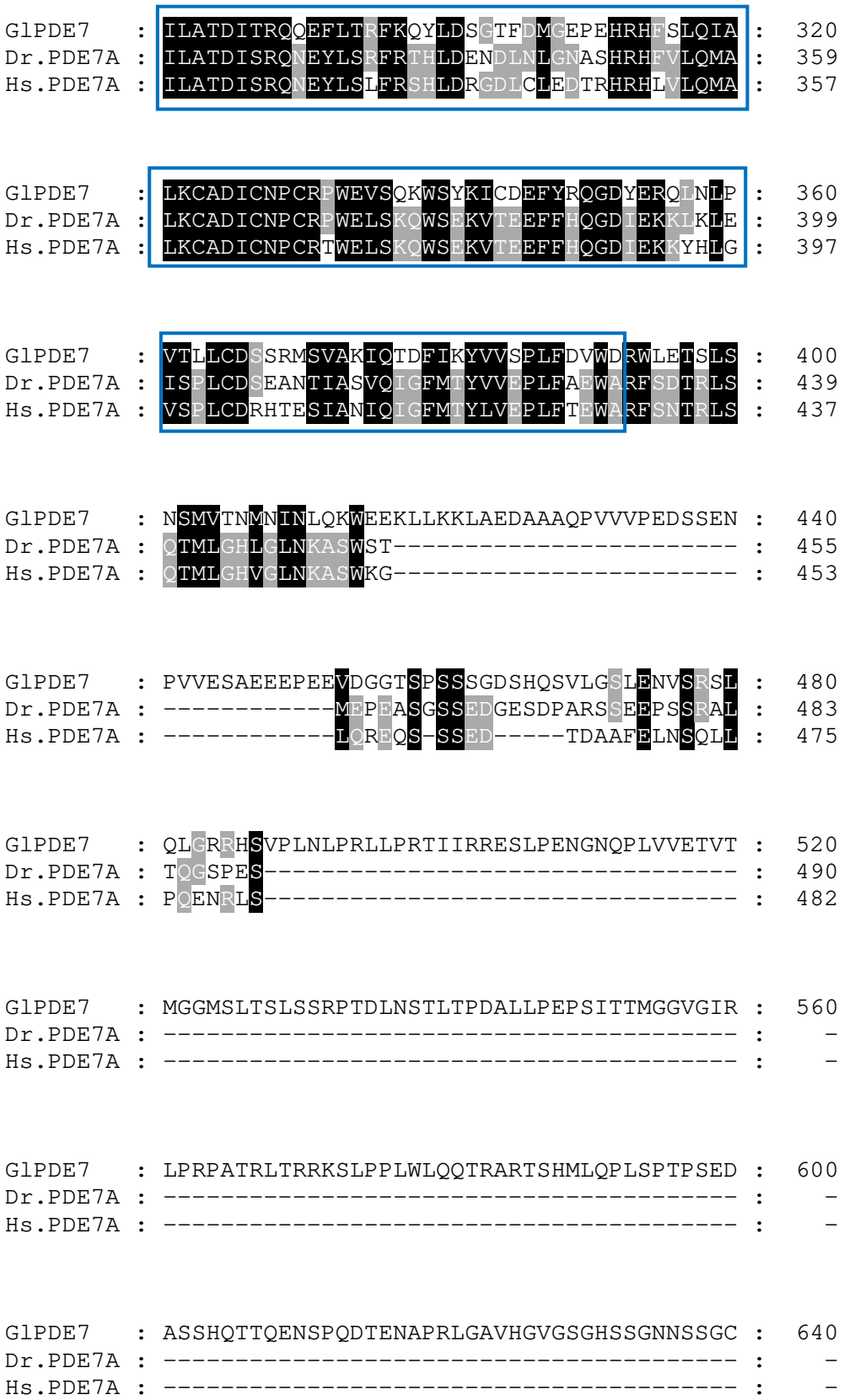
```
G  F  P  S  V  L  P  H  Y  Y  H  E  Y  H  R  G  S  G  G  L   963

GAACTGTTTGCCGGACTGTGGAGATCACACTTGGAACCTGAGAGCTCAAGTTCAGTGTTC 3721
 E  L  F  A  G  L  W  R  S  H  L  E  P  E  S  S  S  S  V  F   983
GTGGATGGTGACCTTTGTTCCCAGTCTTCTGGTGGCCTAACCCCCAGCACTCCTGGTGCT 3781
 V  D  G  D  L  C  S  Q  S  S  G  G  L  T  P  S  T  P  G  A  1003
GTGCTTCCACCCCACCGCCCCCTTCCCCACACCCTCCACAGGCGGGGCTCACTGCCAACT 3841
 V  L  P  P  H  R  P  L  P  H  T  L  H  R  R  G  S  L  P  T  1023
GACCTCTTCTACTCAGGGGACTTCAGTGTGTGGGATTGCGAGGGGACATAGCCACTTCAG 3901
 D  L  F  Y  S  G  D  F  S  V  W  D  C  E  G  T  -           1039
TTCTCAGGCTCAGCGCTTCCGCCTCAGTCCAATAATTCTGCCAGCCTCCAGTGCCAGCCC 3961
CCACCCCCCTTTTACCTTCTCAGCCTGCCTGTGAGCCATGTGTACTCATGTCTTATGACT 4021
CTGTTACCCAAAACCTTCAAGTACAGGATGCAGATGAACAAAATTATTGTAAGTTCAATT 4081
GATATGTCGTTGTCTAGCATTCTCAAGAAATGAGAACTTTGTGCTTCTTATGTATATAGT 4141
TTTGTAGCATTGGAAAAGTAATACTGTATATGTTTGACCTTTGTTCCTTAATGTGTTTAA 4201
AGATTACACGTGGAAAAGTATTTAGATACAATTTTTATTCTAGAACCATGTAGTCCTATA 4261
CAGAGATGTTAATTTAATTAACATTAGTCATAGAAATATATATTGTACAACCTTTTTGTT 4321
TGTATAGTTCATTGCACAGACATGAATAGCTAAATGCAGTTTGTCCTGGTGTTCATGTGT 4381
TTGCTCTTGGCTGCTACGGCAACATCCTCTGATCGTCAGGCTGTGATGAGCCAGCAAGGT 4441
CTGCAGGCCCAGGGGTCACTGGGCCTGGAGCAGCAGTCTTCAAGTTTGGACCATTTTTTG 4501
TATTAAATAATGCTTGAGTAAATGAATTCAGATATTTTGGTCAGAAGTTATTTTTATTAC 4561
TGTGAATATAGATGATAAAAGTCTTAAGCTTATACATAATCAATTAGTTTTTCATGAACT 4621
TTAATGGTGAATATAATATTTGTATTAATAAATTCTAGTTTTTACAATGTCGGGAGACTT 4681
GAAAAGCTCAATCAAGCTCAATTTTATGGTGACGTCCAGTACTGTACATTTACAATGAAG 4741
CTTCACCTAGACAATCCATGTTCTGTTTCATTTGTGTATGAGTAAGGATCAATGTCTCTG 4801
TTTTGTTTAGAACACAAGTAATGCCACAGTGCAGTACATTCTAAATGGAAACTGCAAAAT 4861
ATAAACTAAATTTTTCTGCTGTTGAAGTAACTTAAATGGTGTCTTCCTTTCCCATTTCAC 4921
CACGTCAGTGTCAGTGTCACTCGTCCAAATCATGAAATTTGGACCAATTTTGTAGGCTCC 4981
TCAATATGAAATTGTTCCATTGTGTCATTTGCAGAAGTTTACCTTAGATGTCTTCGGCAC 5041
ATTTCAGTGTTTGGGATGGTGAACAATCTGTTTATTGTGGGTGACACTAACATAGTATTG 5101
TGATTTACCACTCTAGTCAGTTTGGAAGGTGATGGGAGAAGGTACCTTTACTGCCCTCAT 5161
CCCCTGGAGGGGCTGAGCAGCTGTGAGGGATCATCATGTGTTTCCATACTTCCTTCTGGG 5221
TGACAAGCCTTCTTGTCACTGCTCTTAGGTGTTGGGTAACCACATTATTATGATTGTTTT 5281
ATTTTCTTTTGGACAAGAAAACTTTCTTCGTAACATGTAATATCTCATTGAACTCAAATG 5341
AAAATTCTGCCTCACAAATTCTATTATGCCAAAAAATGCATTGTGTAGTATACTGTCCAT 5401
GTATCTTTTATTTGCATAACCAGTAGCTATGCAACTACTGTATGATCAAGATGAGCAAAA 5461
CACTTTAAGCTGTTTAATAATATGATTATATTTTATTTCTTAATTACTATTCATTATCTG 5521
TAATTTAATTTTGTCTTCCCCGGACTATTTGCTGTGATATTTTATAAAGATTTGTCCCAT 5581
ACACACATAGTATGCCAGAGAGTTTCCAAAATATATCATACAGACATAAAATAATCAAAA 5641
TCCAAGCATGTGCCAAATGTGTTAAGTTCTCAGGAGCTCCTGTCTGCTGTAGGGTGGCAG 5701
CCAAATTTGGCAGCAGTGACCTCAGAATGTTCATTCAGACAGACAGACAATACATCAAAA 5761
GTGATAAAGTAAATATACCCCACTGTGTGCCAAGACTTGAGGCCTAGCACTCTTCAGGAC 5821
TTAGTTACTGATGGCACCAGAAGATGGTGCCATGTAAGTGCTGCTGCTTCCCTCACCATA 5881
AAGTATGTTCAGTATAATTATAGTGAAAATGTCAGGAAATACAGTTATCAATTAAATTCA 5941
TTACTATCAGATAAAGTAGTGTGTTAAAGCTGAAACAAGATTTCTAAGAACGCTACTGCA 6001
ATCTTTAACAAGGGGGAAGGAATGCATGGGTCAGCTCAGTACTAGTGTTTGTGAAGTGAC 6061
AATGGCTGTGATGAAAATTTTCCTACTATGTATGCTTTTTAAGAAAAAACTTTATTTTGT 6121
GAAATGGCATGTTGTAATAGCTTAAAGCTTTTGCTATGAGAAAAACTTAACAGTGCAGAT 6181
ATCATTAGAAGAATGTTTCTAATTTTGAAAAATGTATGCACTTACTGCACCTCACATTGT 6241
TTGGTTAACAGTCTTAGTACTGTAACTCACATTGTTTGGTAGTCAGTCTTTCAAAGGAAA 6301
TAACTCCAAACTAGATGGGGGAATGTTCACATCCCAAGTTCCTTGCAGTGGAAATCCCCT 6361
GAAAACCTAGTACATACTTGCAGAGCACACAGTCAATGTTACATGCCAATAGATTTTGAA 6421
GTGCTCTTCTTTGCACATCATTCTTCAAACCAGCTTGTACTTTCATCTAATGGCCTGTGG 6481
AGGATGATGCACAACATTTTATTTTGTAAATTGAAAGGATCCATGGAAGGCATTTGGCTA 6541
AGACATTCTCCTCTTGCCACAGTGGCGGCCCTTGTTTTAGAGCAGTGCTCACACCCCACT 6601
TTTGAGAACAGATAATAAAATTTGCACCATTACTACACAAAATGCGTATTCAGTTCAAAT 6661
CTCTTGAACATCCATGCCAAATTTAACTTGTTGAACCTAACAAATCTCTCATTCCCCAAA 6721
```

```
CTAAGTATTGTTTAAATCTTGAAGCAGTTTTCTCTTGTATTAATATATTTACACTGATAA 6781
TTGTTATAACATATGACTACAAACTGATCTCCCATGGAAGCAAGGCTACCAGTATAACCA 6841
AACTTAAATCTTTAAGCAATTTTCTTCTTTCTTAGCACATTGACACTAGCAATTGTCAAA 6901
ATGTGTGACTGTAGACTCACCTCCAACTGAAGCATTGTGGGAACAAAGATCATCAGTATA 6961
ATTAAGGCATATCCTTGGATTTTGGTTTAAGTTCCTGCAGAAGGACTGAAACACTTTGGC 7021
AGAACTCACTTAAGAGTGGTAAATCACACCCAAGTATTACCTGCTGTGTGGTAGGGTTGT 7081
GGCATAGTGGATCAACTTGCTGGAGTCTTGAGAACATTGCAGGCACAAAACAGTAATCGT 7141
AGAAACACTGGCAAGACTTACTTAAGAGTGGTAAATCACACCTGAGTATTACCTGCTGTG 7201
AGGTGGATCAAAGCAAAATTAATTAACTTGTTAGAATCTTGAGAGCAGTGCAGGCACAAA 7261
ATATTCTCAATAATTTTGTACATATGCCATTGGTCTGTAACTGTCTTCATATTTATAGCA 7321
AGCTGTTTATGCTGTCATGGTTGACAGGAATTTGCTTCAAAATTATAGCCACATCAAGAT 7381
AAGACAGCATGTTAGATTGTTATAATATGCAATTTCTGAACATATGTAGTGATGAGGTGC 7441
ATTAATTTGTATCAAACTGAAAAACATCAGTATTTTTTATATGTTGTGAAGGATTAATGT 7501
AGTACATATTATTATTATTATTATTA                                     7531
```

Figure 2.11. Nucleotide and amino acid sequence of cDNA encoding Gl-PDE7.

A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.


GlPDE7 : ----------MVRMLGDVKGNTTVETSLAATVGPS : 25
Dr.PDE7A : MELCYQLPVLPLDRPVPKHVLSRRGAISFSSSSSLFGAPD : 40
Hs.PDE7A : MEVCYQLPVLPLDRPVPQHVLSRRGAISFSSSSALFGCPN : 40
GlPDE7 : -RSTDARLDAANTESRARATLTMNYSLGDMNVGEAEQRLL : 64
Dr.PDE7A : PRQLSQRRGAISYDSSDQTALYIR-MLGDVRVRSQVGFEP : 79
Hs.PDE7A : PRQLSQRRGAISYDSSDQTALYIR-MLGDVRVRSRAGFES : 79
GlPDE7 : GGAGRGPY--SRFLTLHRRR----------RRK : 85
Dr.PDE7A : ERRGSHPYLGIDFRTLHSRAESAGSIPARRIRRLFSFQRH : 119
Hs.PDE7A : ERRGSHPY--IDFRIFHSQSEIEVSVSARNIRRLLSFQRY : 117
GlPDE7 : LPTRRLDK-----QHQALLDDLYNGLTQYLLSRVGVWGFN : 120
Dr.PDE7A : LLSSRLLRGAPHLNPLHILDEDYCGQAKCMLEKVGSWNFD : 159
Hs.PDE7A : LRSSRFFRGTAVSNSLNILDDDYNGQAKCMLEKVGNWNFD : 157
GlPDE7 : AFTLDSVCGGRPVSVLCVFLLHEYGLIEHFKFDTVTVWKC : 160
Dr.PDE7A : IFLFDRLTNGNSLVFLTFHLLSQYGLIELFQLDMVKVRRF : 199
Hs.PDE7A : IFLFDRLTNGNSLVSLTFHLFSLHGLIEYFHLDMMKLRRF : 197
***
GlPDE7 : FSLIEDGYHASNPYHNAIHAADVTQAMHCYLQEEKIHQHL : 200
Dr.PDE7A : LVLVQEDYHNQNPYHNAVHAADVTQAMHCYLREPKLAQSL : 239
Hs.PDE7A : LVMIQEDYHSQNPYHNAVHAADVTQAMHCYLKEPKLANSV : 237
****
GlPDE7 : TPLEMLAALIAAVCHDLDHPGVNQPFLIATDNHLAALYKN : 240
Dr.PDE7A : TSFDILLGLLAAATHDLDHPGVNQPFLIKTNHYLAALYRN : 279
Hs.PDE7A : TPWDILLSLIAAATHDLDHPGVNQPFLIKTNHYLATLYKN : 277
GlPDE7 : FSVLENHHWRCAMGCLWESGLLDSWDPDDVATLQDMLRSL : 280
Dr.PDE7A : TSVLENHHWRSAVGLLRETELFSHLPAEDSLSIERQLGSL : 319
Hs.PDE7A : TSVLENHHWRSAVGLLRESGLFSHLPLESRQQMETQICAL : 317


GlPDE7 : ILATDITRQQEFLTRFKQYLDSGTFDMGEPEHRHFSLQIA : 320
Dr.PDE7A : ILATDISRQNEYLSRFRTHLDENDLNLGNASHRHFVLQMA : 359
Hs.PDE7A : ILATDISRQNEYLSLFRSHLDRGDICLEDTRHRHLVLQMA : 357
GlPDE7 : LKCADICNPCRPWEVSQKWSYKICDEFYRQGDYERQLNLP : 360
Dr.PDE7A : LKCADICNPCRPWELSKQWSEKVTEEFFHQGDIEKKLKLE : 399
Hs.PDE7A : LKCADICNPCRTWELSKQWSEKVTEEFFHQGDIEKKYHLG : 397
GlPDE7 : VTLLCDSSRMSVAKIQTDFIKYVVSPLFDVWDRWLETSLS : 400
Dr.PDE7A : ISPLCDSEANTIASVQIGFMIYVVEPLFAEWARFSDTRLS : 439
Hs.PDE7A : VSPLCDRHTESIANIQIGFMIYLVEPLFTEWARFSNTRLS : 437
GlPDE7 : NSMVTNMNINLQKWEEKLLKKLAEDAAAQPVVVPEDSSEN : 440
Dr.PDE7A : QTMLGHLGLNKASWST---------- : 455
Hs.PDE7A : QTMLGHVGLNKASWKG---------- : 453
GlPDE7 : PVVESAEEEPEEVDGGTSPSSSGDSHQSVLGSLENVSRSL : 480
Dr.PDE7A : ----------MEPEASGSSEDGESDPARSSEEPSSRAL : 483
Hs.PDE7A : ----------LQREQS-SSED-----TDAAFELNSQLL : 475
GlPDE7 : QLGRRHSVPLNLPRLLPRTIIRRESLPENGNQPLVVETVT : 520
Dr.PDE7A : TQGSPES---------- : 490
Hs.PDE7A : PQENRLS---------- : 482
GlPDE7 : MGGMSLTSLSSRPTDLNSTLTPDALLPEPSITTMGGVGIR : 560
Dr.PDE7A : ---------- : -
Hs.PDE7A : ---------- : -
GlPDE7 : LPRPATRLTRRKSLPPLWLQQTRARTSHMLQPLSPTPSED : 600
Dr.PDE7A : ---------- : -
Hs.PDE7A : ---------- : -
GlPDE7 : ASSHQTTQENSPQDTENAPRLGAVHGVGSGHSSGNNSSGC : 640
Dr.PDE7A : ---------- : -
Hs.PDE7A : ---------- : -

```
GlPDE7   : GGSGGCDRVKSGGGECSDPVEPIHHPIRSDTDPLTAAQDV :  680
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -

GlPDE7   : GVHGRKCVSNHQGATTFLHADCLDNHPYRPFIGRGLVRRA :  720
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -

GlPDE7   : SLDSTSISSKREFLDRLLHESSKFPRVDRTNSELDKENLV :  760
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -

GlPDE7   : PREALTYREQQQQQQSSWKTRAWRSLNCDDENVCDPREKL :  800
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -

GlPDE7   : MKPGMYKLNQSQGSLYAHGRRGSAPLLRPEELLGGLRGGA :  840
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -

GlPDE7   : ERPDYNYLSLRRGSAPSQANQKGCDVETGSEVGGRHTPLT :  880
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -

GlPDE7   : STENLPFSEYVSSHSRLRGRRRGSIPFDHPGLCRQGSGGV :  920
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -

GlPDE7   : EVLPSRTGVGGNLAVSGGGSGAAGFPSVLPHYYHEYHRGS :  960
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -
```

```
GlPDE7   : GGLELFAGLWRSHLEPESSSSVFVDGDLCSQSSGGLTPST : 1000
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -


GlPDE7   : PGAVLPPHRPLPHTLHRRGSLPTDLFYSGDFSVWDCEGT : 1039
Dr.PDE7A : ---------------------------------------- :    -
Hs.PDE7A : ---------------------------------------- :    -
```

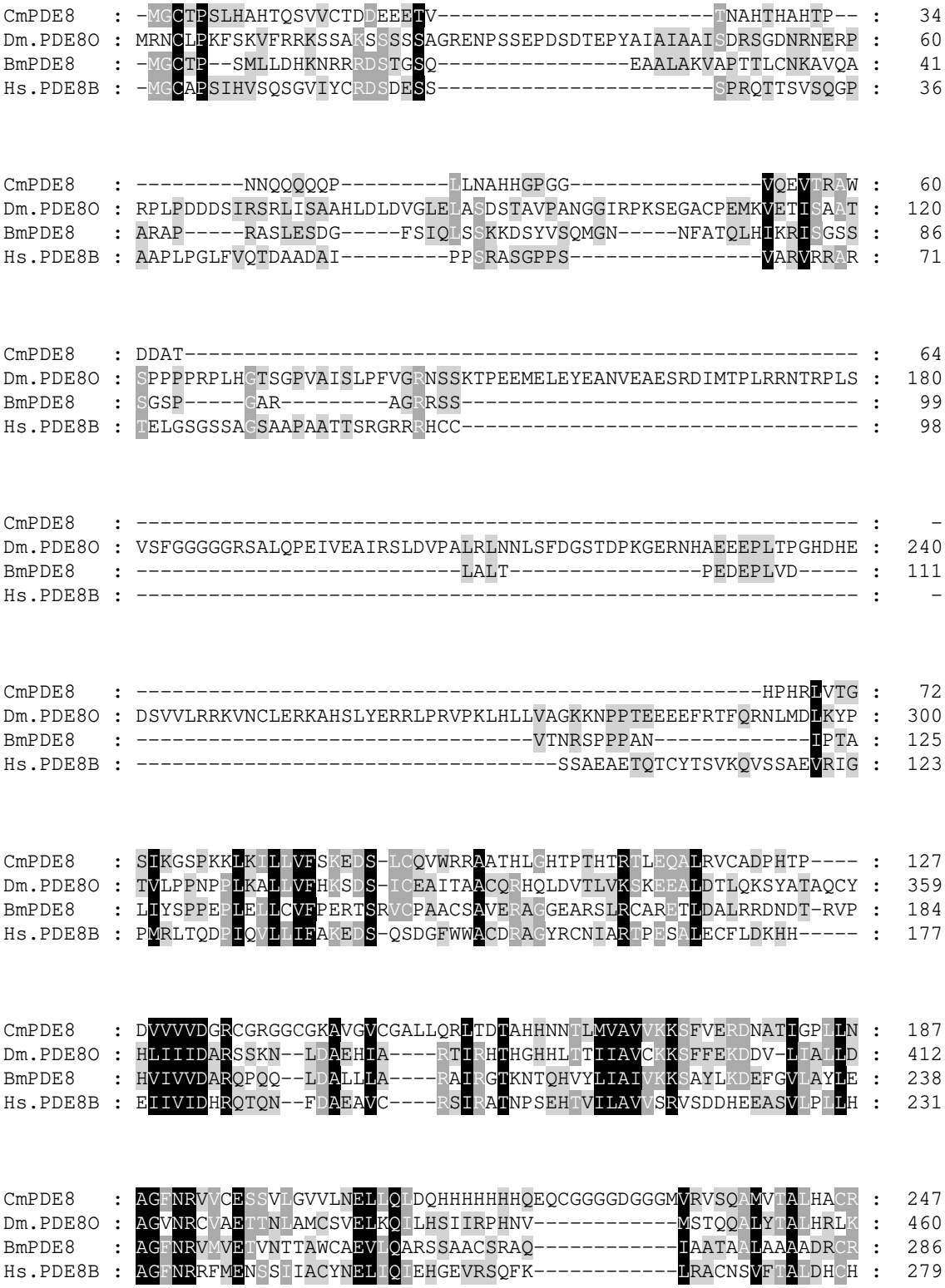
Figure 2.12. Multiple alignment of deduced amino acid sequences of PDE7 proteins in one crustacean species, one fish species, and one mammal species. Abbreviations: Gl: *G lateralis*; Dr: *D rerio*; Hs: *H sapiens*. Black shading indicates that amino acid residues that are identical or similar in all sequences; gray shading indicates identical or similar amino acids in most of the sequences. Dashes indicate gaps introduced to optimize the alignment and the boxes indicate highly conserved domains. Blue asterisks indicate the signature sequences in the catalytic domain. The colors of the boxes correspond to the colors of the domains in Fig. 2. 9.

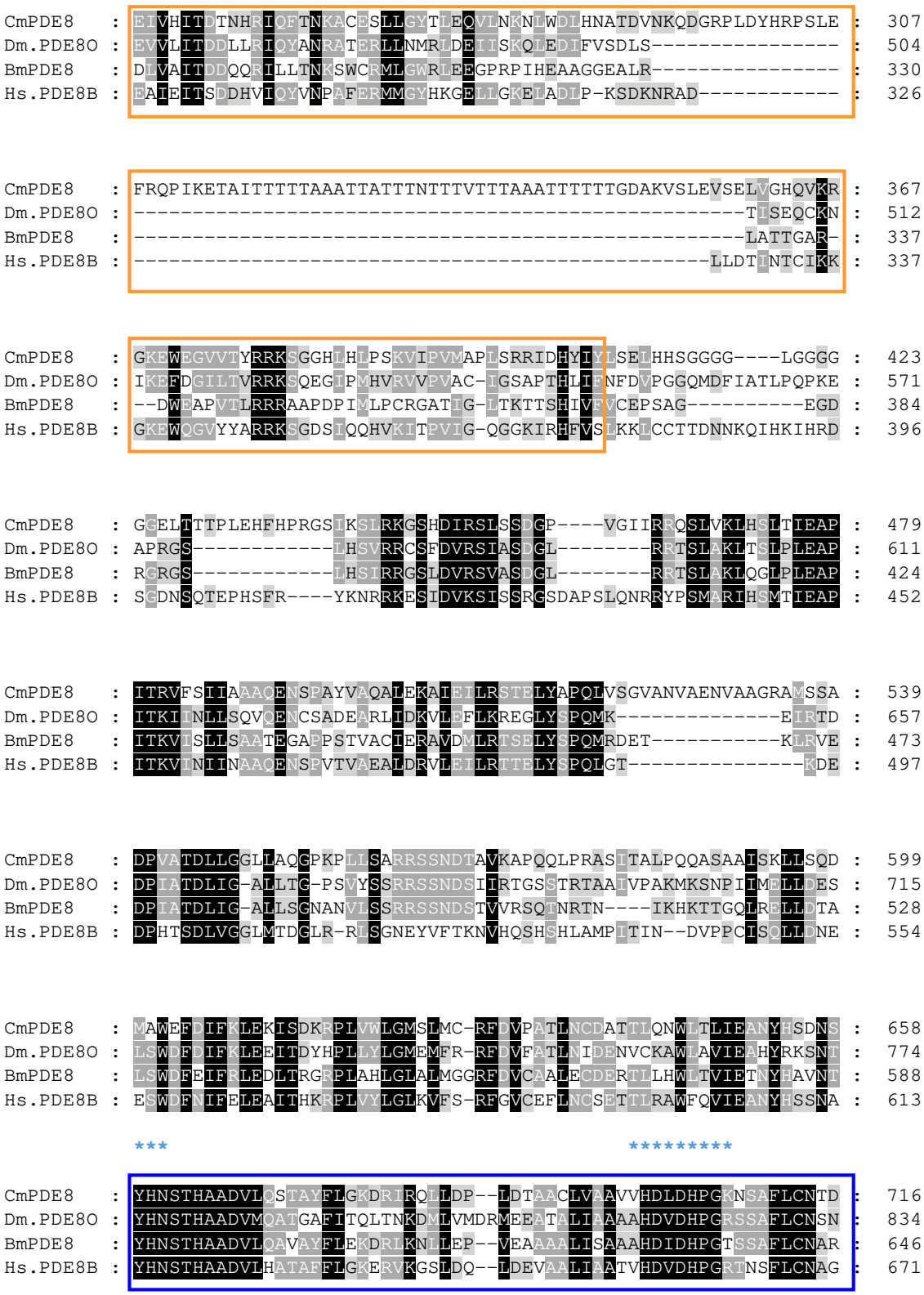
CmPDE8 3007 nucleotides

```
   1                                                    M  G  C  T
   1 ACAACTACTACAACTACTACTACAACTACTACTACTACAGTGTGAGGGATGGGGTGCACT
   5 P  S  L  H  A  H  T  Q  S  V  V  C  T  D  D  E  E  E  T  V
  61 CCCAGCCTACACGCGCACACCCAGTCTGTGGTGTGCACGGACGATGAAGAAGAAACAGTC
  25 T  N  A  H  T  H  A  H  T  P  N  N  Q  Q  Q  Q  Q  P  L  L
 121 ACAAATGCGCACACACACGCACACACGCCCAATAATCAACAACAACAACAGCCGCTGCTT
  45 N  A  H  H  G  P  G  G  V  Q  E  V  T  R  A  W  D  D  A  T
 181 AACGCACATCATGGTCCCGGAGGAGTACAGGAGGTCACCCGCGCCTGGGATGACGCCACA
  65 H  P  H  R  L  V  T  G  S  I  K  G  S  P  K  K  L  K  I  L
 241 CACCCACACCGGTTGGTGACAGGATCTATAAAAGGCTCCCCTAAGAAACTCAAAATCTTA
  85 L  V  F  S  K  E  D  S  L  C  Q  V  W  R  R  A  A  T  H  L
 301 TTAGTATTCAGTAAGGAGGACTCACTGTGTCAGGTGTGGAGGAGGGCCGCTACCCACCTG
 105 G  H  T  P  T  H  T  R  T  L  E  Q  A  L  R  V  C  A  D  P
 361 GGCCACACCCCCACACACACCCGCACCTTGGAACAGGCTCTGCGGGTGTGTGCTGACCCC
 125 H  T  P  D  V  V  V  V  D  G  R  C  G  R  G  G  C  G  K  A
 421 CACACACCCGACGTGGTGGTGGTGGATGGCAGGTGTGGCCGTGGCGGGTGTGGCAAGGCT
 145 V  G  V  C  G  A  L  L  Q  R  L  T  D  T  A  H  H  N  N  T
 481 GTGGGGGTGTGTGGTGCGCTGTTACAAAGACTGACTGACACTGCTCATCACAACAACACT
 165 L  M  V  A  V  V  K  K  S  F  V  E  R  D  N  A  T  I  G  P
 541 CTCATGGTGGCGGTGGTGAAGAAAAGTTTTGTGGAGAGAGACAATGCAACGATTGGCCCA
 185 L  L  N  A  G  F  N  R  V  V  C  E  S  S  V  L  G  V  V  L
 601 TTACTGAATGCTGGCTTTAATAGGGTTGTGTGTGAGAGCAGTGTGCTGGGAGTAGTGCTG
 205 N  E  L  L  Q  L  D  Q  H  H  H  H  H  H  H  Q  E  Q  C  G
 661 AATGAGCTGCTACAACTAGACCAGCACCACCACCATCATCATCATCAGGAGCAGTGTGGT
 225 G  G  G  D  G  G  G  M  V  R  V  S  Q  A  M  V  T  A  L  H
 721 GGTGGTGGTGATGGTGGTGGTATGGTGAGGGTGTCCCAGGCTATGGTGACGGCTCTTCAT
 245 A  C  R  E  I  V  H  I  T  D  T  N  H  R  I  Q  F  T  N  K
 781 GCTTGCCGGGAGATTGTGCATATTACTGATACCAACCATAGGATACAGTTCACCAACAAG
 265 A  C  E  S  L  L  G  Y  T  L  E  Q  V  L  N  K  N  L  W  D
 841 GCGTGTGAGTCGTTGTTGGGCTACACTCTAGAACAAGTGTTGAACAAGAACCTTTGGGAC
 285 L  H  N  A  T  D  V  N  K  Q  D  G  R  P  L  D  Y  H  R  P
 901 CTTCACAACGCCACCGATGTAAACAAACAAGATGGCCGACCCCTAGACTACCACAGACCA
 305 S  L  E  F  R  Q  P  I  K  E  T  A  I  T  T  T  T  T  A  A
 961 TCCCTAGAGTTCAGACAACCTATAAAAGAAACTGCTATTACTACTACTACTACTGCTGCT
 325 A  T  T  A  T  T  T  N  T  T  T  V  T  T  T  A  A  A  T  T
1021 GCTACTACTGCTACTACAACGAATACTACTACTGTGACTACTACTGCTGCTGCCACGACT
 345 T  T  T  T  G  D  A  K  V  S  L  E  V  S  E  L  V  G  H  Q
1081 ACTACTACTACTGGTGATGCTAAGGTGTCATTGGAGGTGAGTGAGTTGGTGGGTCATCAA
 365 V  K  R  G  K  E  W  E  G  V  V  T  Y  R  R  K  S  G  G  H
1141 GTGAAGCGAGGCAAGGAGTGGGAGGGAGTGGTGACCTATCGCAGGAAGTCTGGAGGTCAT
 385 L  H  L  P  S  K  V  I  P  V  M  A  P  L  S  R  R  I  D  H
1201 CTTCACCTACCATCTAAGGTCATTCCTGTCATGGCTCCTCTCTCAAGACGCATTGACCAC
 405 Y  I  Y  L  S  E  L  H  H  S  G  G  G  G  L  G  G  G  G  G
1261 TACATCTACCTGAGTGAGCTGCATCATAGTGGTGGTGGTGGCCTGGGTGGTGGTGGTGGT
 425 G  E  L  T  T  T  P  L  E  H  F  H  P  R  G  S  I  K  S  L
1321 GGCGAGTTGACCACTACACCACTGGAACACTTTCACCCGAGAGGTTCCATCAAGTCTCTG
 445 R  K  G  S  H  D  I  R  S  L  S  S  D  G  P  V  G  I  I  R
1381 AGGAAAGGCTCACATGATATAAGATCCCTTAGTAGTGATGGTCCAGTTGGTATAATTCGT
 465 R  Q  S  L  V  K  L  H  S  L  T  I  E  A  P  I  T  R  V  F
```

```
1441 CGGCAGAGTCTGGTGAAGCTTCATTCGCTCACTATTGAAGCTCCGATCACCCGAGTGTTC
 485 S  I  I  A  A  A  Q  E  N  S  P  A  Y  V  A  Q  A  L  E  K
1501 TCCATCATCGCGGCCGCCCAGGAGAACAGCCCGGCTTATGTGGCACAGGCTTTGGAGAAA
 505 A  I  E  I  L  R  S  T  E  L  Y  A  P  Q  L  V  S  G  V  A
1561 GCTATAGAGATTCTCCGCTCCACAGAACTCTATGCACCACAGCTCGTGTCTGGTGTGGCT
 525 N  V  A  E  N  V  A  A  G  R  A  M  S  S  A  D  P  V  A  T
1621 AATGTGGCTGAGAATGTGGCTGCCGGGAGGGCTATGTCGTCTGCTGATCCTGTGGCCACT
 545 D  L  L  G  G  L  L  A  Q  G  P  K  P  L  L  S  A  R  R  S
1681 GATCTGTTGGGGGGTCTGCTTGCGCAAGGGCCCAAGCCACTGCTGTCAGCGCGCCGCAGC
 565 S  N  D  T  A  V  K  A  P  Q  Q  L  P  R  A  S  I  T  A  L
1741 AGCAACGACACAGCTGTCAAGGCCCCACAGCAGCTGCCAAGAGCCTCGATAACAGCTTTA
 585 P  Q  Q  A  S  A  A  I  S  K  L  L  S  Q  D  M  A  W  E  F
1801 CCACAGCAAGCCTCGGCAGCCATTAGCAAGCTCCTCTCTCAGGACATGGCCTGGGAATTC
 605 D  I  F  K  L  E  K  I  S  D  K  R  P  L  V  W  L  G  M  S
1861 GATATTTTTAAACTCGAGAAAATATCCGATAAAAGACCGCTGGTGTGGCTGGGCATGTCC
 625 L  M  C  R  F  D  V  P  A  T  L  N  C  D  A  T  T  L  Q  N
1921 CTGATGTGCCGCTTCGACGTGCCTGCCACACTCAACTGTGACGCCACCACACTGCAGAAC
 645 W  L  T  L  I  E  A  N  Y  H  S  D  N  S  Y  H  N  S  T  H
1981 TGGCTCACCCTTATAGAGGCAAACTACCACAGTGATAACTCCTACCACAACTCCACGCAC
 665 A  A  D  V  L  Q  S  T  A  Y  F  L  G  K  D  R  I  R  Q  L
2041 GCTGCCGACGTCCTCCAGTCCACCGCTTACTTCTTGGGCAAGGACAGGATAAGGCAGCTG
 685 L  D  P  L  D  T  A  A  C  L  V  A  A  V  V  H  D  L  D  H
2101 CTGGACCCGTTAGATACAGCTGCGTGTCTGGTGGCAGCTGTGGTACACGACCTAGACCAT
 705 P  G  K  N  S  A  F  L  C  N  T  D  N  E  L  A  I  L  Y  N
2161 CCTGGGAAGAACAGCGCCTTCCTTTGCAACACTGATAATGAGTTGGCAATACTGTACAAT
 725 D  V  S  V  L  E  C  H  H  V  A  V  S  F  K  H  T  R  S  D
2221 GATGTGAGTGTGTTGGAGTGTCACCACGTGGCTGTGTCCTTCAAGCACACTCGCTCCGAT
 745 D  R  V  N  I  Y  K  G  L  D  R  D  T  Y  K  H  L  R  K  S
2281 GATAGGGTCAATATCTATAAAGGCCTGGACCGTGACACTTACAAACACTTGAGGAAAAGT
 765 I  I  D  M  V  L  A  T  D  M  T  R  H  F  E  H  L  S  K  F
2341 ATTATAGACATGGTGTTGGCAACAGACATGACCAGACACTTTGAACACTTAAGTAAATTT
 785 V  N  M  A  A  T  T  T  T  T  T  T  A  D  G  G  D  D  S
2401 GTCAACATGGCTGCCACTACTACTACTACTACTACTGCTGATGGTGGCGATGATAGT
 805 D  V  F  D  E  E  T  A  A  A  A  S  D  L  L  S  F  N  T  P
2461 GATGTTTTTGATGAGGAGACAGCAGCAGCAGCATCAGACCTACTGTCCTTCAACACACCC
 825 E  N  I  V  I  I  K  R  M  L  I  K  C  A  D  V  S  N  P  L
2521 GAGAACATCGTCATCATCAAGAGAATGTTAATTAAGTGTGCTGACGTAAGCAATCCTCTT
 845 R  P  T  H  L  S  I  D  W  A  Y  R  I  A  N  E  Y  F  N  Q
2581 AGGCCTACACACCTCTCTATTGACTGGGCCTATAGGATTGCTAACGAGTACTTCAACCAG
 865 T  E  E  E  K  V  R  E  L  P  I  V  M  P  Q  F  D  R  T  T
2641 ACGGAGGAGGAGAAGGTGCGTGAGCTGCCCATAGTGATGCCACAGTTTGACAGAACCACT
 885 C  S  I  P  K  S  Q  I  G  F  I  D  F  F  I  N  D  M  F  D
2701 TGCTCCATTCCCAAGTCTCAAATTGGCTTCATTGACTTCTTCATCAACGACATGTTTGAT
 905 A  W  D  A  L  A  D  I  S  E  L  L  E  H  L  R  T  N  Y  L
2761 GCTTGGGACGCTTTGGCCGACATCTCAGAACTTCTGGAACACTTAAGAACAAATTATCTC
 925 Y  W  K  E  Q  E  E  Q  E  Q  T  T  I  T  T  T  T  A  T  A
2821 TATTGGAAAGAACAAGAAGAACAAGAACAAACAACAATAACAACAACAACTGCTACTGCT
 945 A  A  T  E  P  P  P  P  P  P  P  P  P  P  P  E  E  L  H  I
2881 GCTGCTACTGAACCACCACCACCACCACCACCACCACCTCCTGAAGAACTACATATT
 965 K  E  E  E  E  E  E  E  E  K  S  *  K  R  R  R  R  R  K  R
2941 AAAGAAGAGGAGGAGGAGGAGGAGGAAAAGAGTTAAAAGAGGAGAAGGAGGAGAAAGAGG
 985 R  R
3001 AGGAGAA
```

Figure 2.13. Nucleotide and amino acid sequence of cDNA encoding Cm-PDE8.
A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue. The regulatory region, the PAS domain, is shown in an orange box.


CmPDE8 : -MGCTPSLHAHTQSVVCTDDEEETV----------INAHTHAHTP-- : 34
Dm.PDE8O : MRNCLPKFSKVFRRKSSAKSSSSSAGRENPSSEPDSDTEPYAIAIAAISDRSGDNRNERP : 60
BmPDE8 : -MGCTP--SMLLDHKNRRRDSTGSQ----------EAALAKVAPTTLCNKAVQA : 41
Hs.PDE8B : -MGCAPSIHVSQSGVIYCRDSDESS----------SPRQTTSVSQGP : 36
CmPDE8 : ---------NNQQQQQP----------LLNAHHGPGG----------VQEVTRAW : 60
Dm.PDE8O : RPLPDDDSIRSRLISAAHLDLDVGLELASDSTAVPANGGIRPKSEGACPEMKVETISAAT : 120
BmPDE8 : ARAP-----RASLESDG-----FSIQLSSKKDSYVSQMGN-----NFATQLHIKRISGSS : 86
Hs.PDE8B : AAPLPGLFVQTDAADAI---------PPSRASGPPS----------VARVRRAR : 71
CmPDE8 : DDAT---------- : 64
Dm.PDE8O : SPPPPRPLHGTSGPVAISLPFVGRNSSKTPEEMELEYEANVEAESRDIMTPLRRNTRPLS : 180
BmPDE8 : SGSP-----GAR----------AGRRSS---------- : 99
Hs.PDE8B : TELGSGSSAGSAAPAATTSRGRRRHCC---------- : 98
CmPDE8 : ---------- : -
Dm.PDE8O : VSFGGGGGRSALQPEIVEAIRSLDVPALRLNNLSFDGSTDPKGERNHAEEEPLTPGHDHE : 240
BmPDE8 : ----------LALT----------PEDEPLVD----- : 111
Hs.PDE8B : ---------- : -
CmPDE8 : ----------HPHRLVTG : 72
Dm.PDE8O : DSVVLRRKVNCLERKAHSLYERRLPRVPKLHLLVAGKKNPPTEEEEFRTFQRNLMDLKYP : 300
BmPDE8 : ----------VTNRSPPPAN----------IPTA : 125
Hs.PDE8B : ----------SSAEAETQTCYTSVKQVSSAEVRIG : 123
CmPDE8 : SIKGSPKKLKILLVFSKEDS-LCQVWRRAATHLGHTPTHTRILEQALRVCADPHTP---- : 127
Dm.PDE8O : TVLPPNPPLKALLVFHKSDS-ICEAITAACQRHQLDVTLVKSKEEALDILQKSYATAQCY : 359
BmPDE8 : LIYSPPEPLELLCVFPERTSRVCPAACSAVERAGGEARSLRCARETLDALRRDNDT-RVP : 184
Hs.PDE8B : PMRLTQDPIQVLLIFAKEDS-QSDGFWWACDRAGYRCNIARIPESAIECFLDKHH----- : 177
CmPDE8 : DVVVVDGRCGRGGCGKAVGVCGALLQRLTDTAHHNNTLMVAVVKKSFVERDNATIGPLLN : 187
Dm.PDE8O : HLIIIDARSSKN--LDAEHIA----RTIRHTHGHHLTTIIAVCKKSFFEKDDV-LIALLD : 412
BmPDE8 : HVIVVDARQPQQ--LDALLLA----RAIRGTKNTQHVYLIAIVKKSAYLKDEFGVLAYLE : 238
Hs.PDE8B : EIIVIDHRQTQN--FDAEAVC----RSIRATNPSEHTVILAVVSRVSDDHEEASVLPLLH : 231
CmPDE8 : AGFNRVVCESSVLGVVLNELLQLDQHHHHHHHQEQCGGGGDGGGMVRVSQAMVLALHACR : 247
Dm.PDE8O : AGVNRCVAETTNLAMCSVELKQILHSIIRPHNV----------MSTQQALYLALHRLK : 460
BmPDE8 : AGFNRVMVETVNTTAWCAEVLQARSSAACSRAQ----------IAATAALAAAADRCR : 286
Hs.PDE8B : AGFNRRFMENSSIIACYNELIQIEHGEVRSQFK----------LRACNSVFIALDHCH : 279


CmPDE8 : EIVHITDTNHRIQFTNKACESLLGYTLEQVLNKNLWDIHNATDVNKQDGRPLDYHRPSLE : 307
Dm.PDE8O : EVVLITDDLLRIQYANRATERLLNMRLDEIISKQLEDIFVSDLS---------- : 504
BmPDE8 : DLVAITDDQQRILLTNKSWCRMLGWRLEEGPRPIHEAAGGEALR---------- : 330
Hs.PDE8B : EATEITSDDHVIQYVNPAFERMMGYHKGELLGKELADLP-KSDKNRAD---------- : 326
CmPDE8 : FRQPIKETAITTTTTAAATTATTTNTTTVTTTAAATTTTTTGDAKVSLEVSELVGHQVKR : 367
Dm.PDE8O : ----------TISEQCKN : 512
BmPDE8 : ----------LATTGAR- : 337
Hs.PDE8B : ----------LLDTINTCIKK : 337
CmPDE8 : GKEWEGVVTYRRKSGGHILHLPSKVIPVMAPLSRRIDHYIYLSELHHSGGGG----LGGGG : 423
Dm.PDE8O : IKEFDGILTVRRKSQEGIPMHVRVVPVAC-IGSAPTHLIFNFDVPGGQMDFIATLPQPKE : 571
BmPDE8 : --DWEAPVTLRRRAAPDPIMLPCRGATIG-LTKTTSHIVFVCEPSAG----------EGD : 384
Hs.PDE8B : GKEWQGVYYARRKSGDSIQQHVKITPVIG-QGGKIRHFVSIKKLCCTTDNNKQIHKIHRD : 396
CmPDE8 : GGELTTTPLEHFHPRGSTKSLRKGSHDIRSLSSDGP----VGIIRRQSLVKLHSLTIEAP : 479
Dm.PDE8O : APRGS----------LHSVRRCSFDVRSIASDGL--------RRTSLAKLTSLPLEAP : 611
BmPDE8 : RGRGS----------LHSIRRGSLDVRSVASDGL--------RRTSLAKLQGLPLEAP : 424
Hs.PDE8B : SGDNSQTEPHSFR----YKNRRKESIDVKSISSRGSDAPSLQNRRYPSMARIHSMTIEAP : 452
CmPDE8 : ITRVFSIIAAAQENSPAYVAQALEKAIEILRSTELYAPQIVSGVANVAENVAAGRAMSSA : 539
Dm.PDE8O : ITKIINLLSQVQENCSADEARLIDKVLEFLKREGLYSPQMK----------EIRTD : 657
BmPDE8 : ITKVISLLSAATEGAPPSTVACIERAVDMLRTSELYSPQMRDET----------KLRVE : 473
Hs.PDE8B : ITKVINIINAAQENSPVTVAEALDRVLEILRTTELYSPQIGT----------KDE : 497
CmPDE8 : DPVATDLLGGLLAQGPKPLLSARRSSNDTAVKAPQQLPRASITALPQQASAAISKLLSQD : 599
Dm.PDE8O : DPIATDLIG-ALLTG-PSVYSSRRSSNDSIIRTGSSTRTAAIVPAKMKSNPIIMSLLDES : 715
BmPDE8 : DPIATDLIG-ALLSGNANVLSSRRSSNDSTVVRSQTNRTN----IKHKTTGQLRSLLDTA : 528
Hs.PDE8B : DPHTSDLVGGLMTDGLR-RLSGNEYVFTKNVHQSHSHLAMPITIN--DVPPCISQLLDNE : 554
CmPDE8 : MAWEFDIFKLEKISDKRPLVWLGMSLMC-RFDVPATLNCDATTIQNWLTLIEANYHSDNS : 658
Dm.PDE8O : LSWDFDIFKLEEITDYHPLLYLGMEMFR-RFDVFATLNIDENVCKAWLAVIEAHYRKSNT : 774
BmPDE8 : LSWDFEIFRLEDLTRGRPLAHLGLALMGGRFDVCAALECDERTILHWLTVIETNYHAVNT : 588
Hs.PDE8B : ESWDFNIFELEAITHKRPLVYLGLKVFS-RFGVCEFLNCSETTIRAWFQVIEANYHSSNA : 613
CmPDE8 : YHNSTHAADVLQSTAYFLGKDRIRQLLDP--LDTAACLVAAVVHDLDHPGKNSAFLCNTD : 716
Dm.PDE8O : YHNSTHAADVMQATGAFITQLTNKDMLVMDRMEEATALIAAAAHDVDHPGRSSAFLCNSN : 834
BmPDE8 : YHNSTHAADVLQAVAYFLEKDRLKNLLEP--VEAAAALISAAAHDIDHPGTSSAFLCNAR : 646
Hs.PDE8B : YHNSTHAADVLHATAFFLGKERVKGSLDQ--LDEVAALIAATVHDVDHPGRTNSFLCNAG : 671

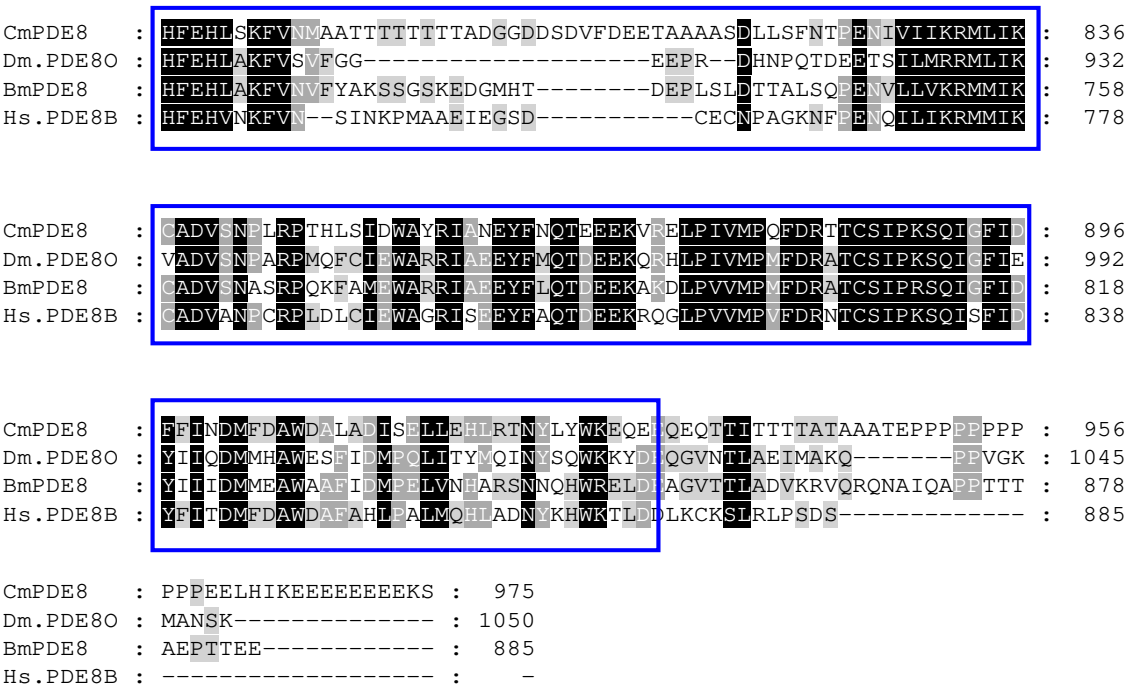


Figure 2.14. Multiple alignment of deduced amino acid sequences of PDE7 proteins in one crustacean species, two insect species, and one mammal species. Abbreviations: Cm: *C maenas*; Dm: *D melanogaster*; Bm: *B mori*; Hs: *H sapiens*. Black shading indicates that amino acid residues that are identical or similar in all sequences; gray shading indicates identical or similar amino acids in most of the sequences. Dashes indicate gaps introduced to optimize the alignment and the boxes indicate highly conserved domains. Blue asterisks indicate the signature sequences in the catalytic domain. The colors of the boxes correspond to the colors of the domains in Fig. 2. 13.

GlPDE9 4111 nucleotides, structure G sequence

```
GACCGTCGACGTGGAGGGAGCGCGTTCGTTGTCTTAGTTTCTCGGACTGCGTGGGACATT
GGCCAAACTTTTTACCCGTAAAATCTCCCCTAGTGACCAGGCATTTCGTGACAGACGTTA
GTGACCCAGGTGCTGTGCAATGGGTCAGCATCAGCAATGATCGGCTTCCCCAGTGTGGTA
TTCGTGTTATAAGGAACAAAAATATATCGAAGTTAAATCTGGATACATATAGTATTGGTA
AACTAAGTGTTATCTTTGATTTGTACGTGTGCTGTGTGAGGTGAGGCCTGGCAGCTGCAC
CTCGCCTGGCCACACAACGAGCGGCAGCTCCACTCACAGCAACAGCGGCACTAAACGAGC
CTAGCAGCAAGGCGGCGGCAGAGCGACCAACCACAGCCTGACGTCACGTCACTGTCAAGG
TGTGGGGGTGAGGATGCCCGCATGGAGAGGTGCGCCAGGACCGTCTACTTCACAGTGGGA
GACAGACAGGACTCCGCCAGCCTCCACCCTGATGCCCGCCCCGAGGATGTCAGAGAGCTG
TTCAGGAGCGCGTGTGGGGCGGGGCCTAGTGACGTCATCAAGCTGTACACCAGTGAAGGG
AGTCTGCTGAACGCCGGAGCCCACCTGCCTGCCAACACCCCTGCCACCCCCTACCGCCTG
GACGTAGCTGCCACCCCCTGCAATGGAGAGAAGCTGAACCATCTGGGGATTGACCTTATG
GACCTGGAGCAGAGGCTGTGCGAGGTCGAGCGAAGCGTGGCTGCCCTGCGAGCAGACCTG
CCGCCCGTGGTCGAGGAGCTGAGGCGCGCCGTGGACACATTCAAGATGAGGTTGGAGACC
ACTGAGCACCTGTCCTGGCTGGGCCACCAAGAAAGCATGCAGTAGTTCCTCCCGTCACAG
ATTCTTCCCGCGGTTGTCCCTTCATGCTTCCTTGTCCTTACAAGTAGACAGACCCACAGC
CATGCACCGTCTCCTGCAGGGTTCTACAAGGAGGGGATTGGAGAGCCTCTTGGGACTCTG 1
GGCAACTCTCATCCGTGCTGGAAAAACGTTCAGTACCGGAGGAAGGCGGAGGAGGAGGTG 1
GCAACTGTGTGCAACAAGTTTAGAGAAATATGTGACGGCGTGGTGAGCGTGGACACCCAA 1
GAGCTCCTGCGGCTGCCTTCGTTTAACAACTGGGCGTGGGAAGACTGGGAAATTCTCCTT 1
CTCCTCCAGCACATGTACAAGGATCTCGGGCTCCTGTCGAAGTTCGGCATCGAGTTGGAG 1
                M  Y  K  D  L  G  L  L  S  K  F  G  I  E  L  E
GTGTTCCGCAGCTTCTTGTGTCGTGTGTACCACTGCTACAACCAAGTTCCCTTCCACAAC 1
 V  F  R  S  F  L  C  R  V  Y  H  C  Y  N  Q  V  P  F  H  N
TTCATGCACGCCTTCTGTGTCACTCAGATGATGTACGGGATGATCTGCAAATGTGACCTG 1
 F  M  H  A  F  C  V  T  Q  M  M  Y  G  M  I  C  K  C  D  L
CAGCGGCGACTGGGTGACCTCGATTGCCTCATCCTGCTCACGTCCTGCATCTGCCACGAC 1
 Q  R  R  L  G  D  L  D  C  L  I  L  L  T  S  C  I  C  H  D
CTAGACCATCCGGGCTTCAACAACATTTACCAAATCAATGCCAGGACCGAACTCGCCCTC 1
 L  D  H  P  G  F  N  N  I  Y  Q  I  N  A  R  T  E  L  A  L
CGCTACAATGATATCTCGCCGCTTGAGAACCACCACTGCTCCGTTGCCTTCAGCGTCCTC 1
 R  Y  N  D  I  S  P  L  E  N  H  H  C  S  V  A  F  S  V  L
GAACGCAACGAGTGCAATATATTCAGAAACATGCCCCCGGAGGACTACAAGAAGGTGCGG 1
 E  R  N  E  C  N  I  F  R  N  M  P  P  E  D  Y  K  K  V  R
GAGGGTATGATCCGCTGCATCCTCGCCACGGACATGGCGCGTCACAATGAAATCCTCTCA 1
 E  G  M  I  R  C  I  L  A  T  D  M  A  R  H  N  E  I  L  S
GACTTTCGAGAGATCATGCCAGAATTCTCTTACGAGAACCGAGCACACGTCAATGTGCTG 1
 D  F  R  E  I  M  P  E  F  S  Y  E  N  R  A  H  V  N  V  L
TCCATGGTGCTGATCAAGGTGGCTGACATTAGCAACGAGGCACGCCCGCTGGACATTGCT 1
 S  M  V  L  I  K  V  A  D  I  S  N  E  A  R  P  L  D  I  A
GAGCCCTGGCTGGAGTGTCTCATGCAGGAATTCTTCAATCAAAGCGATCTTGAGAAGCTT 1
 E  P  W  L  E  C  L  M  Q  E  F  F  N  Q  S  D  L  E  K  L
GAGGGGCTGCCGGTGTCCCCCTTCATGGACCGGGAGAAGGTTACCAAACCCTCCTCCCAG 1
 E  G  L  P  V  S  P  F  M  D  R  E  K  V  T  K  P  S  S  Q
TGCTCCTTCATCGGCTTTGTCTTACTACCTCTGTTTGAAGCTCTCGGGAAGGTCCTCCCA 1
 C  S  F  I  G  F  V  L  L  P  L  F  E  A  L  G  K  V  L  P
GAGCTAGATGACCTGATCATTCAGCCAGTGAGGTTTGCATTGGAACACTACAGAAAATTG 2
 E  L  D  D  L  I  I  Q  P  V  R  F  A  L  E  H  Y  R  K  L
AATGAAGCTGCAAAAAAAGCTTCAGAAGAGCAAGAAGCTACCTTAGAGCCCACGGTTGAA 2
 N  E  A  A  K  K  A  S  E  E  Q  E  A  T  L  E  P  T  V  E
GAGGAGGAGCTGCAAGTAGAAAGACCTAACAGTCGCCTTTCTCGGGAAAACTCTAAAAAG 2
 E  E  E  L  Q  V  E  R  P  N  S  R  L  S  R  E  N  S  K  K
GTTGTGCAAAAGACTGAAAGCTCTTTCAGCATTGGAAGTAGAGCTTCATCACGCATCTCC 2
 V  V  Q  K  T  E  S  S  F  S  I  G  S  R  A  S  S  R  I  S
```

```
ATGTACCGATCCAGCACATGCGATTACGGTGATGGTGCAGAGTTGGACACAGAAACAGAA 2281
 M  Y  R  S  S  T  C  D  Y  G  D  G  A  E  L  D  T  E  T  E   356
GTTGATGTGAGTGAAAGGACATCAAGATTTAAGATCGCCACAGACATTCACATATCTCCA 2341
 V  D  V  S  E  R  T  S  R  F  K  I  A  T  D  I  H  I  S  P   376
TCACGGAGAAACAGCTCGGAGCGACGCAGTAGTGTTGGTGGGCGATCTAGTTGTGAGCGA 2401
 S  R  R  N  S  S  E  R  R  S  S  V  G  G  R  S  S  C  E  R   396
ACTGTGTCGCCTAGAACCCTCGAGGAGCGGCTCCTGCCTCACGCTGAAGCCAAGAATGAA 2461
 T  V  S  P  R  T  L  E  E  R  L  L  P  H  A  E  A  K  N  E   416
CCTGAGGATGGTGAGGTACATCCAAAGAAAAGTGAAAAGGAATCACTGTTTGCTCGGTTT 2521
 P  E  D  G  E  V  H  P  K  K  S  E  K  E  S  L  F  A  R  F   436
AGAATTTTCTCTGAACGTCTCTCTTCCAGTGACAAGGAGAGGACCTCCAATGGGTCAACC 2581
 R  I  F  S  E  R  L  S  S  S  D  K  E  R  T  S  N  G  S  T   456
ATGATGGGTAGTAGGAGCACTAGATGTAAACAGAGTGGTCTGCAAAGTGTTCTGAAACGT 2641
 M  M  G  S  R  S  T  R  C  K  Q  S  G  L  Q  S  V  L  K  R   476
AGTAGGAGTAAATCTGAACCTACGAAGAGCAGACACCATCGAACATTTCATATATCACGG 2701
 S  R  S  K  S  E  P  T  K  S  R  H  H  R  T  F  H  I  S  R   496
CCAAGAATTAGCTTTGGTACAAATCCCCAGAAAATGCAGTGTGGTGAAAATATGTTTAAT 2761
 P  R  I  S  F  G  T  N  P  Q  K  M  Q  C  G  E  N  M  F  N   516
AATCATGATAAAGAGAAAACAAAAGACAAATGTAAGGAATCATCATCTGAGGGTCTCATC 2821
 N  H  D  K  E  K  T  K  D  K  C  K  E  S  S  S  E  G  L  I   536
TCTTCATTAGAAATTAAGACTTCTACCTCCTTTGAACTTGTAGAACCAAAAAGAAAACCT 2881
 S  S  L  E  I  K  T  S  T  S  F  E  L  V  E  P  K  R  K  P   556
TGTGTGTCATCCAGTGCCCAGCCCTCTCCAACACTCTCTGAAAAGTGTGTGTTAGTTACA 2941
 C  V  S  S  S  A  Q  P  S  P  T  L  S  E  K  C  V  L  V  T   576
AACTCTATCTTAAAGCACAAAGGATTTAGTTTATCTCTTGATGTACTGCCAAGAAAGAAT 3001
 N  S  I  L  K  H  K  G  F  S  L  S  L  D  V  L  P  R  K  N   596
GGTCGATTGAGGAGGTCGGCAGCAACCGAAACCCATACAGCAGAAAACACTCCAGGTAGC 3061
 G  R  L  R  R  S  A  A  T  E  T  H  T  A  E  N  T  P  G  S   616
TCTGAGGACAACCTCTTAGAGGAGTCCAAAAGGCAGCAGGTGGATGGAGCTGCCAGATTC 3121
 S  E  D  N  L  L  E  E  S  K  R  Q  Q  V  D  G  A  A  R  F   636
AGTCGACATCAAACATTCTTCAGCCAGACTAAGGGGGTTCACTCTCCTGAAGGACTCAAG 3181
 S  R  H  Q  T  F  F  S  Q  T  K  G  V  H  S  P  E  G  L  K   656
GGATTTAAAAAGAAACCAGAGTCAACTAGAGCTTTACTCTTCAAGTCATTGTCATTCAGA 3241
 G  F  K  K  K  P  E  S  T  R  A  L  L  F  K  S  L  S  F  R   676
AAGAAATCTCCAACCAAAGATGATGAAATGAGGCATAGTGATTCATGTGGTCCCAGTGAG 3301
 K  K  S  P  T  K  D  D  E  M  R  H  S  D  S  C  G  P  S  E   696
GAGAGGTTATCACAGGCAGGAACATGTTTTTGTTTTTAAAACTTATTTAAGTTAGGTAAA 3361
 E  R  L                                                       699
ACCACTGAGGAGTAAAGGGATGATTAGGGTCAAGGTATATGAGGGTTGAGAGTCTTCCAC 3421
AGTGGACCATTCCTCACTTCAATTGCAAATATTTTTGAGATACTTAGAGAGTTAAGGTAA 3481
ACTAAAGGGAATCTCTTCAAATGCATTATGAACTGATTACACTTCAACTTGATTTTGAAA 3541
GAATTTATAACAAAGAAAGTTCACTAAGCTTCAGGAAACATAAGTGTTTTGGGTAACATT 3601
GGGGAGGAAGTCCCAGGATGGGTGGGCTCTATGTAGCTTAGGATAATTTTAGCTGCAGTT 3661
GCCAAAAACACCCTCTCCTATCCTCACCTTTCCTTGCCGCACCTCTGCTTGGGACTGACT 3721
GGCCATGTGGGTGCAGTTAAAAAGTACCTTAATTACTGAGGGTTGTAGTATTTAAATGTT 3781
TAGATTATATATAAATAAATGTATTTTCATAGTGTTGTACAAACATTTGAAAGATTTTAT 3841
TGCAAGGGATGAATGACCATGTTATAAAACAAACTAATGTGTGGCAAAAGACCAGTTATA 3901
AAATAGTGAAATATAGATTAGAGCCTAATATTTATTTGTGTAGAAAATGTACAGATATAA 3961
ACTTAGCTTCTTCATGCTCAAAACAAATTTATAATGCCAATGATATAGGAAAGCCCTTCA 4021
AGATTTTATCTGCAAAGTATTTAATAATTTTATATCTAACAGGAAGGTATTCTTTGACTA 4081
AAAGTCAGTGTGTGTGTGTATATATATA                                   4111
```

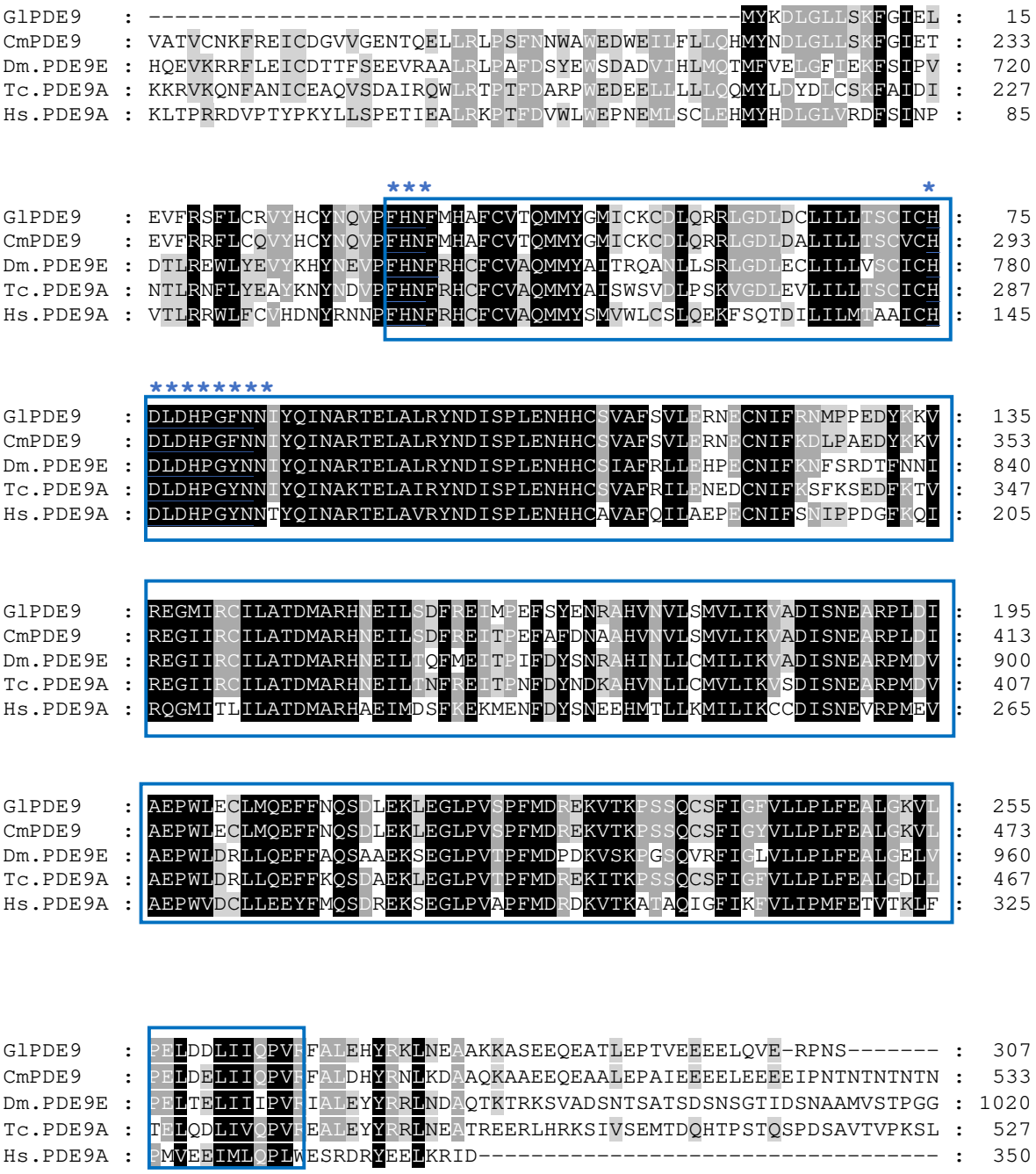
Figure 2.15. Nucleotide and amino acid sequence of cDNA encoding Gl-PDE9. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. The conserved catalytic domain is located within the blue boxes started with the initiating (FHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.

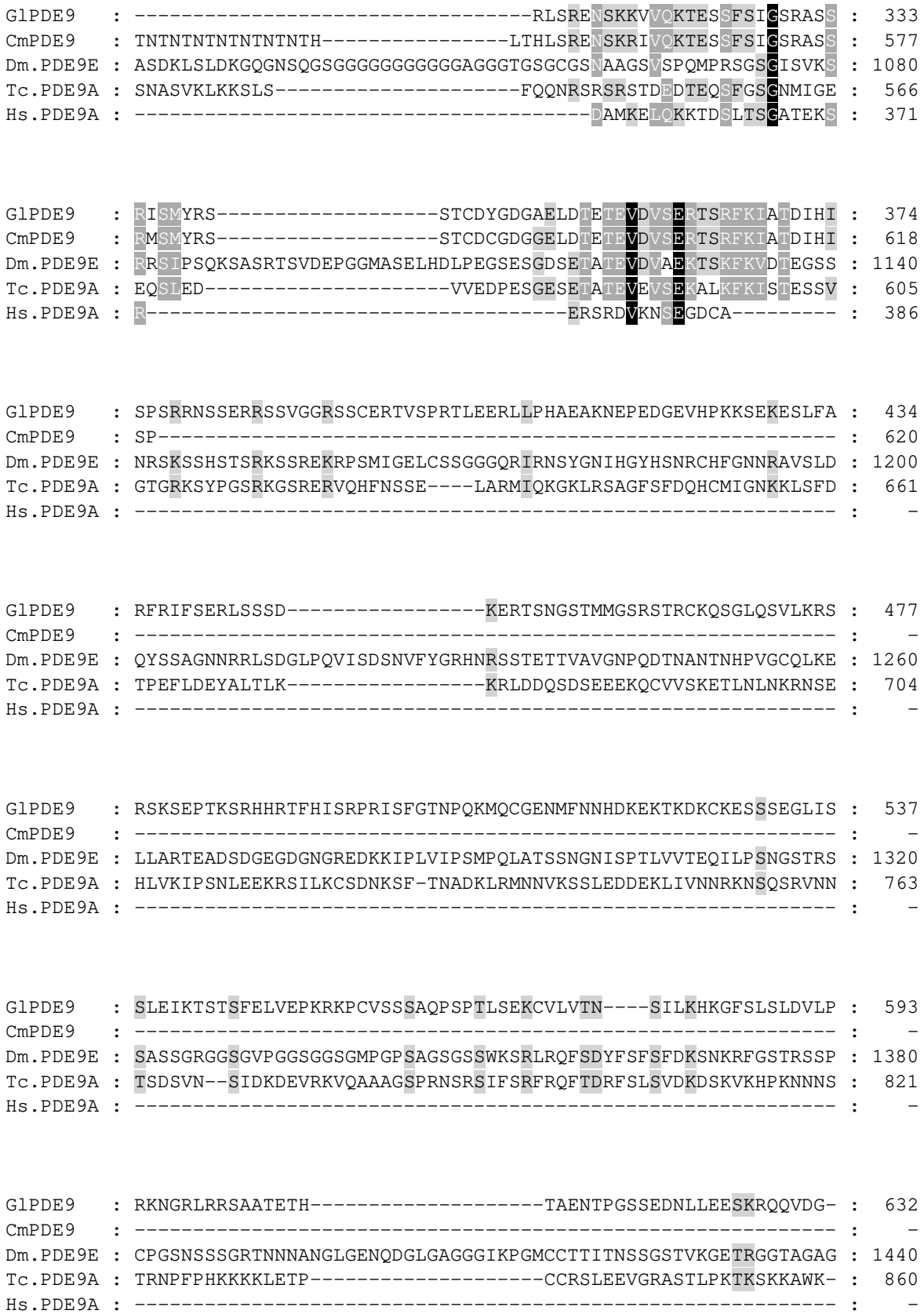
CmPDE9 4356 nucleotides

```
   1 GCTGAGCGGGGCATTGGCCTAACTTTTAACCCGCTAAACCGTCCCTTTCGACTCAGTATT
  61 CAGTGACAGTCATTGGTGACCAGAGTGCAATACGATCGCTCGCCACCGGCAATGAGCGGC
 121 TCCGGCAGTGTTGTAATCGTGTAATAAGGAACAGGATAAAAATACCTAAGTTTGATCTAG
 181 ATGGATAAAGTCTGGGTCAACTGGGAGTACTGTCTTGGGTGACCCATGTACTGTGTGGGC
 241 TGAGGCCGGGGAGCTGCTGCCTCGACTGGCCTCACGAGCACCACGGGGCGGCTCCACACA
 301 GTAACACCAGTGGCACTGATCCAGCCTAGCAGCAAGGCAGCGGCACACCACACAACGGCA
   1                                                   M  E  K  C  V
 361 GCCTCACGTCACGTCACTGCCAAGGTGTCGGAGTGAGGATGCCCGTATGGAGAAGTGCGT
   6  R  T  I  Y  F  T  V  G  G  K  Q  D  S  A  S  L  H  P  D  A
 421 CAGGACCATTTACTTCACTGTGGGAGGCAAACAGGACTCAGCCAGCCTTCACCCTGACGC
  26  R  P  E  D  V  K  E  V  F  R  N  A  C  G  A  G  P  A  D  I
 481 TCGCCCAGAAGATGTCAAAGAGGTGTTCCGTAACGCGTGTGGGGCGGGGCCTGCAGACAT
  46  I  K  L  Y  T  S  E  G  H  V  L  N  A  G  P  H  L  P  A  N
 541 CATCAAGCTGTATACTAGTGAAGGACATGTACTAAACGCAGGGCCTCACCTCCCTGCCAA
  66  T  P  A  N  P  Y  R  L  H  V  A  A  T  P  C  N  G  E  K  L
 601 CACCCCTGCCAACCCCTACCGCCTACACGTCGCCGCCACCCCCTGCAACGGAGAGAAGCT
  86  N  R  L  G  I  D  L  M  D  L  E  Q  R  L  C  E  V  E  R  S
 661 GAACCGTTTGGGGATTGATCTTATGGACCTGGAGCAAAGGCTGTGTGAGGTAGAGCGAAG
 106  V  A  S  L  R  A  D  L  P  P  A  V  E  E  L  R  C  A  V  E
 721 TGTTGCCTCTCTTCGAGCAGACTTGCCTCCAGCGGTCGAGGAACTGAGGTGTGCTGTGGA
 126  T  F  R  M  R  L  E  T  T  E  H  L  S  W  L  G  F  Y  K  K
 781 GACCTTCAGGATGAGGCTGGAGACAACTGAGCATCTGTCCTGGCTGGGGTTCTACAAGAA
 146  G  I  G  E  P  L  G  N  L  G  N  P  H  P  Y  W  K  N  V  E
 841 GGGCATTGGCGAGCCTCTCGGTAACCTGGGCAATCCTCACCCTTACTGGAAGAACGTGGA
 166  Y  R  R  K  A  E  D  E  V  A  T  V  C  N  K  F  R  E  I  C
 901 GTATCGCAGGAAGGCGGAGGACGAGGTGGCTACGGTGTGCAACAAGTTTAGAGAAATATG
 186  D  G  V  V  G  E  N  T  Q  E  L  L  R  L  P  S  F  N  N  W
 961 TGACGGTGTGGTCGGGGAGAACACACAGGAGCTTCTAAGACTGCCCTCCTTCAACAACTG
 206  A  W  E  D  W  E  I  L  F  L  L  Q  H  M  Y  N  D  L  G  L
1021 GGCTTGGGAGGACTGGGAGATTCTGTTCCTCCTCCAGCACATGTACAATGATCTTGGTCT
 226  L  S  K  F  G  I  E  T  E  V  F  R  R  F  L  C  Q  V  Y  H
1081 CCTGTCTAAGTTTGGGATCGAAACGGAGGTGTTCCGAAGGTTCCTGTGTCAAGTGTACCA
 246  C  Y  N  Q  V  P  F  H  N  F  M  H  A  F  C  V  T  Q  M  M
1141 CTGCTATAACCAAGTGCCCTTCCATAACTTCATGCACGCCTTCTGTGTGACCCAGATGAT
 266  Y  G  M  I  C  K  C  D  L  Q  R  R  L  G  D  L  D  A  L  I
1201 GTACGGGATGATCTGCAAGTGTGATCTTCAGAGACGCCTGGGTGACTTAGACGCACTTAT
 286  L  L  T  S  C  V  C  H  D  L  D  H  P  G  F  N  N  I  Y  Q
1261 CCTGTTGACCTCCTGCGTATGCCATGACCTTGACCACCCAGGCTTCAACAATATCTACCA
 306  I  N  A  R  T  E  L  A  L  R  Y  N  D  I  S  P  L  E  N  H
1321 GATCAACGCCAGGACTGAACTCGCCCTGCGCTACAATGACATCTCGCCCCTGGAGAATCA
 326  H  C  S  V  A  F  S  V  L  E  R  N  E  C  N  I  F  K  D  L
1381 TCACTGCTCCGTCGCCTTCAGTGTTCTCGAGCGGAATGAGTGCAATATATTTAAGGATCT
 346  P  A  E  D  Y  K  K  V  R  E  G  I  I  R  C  I  L  A  T  D
1441 GCCGGCCGAGGACTACAAGAAGGTGCGGGAAGGCATCATCCGGTGCATTCTGGCCACGGA
 366  M  A  R  H  N  E  I  L  S  D  F  R  E  I  T  P  E  F  A  F
1501 CATGGCACGTCACAATGAAATCCTCTCAGACTTTAGAGAAATCACACCGGAATTTGCTTT
 386  D  N  A  A  H  V  N  V  L  S  M  V  L  I  K  V  A  D  I  S
1561 TGACAACGCAGCGCATGTCAATGTGCTGTCCATGGTGCTGATCAAGGTGGCTGACATCAG
 406  N  E  A  R  P  L  D  I  A  E  P  W  L  E  C  L  M  Q  E  F
1621 CAACGAGGCACGCCCGCTGGACATCGCTGAGCCGTGGCTGGAGTGTCTCATGCAGGAATT
```

```
1681 CTTCAATCAAAGTGATCTGGAGAAGCTTGAAGGGCTGCCAGTGTCTCCCTTCATGGACAG
 446  E  K  V  T  K  P  S  S  Q  C  S  F  I  G  Y  V  L  L  P  L
1741 AGAGAAAGTGACTAAGCCTTCCTCCCAGTGCTCCTTCATTGGCTATGTGTTGCTGCCTCT
 466  F  E  A  L  G  K  V  L  P  E  L  D  E  L  I  I  Q  P  V  R
1801 CTTTGAAGCTCTCGGCAAGGTCCTCCCTGAGCTAGATGAACTAATCATTCAGCCGGTGAG
 486  F  A  L  D  H  Y  R  N  L  K  D  A  A  Q  K  A  A  E  E  Q
1861 ATTTGCACTGGACCACTACAGGAACTTGAAGGATGCGGCACAAAAGGCAGCAGAGGAGCA
 506  E  A  A  L  E  P  A  I  E  E  E  E  L  E  E  E  E  I  P  N
1921 GGAAGCCGCACTGGAGCCGGCCATTGAAGAGGAGGAGCTGGAGGAGGAGGAGATACCCAA
 526  T  N  T  N  T  N  T  N  T  N  T  N  T  N  T  N  T  N  T  N
1981 CACCAACACCAACACCAACACCAACACCAACACCAACACCAACACCAACACTAACACTAA
 546  T  N  T  H  L  T  H  L  S  R  E  N  S  K  R  I  V  Q  K  T
2041 CACTAACACTCACCTCACTCACCTGTCTCGGGAAAACTCCAAACGGATCGTGCAGAAAAC
 566  E  S  S  F  S  I  G  S  R  A  S  S  R  M  S  M  Y  R  S  S
2101 AGAAAGTTCCTTCAGCATCGGGAGCCGAGCATCATCCCGGATGTCCATGTATCGTTCCAG
 586  T  C  D  C  G  D  G  G  E  L  D  T  E  T  E  V  D  V  S  E
2161 CACCTGTGACTGCGGAGACGGTGGAGAACTGGACACAGAGACGGAAGTGGATGTGAGTGA
 606  R  T  S  R  F  K  I  A  T  D  I  H  I  S  P
2221 AAGAACGTCGAGATTTAAAATTGCGACAGACATTCACATATCCCCATAGCGGAGGAACAG
2281 CTCTGAGCGACGGAGTAGTGTGGGCGGGCGGTCCAGCTGTGAGCGAACTGTGTCTCCGAG
2341 AACCCTGGAGGAGCGGCTTCTCCCACACGCTGAAGCCAAGAATGAACCTGAAGATGGTGA
2401 AGTGCATCCAAAGAAAAGTGAAAAGGAGTCTTTGTTTGCTCGGCTTAGAGAACGTCTCTC
2461 TTCCAGTGACAAGGAGAGAACTTCCAACGGATCGACCTTTGTGGGCAGTCGAAGCACGAG
2521 GTGTAAACAAGGTGGTCTGCAGAGTGTTCTCAAACGTAGTAGGAGTAAATCAGAGCCCAC
2581 TAAGAGTAGACATCACCGAACATTTCATATATCGCGGCCAAGAATTAGCTTTGGTGCAAA
2641 TCCCCAGAAATCGCAGTGTGGTGAAAATATGTTTAATAACCATGAAAAAGAGAAGGCACG
2701 AGACAAGTGCAAGGAATCTTCTTCTGAGGGTCTTATCTCTGCATTAGAAATAAAAACTTC
2761 TACCTCCTTCGAACTTATTGAACAAAAAAAGCCTTGTGTGTCATCTAGTGCTCAACCTTC
2821 ACCAACACTCTCAGAAAAGTGTGTATTAGTTACAAACAATATCCTAAAGCACAAAGGTTT
2881 TAGTTTATCTCTCGACGTATTGCCGAGAAAGAACGGACGACTGAAGAGATCTGAAGCCAC
2941 CGAAACCAACACAACAGAAAATACTCCTGGTGGCTCCGAGGACAACCTTATAGAGGGGAC
3001 CAAGAAGCAGCAGGTGGACGGAGCTGTCACACTAATACGACACCAACCAATGCTTAGCCA
3061 TACCAAAGGAGTTCAGTCATCTGATGCACTAAAGGGATTGAAAAAAAAGCCAGAGTCAAC
3121 CAGGGCTTTACTTTTTAAATCCCTATCCTTCAGAAAGAAATCTCCCACTAAGGAGGACGA
3181 GGTGAGACCGGGTGATTCCTGTGGTCCCAGTGAAGAAAGATTATGACAGGCAGGAACCTG
3241 TTTCTGTTTTTAAGGATTATTTAACGGTTAGGTAGAACCACTGGGAAGATGAAGGATGAT
3301 GCCGGTCACATTGTATGAAGGGTGAGAGAGTCTTCCACAGTGGATCATTCTTCGTTCCAA
3361 TTGCAAATATTTTTCTGAGATACATAAAATGTTTAGTAAAACTCTTAGCAATCATCCCTA
3421 CATCAAAAGACAAACTAGTCAACACTTGTACTGTAGCGAGTGCAGGTATAAACTTCCCAC
3481 AAATTATCTTGGCACTTAATATTCATGAAGGATCTGACGACTAAGGTGCAATACTTTACA
3541 CCTCCATGGCAAGGGATGAATGCCCACCCTAAGCACCCTTATGGCTCGTCATTTATCATG
3601 CCGCTGCTATTAGTGTCATGGGTCCTCTGCAGCATCATATAAATACTGAGGTATTGATCT
3661 AAAGGCTTGATACACTCTGAGTTGTGAGAGGCACTCTAGATGGAAGAATAGAACCCCTGA
3721 CAGGGAAGTATAAAGCACAGCACCTTAAACAGCAGGACCATAATGATCATGAGGTGCCAA
3781 GATCATATTTGGAATCTTTATACCTGTAGTTACTACAGCACTATGACTTGACATGTACTG
3841 TATCCCTTGCAAAGAGTGTTTACTTACCTTCAGGAACAATATACATACATAGATGTTTAG
3901 GATGACAGTGGGGAATAAGTTCCAGGGTGGGTGGGCACTCACTCCTGTATCTTGAGGTCC
3961 ATTGTGTTGCCTCTGCCAGAACACCTTCACTAATCCTCACCTCACCTTGTCTTGCCACAA
4021 CTCTACCAGCGACTCACTCACCAGGTGCAGTGTAGTTATTTTTTAAAAGTACCTTAATTA
4081 CTGAGGGTTGTAATCTTCAAATGCCTAGATTATATATAAACATATAAAAATATGTATTTT
4141 CATAGTATTTTACAAATAATTAAAAGATTTTATTACAAGGGATGAATGACCATGCTATTA
4201 AACATATTACACTATGTTTGCCATGTGACCCCATCTTTAAAATAGTACATCTATAGATTA
4261 GAGGAAAACTATTTATTTGCATAGAAAGATGTACAGCTATAAATTTAGCTTCTTCATGCT
4321 CAAACCAAATTTTTAGTGCCAATTATACTATAGGGA
```

Figure 2.16. Nucleotide and amino acid sequence of cDNA encoding Cm-PDE9. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow and the stop codon highlighted in green. The conserved catalytic domain is located within the blue boxes started with the initiating (FHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.



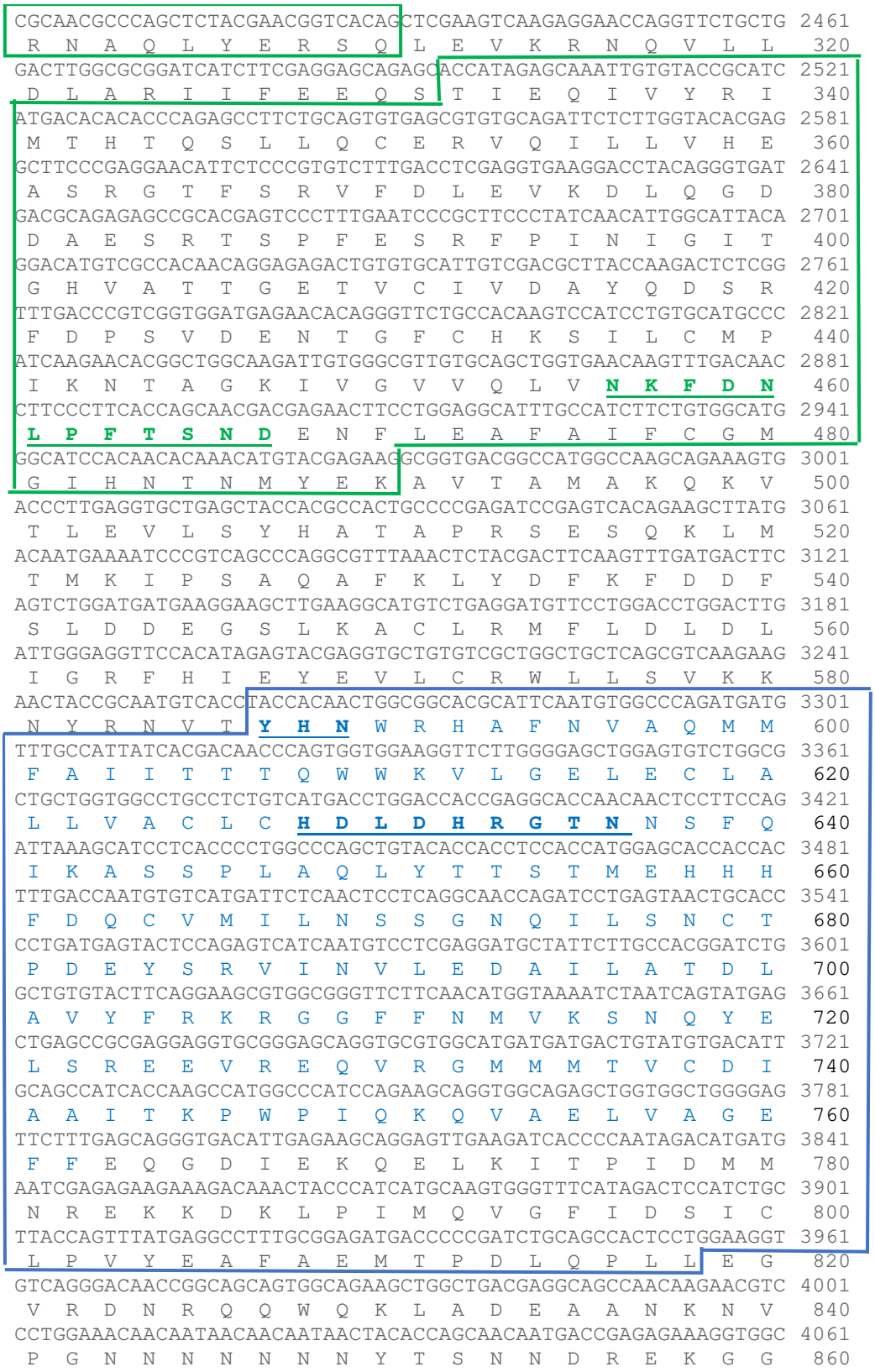

GlPDE9 : ----------RLSRENSKKVVQKTESSFSIGSRASS : 333
CmPDE9 : TNTNTNTNTNTNTNTH----------LTHLSRENSKRIVQKTESSFSIGSRASS : 577
Dm.PDE9E : ASDKLSLDKGQGNSQGSGGGGGGGGGGGAGGGTGSGCGSNAAGSVSPQMPRSGSGISVKS : 1080
Tc.PDE9A : SNASVKLKKSLS----------FQQNRSRSRSTDEDTEQSFGSGNMIGE : 566
Hs.PDE9A : ----------DAMKELQKKTDSLTSGATEKS : 371
GlPDE9 : RISMYRS----------STCDYGDGAELDTETEVDVSERTSRFKIATDIHI : 374
CmPDE9 : RMSMYRS----------STCDCGDGGELDTETEVDVSERTSRFKIATDIHI : 618
Dm.PDE9E : RRSIPSQKSASRTSVDEPGGMASELHDLPEGSESGDSETATEVDVAEKTSKFKVDIEGSS : 1140
Tc.PDE9A : EQSIED----------VVEDPESGESETATEVEVSEKALKFKISTESSV : 605
Hs.PDE9A : R----------ERSRDVKNSEGDCA--------- : 386
GlPDE9 : SPSRRNSSERRSSVGGRSSCERTVSPRTLEERLLPHAEAKNEPEDGEVHPKKSEKESLFA : 434
CmPDE9 : SP---------- : 620
Dm.PDE9E : NRSKSSHSTSRKSSREKRPSMIGELCSSGGGQRIRNSYGNIHGYHSNRCHFGNNRAVSLD : 1200
Tc.PDE9A : GTGRKSYPGSRKGSRERVQHFNSSE----LARMIQKGKLRSAGFSFDQHCMIGNKKLSFD : 661
Hs.PDE9A : ---------- : -
GlPDE9 : RFRIFSERLSSSD----------KERTSNGSTMMGSRSTRCKQSGLQSVLKRS : 477
CmPDE9 : ---------- : -
Dm.PDE9E : QYSSAGNNRRLSDGLPQVISDSNVFYGRHNRSSTETTVAVGNPQDTNANTNHPVGCQLKE : 1260
Tc.PDE9A : TPEFLDEYALTLK----------KRLDDQSDSEEEKQCVVSKETLNLNKRNSE : 704
Hs.PDE9A : ---------- : -
GlPDE9 : RSKSEPTKSRHHRTFHISRPRISFGTNPQKMQCGENMFNNHDKEKTKDKCKESSSEGLIS : 537
CmPDE9 : ---------- : -
Dm.PDE9E : LLARTEADSDGEGDGNGREDKKIPLVIPSMPQLATSSNGNISPTLVVTEQILPSNGSTRS : 1320
Tc.PDE9A : HLVKIPSNLEEKRSILKCSDNKSF-TNADKLRMNNVKSSLEDDEKLIVNNRKNSQSRVNN : 763
Hs.PDE9A : ---------- : -
GlPDE9 : SLEIKTSTSFELVEPKRKPCVSSSAQPSPTLSEKCVLVTN----SILKHKGFSLSLDVLP : 593
CmPDE9 : ---------- : -
Dm.PDE9E : SASSGRGGSGVPGGSGGSGMPGPSAGSGSSWKSRLRQFSDYFSFSFDKSNKRFGSTRSSP : 1380
Tc.PDE9A : TSDSVN--SIDKDEVRKVQAAAGSPRNSRSIFSRFRQFTDRFSLSVDKDSKVKHPKNNNS : 821
Hs.PDE9A : ---------- : -
GlPDE9 : RKNGRLRRSAATETH----------TAENTPGSSEDNLLEESKRQQVDG- : 632
CmPDE9 : ---------- : -
Dm.PDE9E : CPGSNSSSGRTNNNANGLGENQDGLGAGGGIKPGMCCTTITNSSGSTVKGETRGGTAGAG : 1440
Tc.PDE9A : TRNPFPHKKKKLETP----------CCRSLEEVGRASTLPKTKSKKAWK- : 860
Hs.PDE9A : ---------- : -

```
GlPDE9   : -------AARFSRHQTFFSQTKGVHSPEGLKGFKKKPESTRALLFKSLSFRKKSPTKDDE :  685
CmPDE9   : ------------------------------------------------------------ :    -
Dm.PDE9E : GGALTTMTTGNDAHQRHRAYSLDVPGMMRYSSNDSSRHPSNNTLQSAGGGAGLTTGLEVT : 1500
Tc.PDE9A : -------FLVLGKEKDKWASDAAVDKLAVVETDNQSLPSTSQLQSNCASPKTHLEKLEIV :  913
Hs.PDE9A : ------------------------------------------------------------ :    -


GlPDE9   : MRHSDSCGPSEERL------------ :  699
CmPDE9   : ------------------------- :    -
Dm.PDE9E : AQRVPPSLSVEMGLASGSSSEAGPKI : 1526
Tc.PDE9A : SLKEFKVEETVEKTGVEKGEEGGVI- :  938
Hs.PDE9A : ------------------------- :    -
```

Figure 2.17. Multiple alignment of deduced amino acid sequences of PDE9 proteins in two crustacean species, two insect species, and one mammal species. Abbreviations: Gl: *G lateralis*; Cm: *C maenas*; Dm: *D melanogaster*; Tc: *T castaneum*; Hs: *H sapiens*. Black shading indicates that amino acid residues that are identical or similar in all sequences; gray shading indicates identical or similar amino acids in most of the sequences. Dashes indicate gaps introduced to optimize the alignment and the boxes indicate highly conserved domains. The colors of the boxes correspond to the colors of the domains in Fig. 2. 15 & 2.16.

GlPDE11 5752 nucleotides, structure: sequence C

```
GGGAGGGCAGAGGCTGTGCTGGGCTTGGCTGAGGCGCAGTGTGTGTTTCGTAGCTTCACG   61
GGCGGCTGTACGTACGCGTGGCAGACAAACTTGACTTCACGCAGCATCGAGGCAGAGAAA  121
GAATAGAAGAAAATGTCTCAACAGGTTCGTCTTCCCTTCAAAGGAATGATGGCGCCCACC  181
TTTACGCCATTCAAGGCTGATGGGTCCCTCAATCTGGAACTGGTGAAGCCGTACGCAGCA  241
CACCTGAAGGCTTCTGGTGTGAAGGGGGTGTGGGTGAACGGCACGGCCGGCGAGGGCATG  301
TCGCAGACGGTGCTGGAGCGCAAGGCGGTGGCGGAAGCGTGGCTGGCGTGCCGCGGTGAC  361
GTGCCGACAGTGATCGTGCATTGCGGCGCGGGATGCCTCAAGGATACACAGGACCTGGCT  421
CGTCACGCGGAGGAGAAAGGGGCTGATGGAGTGGCCGTGTTGCCCCTCCTCTTCGACCCC  481
CCCAAAACCCCCGACGATCTGGTGGACTACATGGTGGAGGTGGCTAAGGCGTGCCCCTCC  541
AGCCCCCTCTTCTACTACCACATCCCCATGAAGACCGGCGTGAAGTTGTCGATGAGCGAG  601
TTCCTGGAGAAGGGCGTGAAGCGTATACCCACGTTGGCGGGGGTCAAGTTTACCGACGGG  661
GACGTGAGCGGCGAGGGCAGGAAGTGTCTGAAGGTGAACGGCGGCAACCTGACCGTATTT  721
AATGGCTTTGACCAGAGCCTTCAGGAGGCTCTCAGCTTGGGCTTCAACTGTGGCGTCAAC  781
TCGAGCTTCACCTTCCTGCCTCACCTGGCTGGCCGCATCTTCACTCTGATGGAGGCCGGT  841
GACAAGGACGAGGCGGTGAAGGTGCAGCAGAGACTCAGCAGCTGCTTCGACGTCATCTAC  901
AGGCAGAGCGGCGGCATTTTCAGCTCGGCCTGCATGAAGGCAGCGTGCAGCCTCCTGACA  961
GGGCTGCAGTTTGGGCCCACACGCCTCCCCGTGAAGCCCCTGACGGAGGAGATGACGGCC 1021
ACGCTGAAGAGAGACCTTGAGAAACACGGCCTCAAGGTGTACTGAAGGGCGAACGGGAAC 1081
AGCAGAAGGAGGAGAAAGGGAAGGGGAGGAGGAAGAGACGGAGGAGGTTGAAGAGGAAGG 1141
GATGGAAGATAAGGGGGAGGAAGGAATAGAGGAGGAGATGGAAGGGTAAAGGCGAAATTG 1201
GGAGCTGATGTAAGTGGAGGAGAAGGAAGACAAGAGAGGGGAAACAAACGAGGAATATAA 1261
GGAAAAAGAGAGTAAGCTGAAAATTATGCTTAGAAGAGGGAACAGCGTAGAGGGGGGAAG 1321
ACATGAGGGAGTTGGTGACGATGGTGCTCGGTAGGAGGAAGAGGAGGAGGGGGAGGAGGA 1381
GGAGGAGGAGGAGGGGGTGGGGGGGAGCCTCCTGACCCTGCCGAGCTGCGAGGGGGAGGA 1441
GGGGGCCCTCTCCCCCTACACACGCCCCCCCAATACAACTACGACCCGGAGTGTGCCAGA 1501
ATGGAGGCGTGGCTGGACGACCATCAAGATTTCGTGTACGACTACTTCATCAGGAAGGCG 1561
 M  E  A  W  L  D  D  H  Q  D  F  V  Y  D  Y  F  I  R  K  A   20
TCTAGGCACATGGTGGACTCTTGGTTGCTCTCCCATGCCCTGCCTCAGAGCCTCGGGATG 1621
 S  R  H  M  V  D  S  W  L  L  S  H  A  L  P  Q  S  L  G  M   40
GGCGCCGCGGGGTACTGCGGGGCGGGGCCTGAGGCGGGGGCCGGGGCGGGTCTGGCCACC 1681
 G  A  A  G  Y  C  G  A  G  P  E  A  G  A  G  A  G  L  A  T   60
ACCCCGGGACAGCATCAAAACTCCAAGGCGTCGTCTGGAGCTGCCACGCCAGTTCGCAAG 1741
 T  P  G  Q  H  Q  N  S  K  A  S  S  G  A  A  T  P  V  R  K   80
ATTTCAGCCCACGAGTTCGAGAAGGGCGGCCTCCTCAAGCCCATCGTGACCACTATCGAC 1801
 I  S  A  H  E  F  E  K  G  G  L  L  K  P  I  V  T  T  I  D  100
GGGACGCCCACCTTCATATCTCCTGCCGGGGCTGCCGAGAACGTGGCGATACTGGCGAAG 1861
 G  T  P  T  F  I  S  P  A  G  A  A  E  N  V  A  I  L  A  K  120
GTTCGTCGCAAGTCCCGCACGGAGCTCAAAGGCCTCGACGAGCGGCAATTAATCTTCGAA 1921
 V  R  R  K  S  R  T  E  L  K  G  L  D  E  R  Q  L  I  F  E  140
CTCGTGAAGGACATTTGCAACGAGCTCGATGTCCGCCCCCTGTGCCACAAGATCCTGCAG 1981
 L  V  K  D  I  C  N  E  L  D  V  R  P  L  C  H  K  I  L  Q  160
AACGTGTCCATCCTTACCAGCGCTGACAGATGCTCGCTTTTCCTAGTACAAGGGGACAAG 2041
 N  V  S  I  L  T  S  A  D  R  C  S  L  F  L  V  Q  G  D  K  180
GAGACGGAGAACCGCTGCCTCGTCTCCACGCTCTTCGACGTGAACCCAGACTCGACGGTG 2101
 E  T  E  N  R  C  L  V  S  T  L  F  D  V  N  P  D  S  T  V  200
GAGGAGATGGAGGAGAAGGAAGAGATCAGGATAGCGTGGGGTAGTGGCATTGTGGGCTAC 2161
 E  E  M  E  E  K  E  E  I  R  I  A  W  G  S  G  I  V  G  Y  220
ACGGCGCAGAGTGGAGCAATGGTTAACATCCCTGACGCCTATGCTGATGACCGCTTCAAC 2221
 T  A  Q  S  G  A  M  V  N  I  P  D  A  Y  A  D  D  R  F  N  240
TCTGAGATCGACTGCATGACGGGATACAAGACGCGTTCGATGCTGTGTATGCCCATCAAG 2281
 S  E  I  D  C  M  T  G  Y  K  T  R  S  M  L  C  M  P  I  K  260
GACAGTAACGGGGAGGTGATCGGCGTGGCGCAGGTTATCAACAAGCACCAGGGTCAGTCC 2341
 D  S  N  G  E  V  I  G  V  A  Q  V  I  N  K  H  Q  G  Q  S  280
TTCACGACGGCTGACGAGAAGGTGTTTGAGTCCTACCTCCAGTTCTGCGGCATTGGTCTC 2401
 F  T  T  A  D  E  K  V  F  E  S  Y  L  Q  F  C  G  I  G  L  300
```


CGCAACGCCCAGCTCTACGAACGGTCACAGCTCGAAGTCAAGAGGAACCAGGTTCTGCTG 2461
R N A Q L Y E R S Q L E V K R N Q V L L 320
GACTTGGCGCGGATCATCTTCGAGGAGCAGAGCACCATAGAGCAAATTGTGTACCGCATC 2521
D L A R I I F E E Q S T I E Q I V Y R I 340
ATGACACACACCCAGAGCCTTCTGCAGTGTGAGCGTGTGCAGATTCTCTTGGTACACGAG 2581
M T H T Q S L L Q C E R V Q I L L V H E 360
GCTTCCCGAGGAACATTCTCCCGTGTCTTTGACCTCGAGGTGAAGGACCTACAGGGTGAT 2641
A S R G T F S R V F D L E V K D L Q G D 380
GACGCAGAGAGCCGCACGAGTCCCTTTGAATCCCGCTTCCCTATCAACATTGGCATTACA 2701
D A E S R T S P F E S R F P I N I G I T 400
GGACATGTCGCCACAACAGGAGAGACTGTGTGCATTGTCGACGCTTACCAAGACTCTCGG 2761
G H V A T T G E T V C I V D A Y Q D S R 420
TTTGACCCGTCGGTGGATGAGAACACAGGGTTCTGCCACAAGTCCATCCTGTGCATGCCC 2821
F D P S V D E N T G F C H K S I L C M P 440
ATCAAGAACACGGCTGGCAAGATTGTGGGCGTTGTGCAGCTGGTGAACAAGTTTGACAAC 2881
I K N T A G K I V G V V Q L V N K F D N 460
CTTCCCTTCACCAGCAACGACGAGAACTTCCTGGAGGCATTTGCCATCTTCTGTGGCATG 2941
L P F T S N D E N F L E A F A I F C G M 480
GGCATCCACAACACAAACATGTACGAGAAGGCGGTGACGGCCATGGCCAAGCAGAAAGTG 3001
G I H N T N M Y E K A V T A M A K Q K V 500
ACCCTTGAGGTGCTGAGCTACCACGCCACTGCCCCGAGATCCGAGTCACAGAAGCTTATG 3061
T L E V L S Y H A T A P R S E S Q K L M 520
ACAATGAAAATCCCGTCAGCCCAGGCGTTTAAACTCTACGACTTCAAGTTTGATGACTTC 3121
T M K I P S A Q A F K L Y D F K F D D F 540
AGTCTGGATGATGAAGGAAGCTTGAAGGCATGTCTGAGGATGTTCCTGGACCTGGACTTG 3181
S L D D E G S L K A C L R M F L D L D L 560
ATTGGGAGGTTCCACATAGAGTACGAGGTGCTGTGTCGCTGGCTGCTCAGCGTCAAGAAG 3241
I G R F H I E Y E V L C R W L L S V K K 580
AACTACCGCAATGTCACCTACCACAACTGGCGGCACGCATTCAATGTGGCCCAGATGATG 3301
N Y R N V T Y H N W R H A F N V A Q M M 600
TTTGCCATTATCACGACAACCCAGTGGTGGAAGGTTCTTGGGGAGCTGGAGTGTCTGGCG 3361
F A I I T T T Q W W K V L G E L E C L A 620
CTGCTGGTGGCCTGCCTCTGTCATGACCTGGACCACCGAGGCACCAACAACTCCTTCCAG 3421
L L V A C L C H D L D H R G T N N S F Q 640
ATTAAAGCATCCTCACCCCTGGCCCAGCTGTACACCACCTCCACCATGGAGCACCACCAC 3481
I K A S S P L A Q L Y T T S T M E H H H 660
TTTGACCAATGTGTCATGATTCTCAACTCCTCAGGCAACCAGATCCTGAGTAACTGCACC 3541
F D Q C V M I L N S S G N Q I L S N C T 680
CCTGATGAGTACTCCAGAGTCATCAATGTCCTCGAGGATGCTATTCTTGCCACGGATCTG 3601
P D E Y S R V I N V L E D A I L A T D L 700
GCTGTGTACTTCAGGAAGCGTGGCGGGTTCTTCAACATGGTAAAATCTAATCAGTATGAG 3661
A V Y F R K R G G F F N M V K S N Q Y E 720
CTGAGCCGCGAGGAGGTGCGGGAGCAGGTGCGTGGCATGATGATGACTGTATGTGACATT 3721
L S R E E V R E Q V R G M M M T V C D I 740
GCAGCCATCACCAAGCCATGGCCCATCCAGAAGCAGGTGGCAGAGCTGGTGGCTGGGGAG 3781
A A I T K P W P I Q K Q V A E L V A G E 760
TTCTTTGAGCAGGGTGACATTGAGAAGCAGGAGTTGAAGATCACCCCAATAGACATGATG 3841
F F E Q G D I E K Q E L K I T P I D M M 780
AATCGAGAGAAGAAAGACAAACTACCCATCATGCAAGTGGGTTTCATAGACTCCATCTGC 3901
N R E K K D K L P I M Q V G F I D S I C 800
TTACCAGTTTATGAGGCCTTTGCGGAGATGACCCCCGATCTGCAGCCACTCCTGGAAGGT 3961
L P V Y E A F A E M T P D L Q P L L E G 820
GTCAGGGACAACCGGCAGCAGTGGCAGAAGCTGGCTGACGAGGCAGCCAACAAGAACGTC 4001
V R D N R Q Q W Q K L A D E A A N K N V 840
CCTGGAAACAACAATAACAACAATAACTACACCAGCAACAATGACCGAGAGAAAGGTGGC 4061
P G N N N N N N N Y T S N N D R E K G G 860

```
GGGACAAACAATTGAGTGTGTGTGCTGATATGCCAAGTGCCCTTGCCAACCACTGCACAA 4121
 G  T  N  N  -                                                  864
AGTGATGCCGTGGACACAAGTAGAGGAAGTGGTGGATTGTAAGCAAGGTTTTGTGCCGTG 4181
GCTGTGTGTTTCAGTTGGACCCAAGGAGTTCGGGCACAAGTGATGGACTGAAGTGAAGTA 4241
GAATGTGTGAGTGTTCAGTGTCTTGATAATGTACATTGTGGCCTTGACCACCACTCTAAG 4301
TTCTGCTTCTCCTGTGTTGACCTTCTGTACTCGTGTGTGAACAGTTCAAAGGCTATTAAT 4361
AGTATATGTTTTGTACTTCTGACTCTATAAGGTTCATAGATATGTTTAATCCGTGAAATG 4421
AACTTCTGTGCTACATATAAGTGTGAGAAAATATGTATTATCACTTGGTTGTATGACAGA 4481
TATATCTTTCATTTTGGCATAATAATAATATTTAATGCTAGGGTGCTCCCCTGCTGGAGT 4541
AGTGAATGTTTTTATTCAGGAATTTCATCAGGAATGTGCAATATTCAGATAGCATGTATG 4601
GGTCATTATTTATTTGATAGGGACATCTGTTCATCTTCCTTTTGTGTTCATTCAGATTTC 4661
AGCAAGTGGGCCCAAGGGACGCTAAAGGCCTCAATGGGGGTAAAGGGAGGGAGGGGGGCT 4721
GTACTGATGGACCTCCACAGTTCATTCATCTAATAAATAACTGGATACTTCTGTCCTCTC 4781
TTGAGTTGCCTCTTTCCACCCTGCCTCTGCCTCTCCTAACAAGTGAATCATGTCTAGCCA 4841
GGAACACCGAACTTGACACACATTAACATGTTCCCAGTCAAGGCTTGATGCCATATTATA 4901
TAAGCAATCCCCAGGTTATCAATGAGTTTCGCTCCTAAACACAGATGTAAGTTAGATTTG 4961
TCTAAGTCAGAAAGGCAGAAGTGTACTGTTTTACTGTGCCTCAGTAAGCAGTGCAGTGGT 5021
CTGTTACCACAAACCATTGTCTCCAATTAAGATTTTTCAAAATGAAGTCTGTGTATGGGC 5081
TGATCTAGGTTGAAACATTCTTGACCCAGGCACTTACTGTGTTGCAGCAAAGGAGTCTTA 5141
GTGATATGTTCTTTACATCTTTGCATTTTCTTTATACAATAAATTGGATCACACTTTAAA 5201
AATGCATGAAATTATGGTCAAGAGCATACACATAACCAAGTGAATAGTACCATCTCAGTT 5261
GGAATTTAAAAAGTAAGGACAGTGCCACCAGTAATGAAAGTTGAAGTAAAATGGCAAATA 5321
GGCTATTTAGTCACTTAGAAGATACAACTTTACAATTACTTAATTCTACACTGCTTAAAA 5381
TCTCTACTAGGTGTAGTTTTTGTCCTGCCTATCTTATGATCCGGTACTTTATCACTCACC 5441
ACCCTCCATTCTCTTGTGAAAGCTGCAGCAAGCAAGACACCCAGCCAGCAGATCAAAGGG 5501
AGAGCAGCAAAGCCCCTCACACCATCACCACCTTGGGCCCTGTAACAAAAGACACCACAG 5561
TTAATACTGACAAACATACAACTCCCATAGAACCACAAGGCCACAAGTGTCAATTTAAAA 5621
AAAGAAAACTACTGTTAAAGTTTGGCTCATCTAAACGTCCTGTTATGTAATGCAGTTCTG 5681
GACTGTTAGATAATCATAATGCTTCAAGTCACACCTCCACAAGACAGAGGAC          5752
```

Figure 2.18. Nucleotide and amino acid sequence of cDNA encoding Gl-PDE11. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow, the stop codon highlighted in green. Two GAF regulatory domains; GAF-A &GAF-B are indicated by green boxes and contains the (NKxxFDxxE) signature sequence found in all mammalian GAF domains, the sequence is underlined and in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.

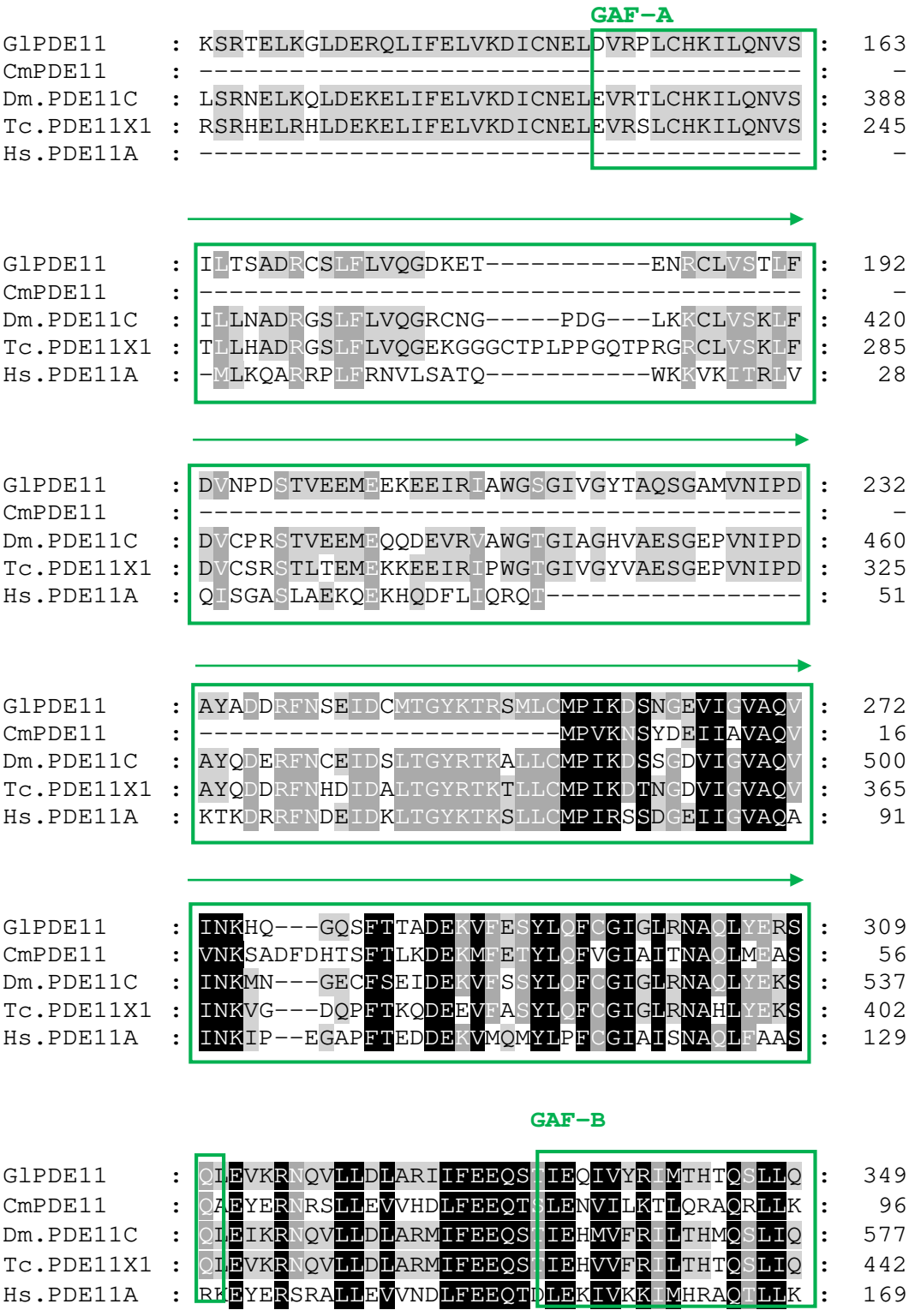
CmPDE11 5839 nucleotides

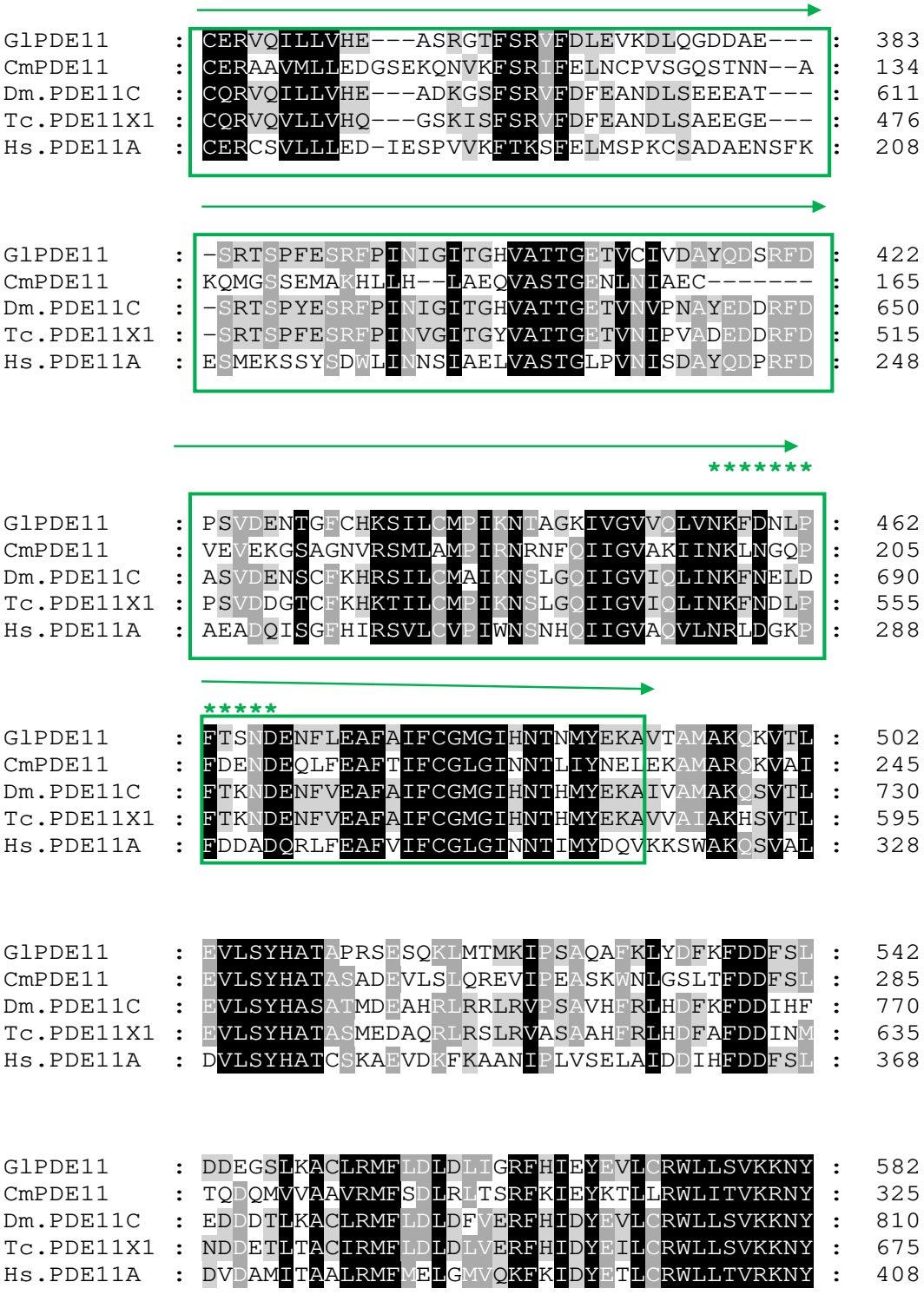
```
   1 GCTGGGGGAGCCACGGCGATGAGACCAAGAACAGCCCGGGGTCAGGTGAGATCAGTGGAG
  61 GGGGCGGTGGGGAGCCTCCAGACCCTTCAGAAGTGCGTGGGGGGGCGCCTCTCCACTCCC
 121 CGCCTCAGTACAACTACGACCCGGAGTGTGCCCGTGTGGAGGCGTGGCTCGACGACCACC
   1                                                 M  V  D  S  W  L
 181 AGGACTTTGTGTACGACTACTTCATCAGGAAGGCGTCTCGACATATGGTGGACTCGTGGC
   7   L  S  H  A  L  P  Q  S  L  G  V  G  A  G  A  Y  C  G  A  G
 241 TTCTCTCCCACGCCCTCCCGCAGAGCCTGGGCGTGGGTGCCGGGGCGTACTGCGGGGCGG
  27   P  G  E  A  G  A  G  P  G  G  I  G  T  A  G  H  Q  N  S  K
 301 GGCCGGGCGAGGCGGGGGCCGGGCCAGGAGGCATTGGCACCGCGGGGCATCAGAACTCCA
  47   A  S  S  G  A  A  T  P  V  R  K  I  S  A  H  E  F  E  K  G
 361 AAGCTTCTTCAGGAGCGGCCACCCCAGTTCGTAAGATTTCGGCGCACGAGTTCGAGAAGG
  67   G  L  L  K  P  I  I  T  T  I  D  G  T  P  T  F  I  S  P  A
 421 GAGGTCTTCTGAAGCCCATCATCACTACCATAGACGGCACGCCCACCTTCATCTCCCCCG
  87   G  A  A  E  N  V  A  I  L  A  K  V  R  R  K  S  R  T  E  L
 481 CTGGCGCCGCTGAGAACGTTGCTATTCTGGCCAAGGTGCGCCGCAAGTCCCGGACGGAGC
 107   K  G  L  D  E  R  Q  L  I  F  E  L  V  K  D  I  C  N  D  L
 541 TCAAGGGATTAGACGAGCGGCAACTTATCTTCGAGCTGGTGAAGGACATCTGCAATGACC
 127   D  V  R  S  L  C  H  K  I  L  Q  N  V  S  I  L  T  N  A  D
 601 TGGATGTGCGTTCCCTGTGCCACAAGATCCTGCAGAACGTGTCCATCCTTACCAATGCCG
 147   R  C  S  L  F  L  V  Q  G  D  K  E  S  D  N  R  C  L  V  S
 661 ACCGTTGCTCACTCTTCCTCGTGCAGGGTGACAAGGAGTCAGACAACCGGTGCCTCGTGT
 167   T  L  F  D  V  N  P  D  S  T  V  E  E  M  E  E  K  E  E  I
 721 CCACGCTGTTTGACGTGAACCCAGACTCGACGGTGGAGGAGATGGAGGAGAAGGAGGAGA
 187   R  I  A  W  G  S  G  I  V  G  Y  T  A  Q  S  G  A  M  L  N
 781 TCAGGATAGCGTGGGGCAGTGGCATTGTGGGTTATACTGCCCAGAGCGGCGCTATGTTGA
 207   I  P  D  A  Y  E  D  D  R  F  N  S  E  I  D  C  M  T  G  Y
 841 ATATTCCTGATGCTTATGAGGATGATCGCTTCAACTCCGAGATTGACTGCATGACAGGCT
 227   K  T  R  S  M  L  C  M  P  I  K  D  S  C  G  E  V  I  G  V
 901 ACAAGACGCGCTCTATGCTGTGTATGCCCATAAAGGACAGTTGTGGAGAGGTGATTGGTGc
 247   A  Q  V  I  N  K  H  Q  G  Q  S  F  T  N  A  D  E  K  V  F
 961 TGGCACAGGTTATCAACAAGCATCAGGGTCAGTCTTTTACCAATGCCGATGAGAAGGTGT
 267   E  S  Y  L  Q  F  C  G  I  G  L  R  N  A  Q  L  Y  E  R  S
1021 TTGAATCCTACCTCCAGTTTTGTGGCATTGGCCTCCGCAATGCTCAGCTGTATGAACGCT
 287   Q  L  E  V  K  R  N  Q  V  L  L  D  L  A  R  I  I  F  E  E
1081 CCCAACTTGAAGTGAAAAGAAATCAGGTTCTTCTGGACTTGGCGAGGATCATCTTTGAAG
 307   Q  S  T  I  E  Q  I  V  Y  R  I  M  T  H  T  Q  S  L  L  Q
1141 AGCAGAGCACTATAGAGCAAATTGTGTACCGCATTATGACACACACCCAGAGCCTCTTGC
 327   C  E  R  V  Q  I  L  L  V  H  E  A  S  R  G  T  F  S  R  V
1201 AATGTGAACGGGTACAGATTCTCTTAGTACATGAAGCGTCTCGGGGAACATTCTCACGTG
 347   F  D  L  E  V  K  D  L  Q  G  D  D  A  E  S  R  T  S  P  F
1261 TGTTTGACTTGGAAGTGAAGGATCTGCAAGGAGATGATGCAGAGAGCCGCACAAGTCCCT
 367   E  S  R  F  P  I  N  V  G  I  T  G  H  A  A  T  T  G  E  T
1321 TTGAGTCCCGGTTCCCCATCAACGTAGGCATAACAGGACATGCGGCCACAACGGGAGAGA
 387   V  C  I  A  D  A  Y  Q  D  S  R  F  D  Q  S  V  D  E  N  T
1381 CTGTGTGCATTGCTGATGCATATCAAGATTCAAGGTTTGACCAATCAGTCGATGAAAACA
 407   G  F  R  H  K  S  I  L  C  M  P  I  K  N  T  A  R  K  I  V
1441 CAGGCTTCCGCCACAAGTCTATCCTATGCATGCCAATCAAGAACACAGCACGCAAAATAG
 427   G  V  V  Q  L  V  N  K  F  D  N  L  P  F  T  S  N  D  E  N
1501 TGGGAGTCGTGCAACTTGTCAACAAATTTGATAACCTTCCCTTTACAAGCAATGATGAAA
 447   F  L  E  A  F  A  I  F  C  G  M  G  I  H  N  T  N  M  Y  E
```

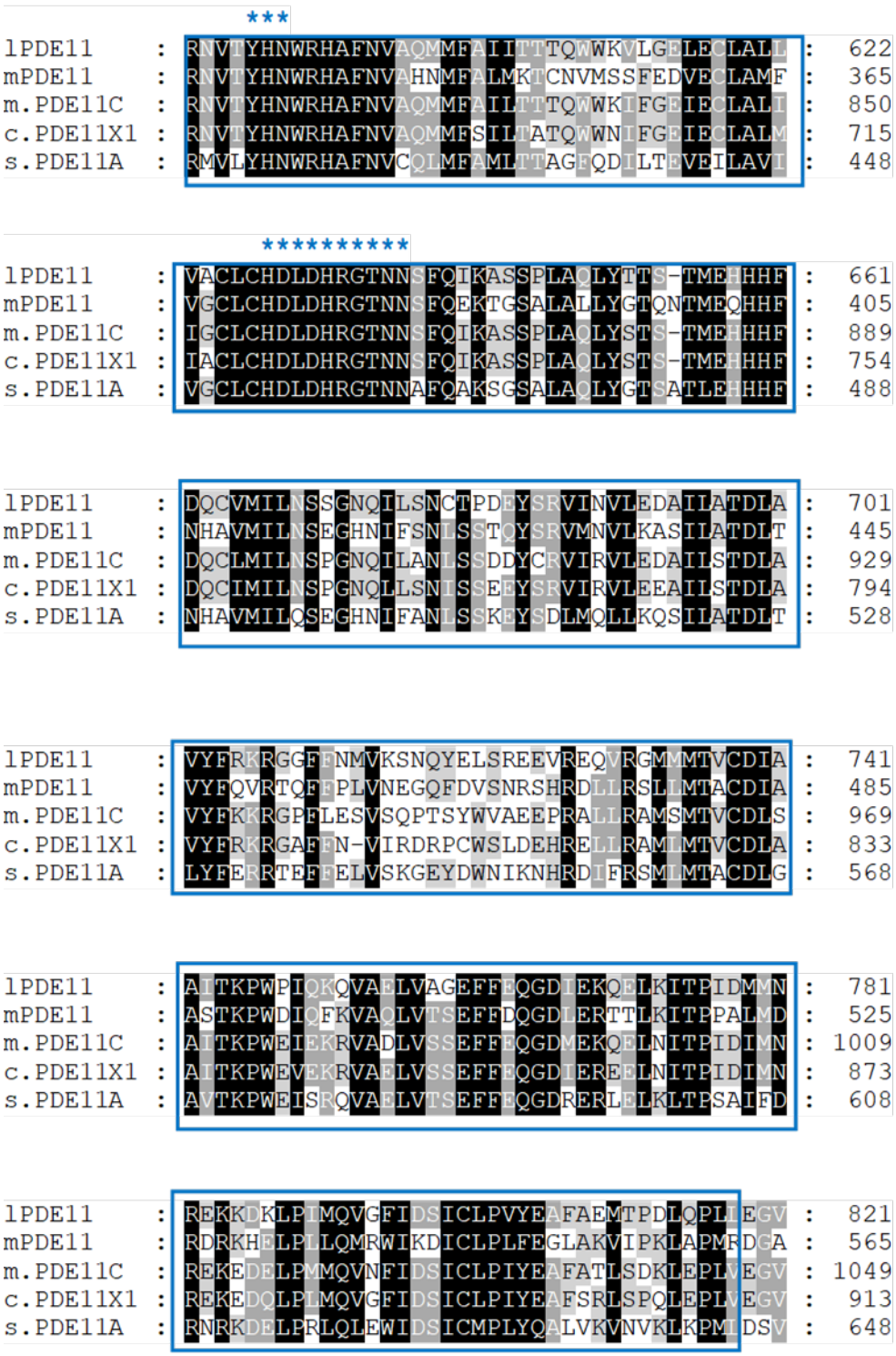
```
1561 ACTTCCTGGAGGCCTTTGCAATATTCTGTGGGATGGGGATCCACAACACCAACATGTACG
 467   K  A  V  T  A  M  A  K  Q  K  V  T  L  E  V  L  S  Y  H  A
1621 AGAAAGCAGTGACAGCAATGGCCAAGCAGAAGGTGACCCTGGAGGTACTGAGCTACCACG
 487   T  A  P  R  T  D  S  Q  K  L  V  K  L  K  I  P  S  A  Q  A
1681 CCACAGCCCCGAGGACAGACTCACAGAAGCTGGTGAAGTTGAAAATACCATCAGCTCAGG
 507   F  Q  L  Y  D  F  K  F  D  D  F  S  L  D  D  D  G  S  L  K
1741 CATTCCAGCTTTACGACTTCAAGTTTGATGACTTCAGTCTTGATGATGATGGAAGTTTGA
 527   A  C  L  R  M  F  L  D  L  D  L  I  G  R  F  H  I  E  Y  D
1801 AGGCCTGTCTGAGAATGTTCCTCGACCTGGACCTGATTGGAAGGTTCCACATTGAGTATG
 547   V  L  C  R  W  L  L  S  V  K  K  N  Y  R  N  V  T  Y  H  N
1861 ATGTGCTGTGCCGTTGGCTGCTCAGCGTCAAGAAGAACTACCGCAATGTCACGTACCATA
 567   W  R  H  A  F  N  V  A  Q  M  M  F  A  I  I  T  T  T  Q  W
1921 ACTGGCGACACGCTTTCAATGTGGCTCAGATGATGTTTGCCATTATCACGACAACACAGT
 587   W  K  V  L  G  E  L  E  C  L  A  L  L  V  A  C  L  C  H  D
1981 GGTGGAAGGTGTTAGGAGAGCTGGAGTGTTTAGCCCTGCTGGTGGCGTGTCTCTGCCATG
 607   L  D  H  R  G  T  N  N  S  F  Q  I  K  A  S  S  P  L  A  Q
2041 ACTTGGATCACCGAGGCACAAACAACTCTTTCCAAATCAAAGCTTCATCTCCATTGGCCC
 627   L  Y  T  T  S  T  M  E  H  H  H  F  D  Q  S  V  M  I  L  N
2101 AGCTGTACACCACCTCTACCATGGAGCACCACCACTTTGACCAGAGTGTCATGATTCTCA
 647   S  C  G  N  Q  I  L  S  N  C  T  P  D  E  Y  S  R  V  I  S
2161 ACTCGTGTGGCAATCAAATCCTAAGTAACTGCACCCCTGATGAGTACTCCCGTGTCATTA
 667   V  L  E  D  A  I  L  A  T  D  L  A  V  Y  F  R  K  R  G  G
2221 GTGTCCTTGAAGACGCTATCCTGGCCACTGACTTGGCCGTGTATTTCAGGAAGCGCGGTG
 687   F  F  N  M  V  K  S  K  Q  C  D  L  N  R  E  D  V  R  E  Q
2281 GATTCTTTAACATGGTGAAGTCAAAACAGTGTGATTTAAACCGGGAGGATGTGCGTGAAC
 707   V  R  G  M  M  M  T  V  C  D  I  A  A  I  T  K  P  W  P  I
2341 AGGTGCGAGGGATGATGATGACAGTGTGTGACATCGCTGCCATTACTAAGCCCTGGCCCA
 727   Q  K  Q  V  A  E  L  V  A  G  E  F  F  E  Q  G  D  I  E  K
2401 TCCAGAAACAGGTTGCAGAATTGGTGGCCGGGGAATTCTTTGAGCAAGGTGACATTGAGA
 747   Q  E  L  K  I  T  P  I  D  M  M  N  R  E  K  K  D  K  L  P
2461 AGCAGGAGTTGAAGATCACCCCAATTGATATGATGAATCGAGAGAAGAAAGACAAACTAC
 767   L  M  Q  V  G  F  I  D  S  I  C  L  P  V  Y  E  A  F  A  D
2521 CATTGATGCAAGTGGGTTTCATAGACTCCATCTGCTTGCCTGTGTATGAGGCCTTTGCAG
 787   M  T  P  D  L  Q  P  L  L  D  G  V  K  E  N  R  Q  N  W  Q
2581 ACATGACCCCAGACCTGCAGCCTCTACTGGACGGTGTGAAGGAAAACCGGCAAAATTGGC
 807   K  L  A  D  E  S  A  S  R  A  M  P  R  N  N  N  N  I  N  N
2641 AGAAGCTGGCGGATGAGTCTGCCAGCAGAGCTATGCCTAGAAACAATAACAATATCAACA
 827   N  N  N  N  N  Y  S  S  N  S  E  R  E  R  G  G  T  N  D
2701 ACAATAACAATAACAACTACTCTAGTAACAGTGAACGAGAAAGAGGTGGGACAAATGATT
2761 AAGTGTGTGCAGATATGTCAAGTGTCCTTGCCACCCATTGCATCAAGTAATGCTGTGGAC
2821 AACTGAAAGTAGAGTTAAGTGATGAATCACTAGCAAGGTTTGTGCAGTGACAATAATGTA
2881 GCAGAAGTACAGATGTTAATGAAATGAGGACTTGAGACAAAAAGGTGCGAGTGTTCAGTG
2941 TGAGTATATGAAGTGTGGCCTTGACCGCTGCTGAAACCATTGCTTCTCCTGTGTGTTGAC
3001 CTCAAGTGTGTGAACATTGCAAACACTAATAGTATATGTTTTATACTTTTTGACTCTGTA
3061 GGTTCAAAAATCTATGTAGTCAGTGAAATGGACATCTGTGCTACCTGTACGTGTGAGAAA
3121 CATACCATCATTGGGTTGTATGATGAGTTTATTGTGGCATAATAATGTTTAATTTAATTA
3181 TAGGATGCTCCAACAACGGAGTATTTAATGTTTTATTCAGGAATTTCATCAGGAATGTGC
3241 AATATTCAAATAGCATGTATGGGTCATTATTTATTTACTAGGCATGTCTGTTCATCTTAA
3301 TGTGTTCACTTAAGGAGTTCAAAAAGTGGGCCCAAGGGATACTTAGGTCCCAAAGTATGT
3361 GGATGGGAGGGAGTGTTATATTGACCTCCATTCATGTAATAAATAACTAGATATTTCTAT
3421 TAACTGTGAAATTATCTCTGCCTTGCATTTCTCACTCCTTGAATAATTTCCCACCATGGC
3481 TACCCAATCTTTCTCGTCACATCAAGAAACATTTGATGTGTGGAAATGCTTTGTTAAAAT
3541 TGAGTCCAAAGAAACAAACTTCTGAAGCTGAATTTCATGAGTCACACAACGCTATCAAGA
```

```
3661 GTACAACTCTTACCATCTTTATGCTCATGGGGAGACTGCAGCCAGCCAGGAGGAAGAGAG
    3721 AAAGAGAGAGAGAGAGAAACAAATCCCCTCCATCCATCTCATCTTGGGCCCTATAACAAA
    3781 AGACAGCACAAGAATTAAACCCTTTTGCTAATTATGATAAGCATATTTTTTTCTATGTTC
    3841 ACCATTTGGATCAAGGAATGTGTATCTAAAATCAGCTGCATATTTCCACCTGTAAATATA
    3901 TAATGCATAAAAGAAATGTGCCATGTCTTTTTTTTTTTCTCAAAAGAAATAATTAGAGCA
    3961 CTCTTGTGTTTCGTGTTTTTTGTTGAGTGAGGCGAGATGAAAGTGTTATGTTAGTAGTGC
    4021 ATCATTGCATCATCCACACTGTGTACCTGCTGCCACTGATGATAGCAAAGCAAGAGTTAT
    4081 GATAGTGTTGCACAATGAGGTGAAGCCAAAGTCATGGATGGGAGGACTATGTTGAGTCAT
    4141 CAGCCATATTCTCTTCCTCTTAAAGTTAGTTTCTCATCAGTCAGTTCTTGCTGCTTGTTA
    4201 CAATTATGAAAGGCAAATGAAAATATGTGAATTTTATATATAAACATCTGCCTCTTCCCC
    4261 TCCCAGTGAATCCCTACAGAGGAGTAAACAAGAAGTTATCCTTTGTCTACGTATTACAAA
    4321 AATTATCACATTAACCAATTGCAGTGTGAGGCAGGAGTTATGTTTTCAGATCAGATTTAC
    4381 CAAACTGTGTCGTTGCCTCAAACCTGTGATAGGAAGAGAATACTGTACGTACATTGCAAG
    4441 GATAACACGGCTCTGGAGGGCAAACTTATCATTGAAGTGAGACAAACAAAGATAAACCTA
    4501 CTGTAAGATTTCATATTAATTCTCACTTTTCTAAATGGGAGCCAATAAGCTGAAGTTTAC
    4561 CTACTAATTAGCGAGACACAGATCCATCTCGGAAAAACATTGATAGATGCAGTATGTACA
    4621 CACTCAACTAAAAGTTATCAAACCTCTTGCAACAGTTGATGCCAAAATTATTTGTGAGTT
    4681 ATGACCACACAATACTTAGTATGCCTGGTTATGATGATGCACATTAAAGTCATGCCACTG
    4741 GCATAGCTACCATCCACACCTTCACACAGAAGGAGTTAAAATTCTTCATATTTAGGCCTA
    4801 TATCTCAGAATGCATGGTGCTTACAGGGTTTGAGAACTTATTGGTGAGAATGTACATATG
    4861 ATTAATGCACTGGTCTCTGGTGCAAGACCCATTAACTACAGCCATTACCAAAGTGAATGC
    4921 CAAACAAATCTCACCACAACCTTTCCATGCTCCCTCATTCCAGTCTACATTTGTAATATT
    4981 GCACAGCTTTGCACAATGCAGCACAATGCTACAGTAAATTGAGAACCAGCCACTGCTGAT
    5041 GATTATATTTTTGTCTGATATATTCTTCACTATTGAAGATAATTATTAACACTACTGAAC
    5101 TGTAGTGGCGTTCAATGTGGTTCCTACTTCTAGTTGGTAAAAACTGACTTCTCAAACAGC
    5161 AGTCTGGTAGTTAGTAGTCATCCCACCTGGGCAAGACTTGTGCTTCTGAATCACCTTGTA
    5221 TGAGGAGCAGAAAACTTGTGTTGGTACTACATGGTCTTAAAAGATACCTCTTACTTGTGT
    5281 CTTTCATTATAACCATTCGTGATTCATAAAGTTTCTGTTCACTTCAGGATATAACCATCT
    5341 TAGATAGAAGTGCTTTTCTTACAATACAAAAGATATTGAGATAAACAGAATGTGTAGCTA
    5401 TATGAGTATAAAATTTTAAAACTTCAGTTGATTAAATAACGTGTAACTTATCAACAATAT
    5461 ATGTAGTGTCAGTAAGCCATTATACTCAAACAAAACACTTTACATTTGGCAGCTGCTTAA
    5521 CAAAAGAAATATTTGATTGAAAGGACATAATTTTTTGTCAGAATAGATTAGACAGTGTGA
    5581 GGTGTGGTTTTATGTAATATGTACGTACTAATAATGTGATGTAAACCACAATCTACAAGG
    5641 GACTATATCACCCATAGTTACCCGTAAGCATCTGCAGATAAAAAAAAAAAAAAGATTATC
    5701 AATTGCTAAACACTCGCAAGCTCTTCTCTATTTCACACTATTTGTTTTCATAATCGATTT
    5761 GTCGGAACACAACCCATTCAAAACTGTAACCATAATATGTTTGCCAATAATTAAAATGAA
    5821 ACAGTAAGTAAGAAAAAAA
```

Figure 2.19. Nucleotide and amino acid sequence of cDNA encoding Cm-PDE11. A full-length open reading frame (ORF) was expressed by the cDNA, the start codon highlighted in yellow, the stop codon highlighted in green, and the promoter element (CAT box) is highlighted in turquoise. Two GAF regulatory domains; GAF-A &GAF-B are indicated by green boxes and contains the (NKxxFDxxE) signature sequence found in all mammalian GAF domains, the sequence is underlined and in green. The conserved catalytic domain is located within the blue boxes started with the initiating (YHN) motif as well as the metal binding motif with a specific sequence signature (HDX_2HX_4N) underlined and in blue.


GAF-A
GlPDE11 : KSRTELKGLDERQLIFELVKDICNELDVRPLCHKILQNVS : 163
CmPDE11 : ---------- : -
Dm.PDE11C : LSRNELKQLDEKELIFELVKDICNELEVRTLCHKILQNVS : 388
Tc.PDE11X1 : RSRHELRHLDEKELIFELVKDICNELEVRSLCHKILQNVS : 245
Hs.PDE11A : ---------- : -
GlPDE11 : ILTSADRCSLFLVQGDKET----------ENRCLVSTLF : 192
CmPDE11 : ---------- : -
Dm.PDE11C : ILLNADRGSLFLVQGRCNG-----PDG---LKKCLVSKLF : 420
Tc.PDE11X1 : TLLHADRGSLFLVQGEKGGGCTPLPPGQTPRGRCLVSKLF : 285
Hs.PDE11A : -MLKQARRPLFRNVLSATQ----------WKKVKITRLV : 28
GlPDE11 : DVNPDSTVEEMEEKEEIRIAWGSGIVGYTAQSGAMVNIPD : 232
CmPDE11 : ---------- : -
Dm.PDE11C : DVCPRSTVEEMEQQDEVRVAWGTGIAGHVAESGEPVNIPD : 460
Tc.PDE11X1 : DVCSRSTLTEMEKKEEIRIPWGTGIVGYVAESGEPVNIPD : 325
Hs.PDE11A : QISGASLAEKQEKHQDFLIQRQT---------- : 51
GlPDE11 : AYADDRFNSEIDCMTGYKTRSMLCMPIKDSNGEVIGVAQV : 272
CmPDE11 : ----------MPVKNSYDEIIAVAQV : 16
Dm.PDE11C : AYQDERFNCEIDSLTGYRTKALLCMPIKDSSGDVIGVAQV : 500
Tc.PDE11X1 : AYQDDRFNHDIDALTGYRTKTLLCMPIKDTNGDVIGVAQV : 365
Hs.PDE11A : KTKDRRFNDEIDKLTGYKTKSLLCMPIRSSDGEIIGVAQA : 91
GlPDE11 : INKHQ---GQSFTTADEKVFESYLQFCGIGLRNAQLYERS : 309
CmPDE11 : VNKSADFDHTSFTLKDEKMFETYLQFVGIAITNAQLMEAS : 56
Dm.PDE11C : INKMN---GECFSEIDEKVFSSYLQFCGIGLRNAQLYEKS : 537
Tc.PDE11X1 : INKVG---DQPFTKQDEEVFASYLQFCGIGLRNAHLYEKS : 402
Hs.PDE11A : INKIP--EGAPFTEDDEKVMQMYLPFCGIAISNAQLFAAS : 129
GAF-B
GlPDE11 : QLEVKRNQVLLDLARIIFEEQSHIEQIVYRIMTHTQSLLQ : 349
CmPDE11 : QAEYERNRSLLEVVHDLFEEQTSLENVILKTLQRAQRLLK : 96
Dm.PDE11C : QLEIKRNQVLLDLARMIFEEQSHIEHMVFRILTHMQSLIQ : 577
Tc.PDE11X1 : QLEVKRNQVLLDLARMIFEEQSHIEHVVFRILTHTQSLIQ : 442
Hs.PDE11A : RKEYERSRALLEVVNDLFEEQTDLEKIVKKIMHRAQTLLK : 169


GlPDE11 : CERVQILLVHE---ASRGTFSRVFDLEVKDLQGDDAE--- : 383
CmPDE11 : CERAAVMLLEDGSEKQNVKFSRIFELNCPVSGQSTNN--A : 134
Dm.PDE11C : CQRVQILLVHE---ADKGSFSRVFDFEANDLSEEEAT--- : 611
Tc.PDE11X1 : CQRVQVLLVHQ---GSKISFSRVFDFEANDLSAEEGE--- : 476
Hs.PDE11A : CERCSVLLLED-IESPVVKFTKSFELMSPKCSADAENSFK : 208
GlPDE11 : -SRTSPFESRFPINIGITGHVATTGETVCIVDAYQDSRFD : 422
CmPDE11 : KQMGSSEMAKHLLH--LAEQVASTGENLNIAEC------- : 165
Dm.PDE11C : -SRTSPYESRFPINIGITGHVATTGETVNVPNAYEDDRFD : 650
Tc.PDE11X1 : -SRTSPFESRFPINVGITGYVATTGETVNIPVADEDDRFD : 515
Hs.PDE11A : ESMEKSSYSDWLINNSIAELVASTGLPVNISDAYQDPRFD : 248
****
GlPDE11 : PSVDENTGFCHKSILCMPIKNTAGKIVGVVQLVNKFDNLP : 462
CmPDE11 : VEVEKGSAGNVRSMLAMPIRNRNFQIIGVAKIINKLNGQP : 205
Dm.PDE11C : ASVDENSCFKHRSILCMAIKNSLGQIIGVIQLINKFNELD : 690
Tc.PDE11X1 : PSVDDGTCFKHKTILCMPIKNSLGQIIGVIQLINKFNDLP : 555
Hs.PDE11A : AEADQISGFHIRSVLCVPIWNSNHQIIGVAQVLNRLDGKP : 288
*****
GlPDE11 : FTSNDENFLEAFAIFCGMGIHNTNMYEKAVTAMAKQKVTL : 502
CmPDE11 : FDENDEQLFEAFTIFCGLGINNTLIYNELEKAMARQKVAI : 245
Dm.PDE11C : FTKNDENFVEAFAIFCGMGIHNTHMYEKAIVAMAKQSVTL : 730
Tc.PDE11X1 : FTKNDENFVEAFAIFCGMGIHNTHMYEKAVVAIAKHSVTL : 595
Hs.PDE11A : FDDADQRLFEAFVIFCGLGINNTIMYDQVKKSWAKQSVAL : 328
GlPDE11 : EVLSYHATAPRSESQKLMTMKIPSAQAFKLYDFKFDDFSL : 542
CmPDE11 : EVLSYHATASADEVLSLQREVIPEASKWNLGSLTFDDFSL : 285
Dm.PDE11C : EVLSYHASATMDEAHRLRRLRVPSAVHFRLHDFKFDDIHF : 770
Tc.PDE11X1 : EVLSYHATASMEDAQRLRSLRVASAAHFRLHDFAFDDINM : 635
Hs.PDE11A : DVLSYHATCSKAEVDKFKAANIPLVSELAIDDIHFDDFSL : 368
GlPDE11 : DDEGSLKACLRMFLDLDLIGRFHIEYEVLCRWLLSVKKNY : 582
CmPDE11 : TQDQMVVAAVRMFSDLRLTSRFKIEYKTLLRWLITVKRNY : 325
Dm.PDE11C : EDDDTLKACLRMFLDLDFVERFHIDYEVLCRWLLSVKKNY : 810
Tc.PDE11X1 : NDDETLTACIRMFLDLDLVERFHIDYEILCRWLLSVKKNY : 675
Hs.PDE11A : DVDAMITAALRMFMELGMVQKFKIDYETLCRWLLTVRKNY : 408



```
GlPDE11     : RDNRQQWQKLAD----EAANKNVP---------------- :  841
CmPDE11     : LKNMAKWSKIA------------------------------ :  576
Dm.PDE11C   : RDNRGHWIDLADVVKTKTSQDQEPEEEQQQQNVISNGDCK : 1089
Tc.PDE11X1  : RKNRVKWLEIA------AAQSLIPPEEQR----------- :  936
Hs.PDE11A   : ATNRSKWEELH------------------------------ :  659


GlPDE11     : ---------------------------------GNNNNNNN :  849
CmPDE11     : ---------------------------------AAQENGA :  583
Dm.PDE11C   : AMSDDDVAASEAEVAVDSPSEKASVNGSNVANNSSNTNKK : 1129
Tc.PDE11X1  : --------------------------------DSNDNLSKP :  945
Hs.PDE11A   : --------------------------------QKRLLAS :  666


GlPDE11     : YTSNNDREKGGGTNN------------------------- :  864
CmPDE11     : CDLLEPPDPPPRVPDG------------------------ :  599
Dm.PDE11C   : IAVASHPTSTQPSDDDNDVDADADDVDEQAAEENGHDAEV : 1169
Tc.PDE11X1  : NTPESNPNASSQISD------------------------- :  960
Hs.PDE11A   : TASSSPASVMVAKEDRN---------------------- :  683
```

Figure 2.20. Multiple alignment of deduced amino acid sequences of PDE11 proteins in two crustacean species, two insect species, and one mammal species. Abbreviations: Gl: *G lateralis*; Cm: *C maenas*; Dm: *D melanogaster*; Tc: *T castaneum*; Hs: *H sapiens*. Black shading indicates that amino acid residues that are identical or similar in all sequences; gray shading indicates identical or similar amino acids in most of the sequences. Dashes indicate gaps introduced to optimize the alignment and the boxes indicate highly conserved domains. Blue and green asterisks indicate the signature sequences in the catalytic domain and GAF domain, respectively.r The colors of the boxes correspond to the colors of the domains in Fig. 2. 13.

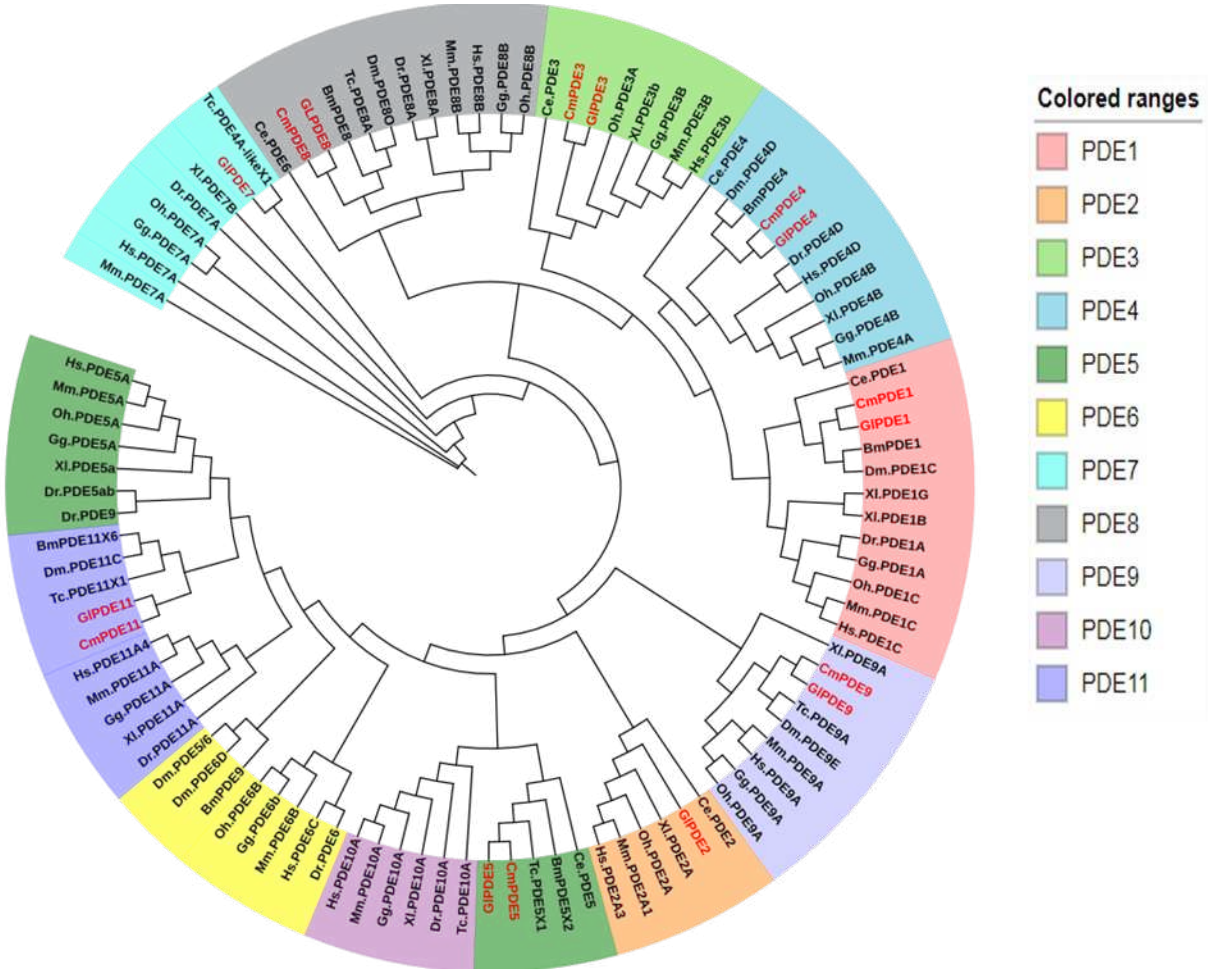
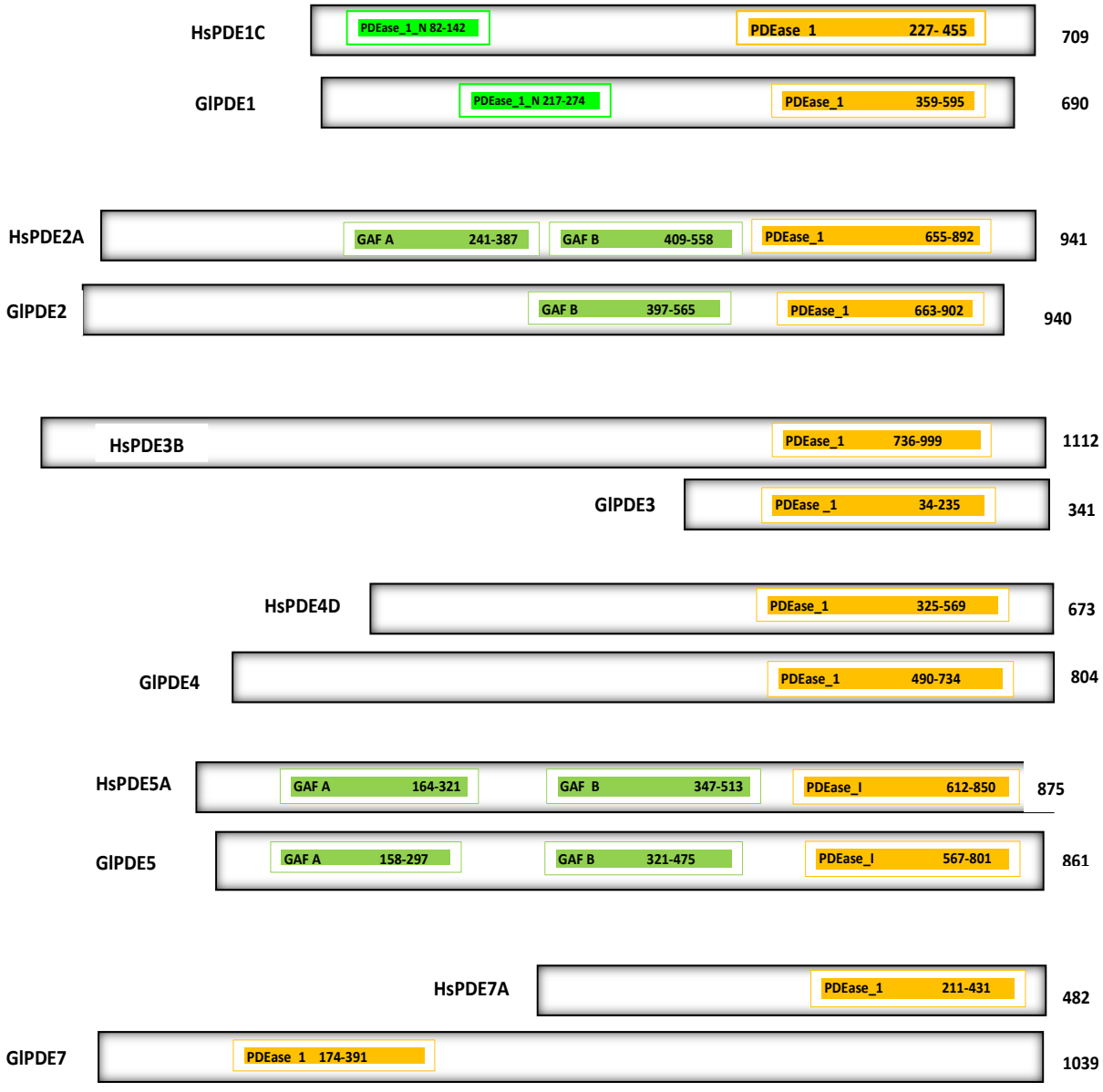


Figure 2.21. Phylogenetic relationships among the different PDEs of *G.lateralis, C. maenas* and orthologs of other species. The 102 deduced amino acid sequences used to generate this tree included the entire open reading frame, catalytic domains, and regulatory domains. *G.lateralis and C. maenas* are shown in red font. Letunic and Bork (2016) *Nucleic Acids Res* **doi: 10.1093/nar/gkw290**


HsPDE1C
PDEase_1_N 82-142
PDEase_1 227- 455
709
GlPDE1
PDEase_1_N 217-274
PDEase_1 359-595
690
HsPDE2A
GAF A 241-387
GAF B 409-558
PDEase_1 655-892
941
GlPDE2
GAF B 397-565
PDEase_1 663-902
940
HsPDE3B
PDEase_1 736-999
1112
GlPDE3
PDEase _1 34-235
341
HsPDE4D
PDEase_1 325-569
673
GlPDE4
PDEase_1 490-734
804
HsPDE5A
GAF A 164-321
GAF B 347-513
PDEase_I 612-850
875
GlPDE5
GAF A 158-297
GAF B 321-475
PDEase_I 567-801
861
HsPDE7A
PDEase_1 211-431
482
GlPDE7
PDEase_1 174-391
1039

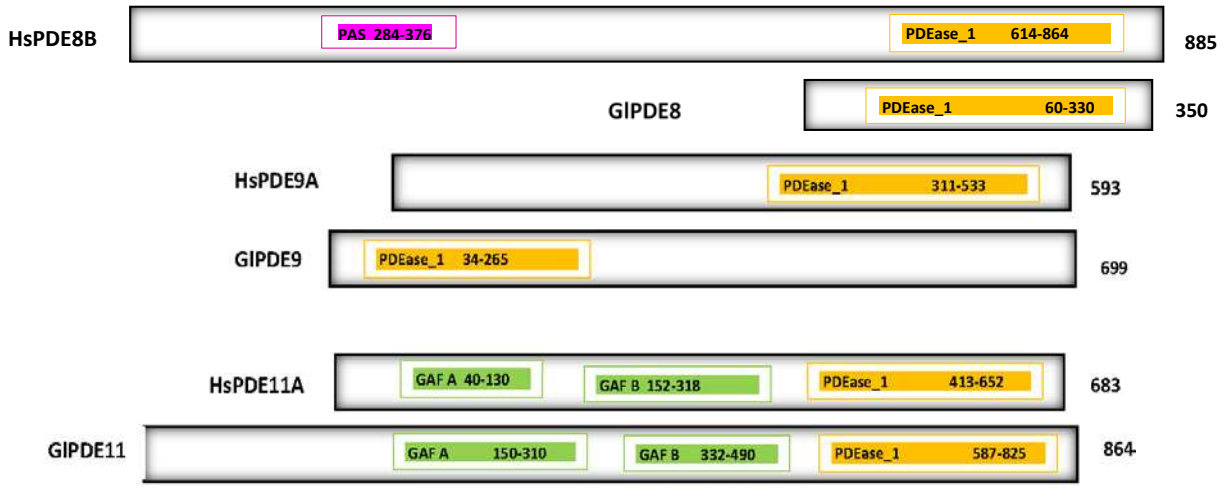


Figure 2.22. A schematic figure showing a comparison of the *G. lateralis* PDEs with the *Homo sapiens* PDEs. Regarding the conserved domains; catalytic domains (PDEase_1) are highly conserved throughout all the PDE families are in yellow boxes. The regulatory domains specific for each family, PDEase_1_N, are shown in bright green boxes, GAF A, or GAF B domains are shown in light green and the PAS domain is shown in purple. The length of each protein in amino acids is shown on the left side of each PDE.

Table 2.4. Effects of PDE inhibitors on the ecdysteroid secretion in the YO of the green shore crab, *Carcinus maenas*. IBMX, a potent and general inhibitor for PDEs, and zaprinast (1 mM) show a very significant effect on ecdysteroidogenesis. Vinpocetine (1mM), EHNA (0.5 mM & 1mM), zaprinast (0.5 mM & 1mM) and zaprinast/rMIH were significant as well. Other inhibitors had no effect on ecdysteroid secretion.

PDE Inhibitor (conc.)	PDE	Ecdysteroid Secretion Mean ± SE		N	% Control	P- value
		Control	Inhibitor			
IBMX (0.5 mM)	General Potent Inhibitor	12.5 ±3.7	7.1±3.0	12	57.2	P<0.001
Vinpocetine (0.1mM)	PDE1	4.6 ± 1.1	1.8± 0.4	13	39.1	P=0.3
Vinpocetine (0.5 mM)	PDE1	3.5±0.7	1.1±0.5	7	31.4	P= 0.06
Vinpocetine (1.0 mM)	PDE1	2.5±0.5	0.4±0.1	8	16.0	P=0.005
EHNA (0.1mM)	PDE2	3.2± 2.3	1.3± 0.9	12	40.6	P=0.5
EHNA (0.5 mM)	PDE2	6.1±2.0	0.5±0.2	8	83	P=0.05
EHNA (1.0 mM)	PDE2	2.1±0.6	0.8±0.2	8	8.2	P=0.02
Rolipram (1 mM)	PDE4	16.1± 5.5	13.5± 3.3	8	84.4	P=0.2
Zaprinast (0.1 mM)	PDE5/PDE11	5.8±2.0	2.5± 1.1	5	43.1	P=0.2
Zaprinast (0.5mM)	PDE5/PDE11	3.9±1.2	0.9±0.1	6	23.1	P=0.05
Zaprinast (1 mM)	PDE5/PDE11	14.5± 4.6	4.4± 2.8	12	30.2	P<0.001
Zaprinast (0.5 mM/rMIH)	PDE5	1.5±0.5	0.5±0.5	8	33.3	P= 0.03
Dipyridamole (0.1mM)	PDE7	8.3±2.2	5.2±1.8	12	62.7	P=0.3
Dipyridamole (0.5mM)	PDE7	4.6±2.2	3.4±2.0	8	74	P=0.6
Dipyridamole (1.0mM)	PDE7	7.9±1.8	6.7±1.9	8	84.8	P=0.7
BC 11-38 (0.1 mM)	PDE11	2.1±0.5	3.1±0.6	8	70.0	P=0.2
BC 11-38 (0.5 mM)	PDE11	3.7±0.5	4.3±0.5	8	86.0	P=0.3
BC 11-38 (1.0 mM)	PDE11	2.9±0.4	2.8±0.3	8	96.0	P=0.9

Table 2.5. Effects of PDE inhibitors on the ecdysteroid secretion in the YO of the blackback land crab, *Gecarcinus lateralis*. All inhibitors had no effect on ecdysteroid secretion as the p-value was higher than 0.05.

PDE Inhibitor (conc.)	PDE	Ecdysteroid Secretion Mean ± SE Control Inhibitor		N	% Control	P- value
IBMX (0.5 mM)	General Potent Inhibitor	3.4 ±1.0	3.1±1.3	8	-6.2	P=0.9
Vinpocetine (0.5 mM)	PDE1	4.5±1.5	3.3±1.1	8	-26.1	P= 0.6
EHNA (0.5 mM)	PDE2	5.1±2.3	4.3±1.6	8	-14.4	P=0.7
Zaprinast (0.5mM)	PDE5/PDE11	1.5±0.8	2.2±0.6	8	46.8	P=0.5
BC 11-38 (0.5mM)	PDE11	7.4± 2.1	4.1± 1.5	8	-44.4	P=0.3

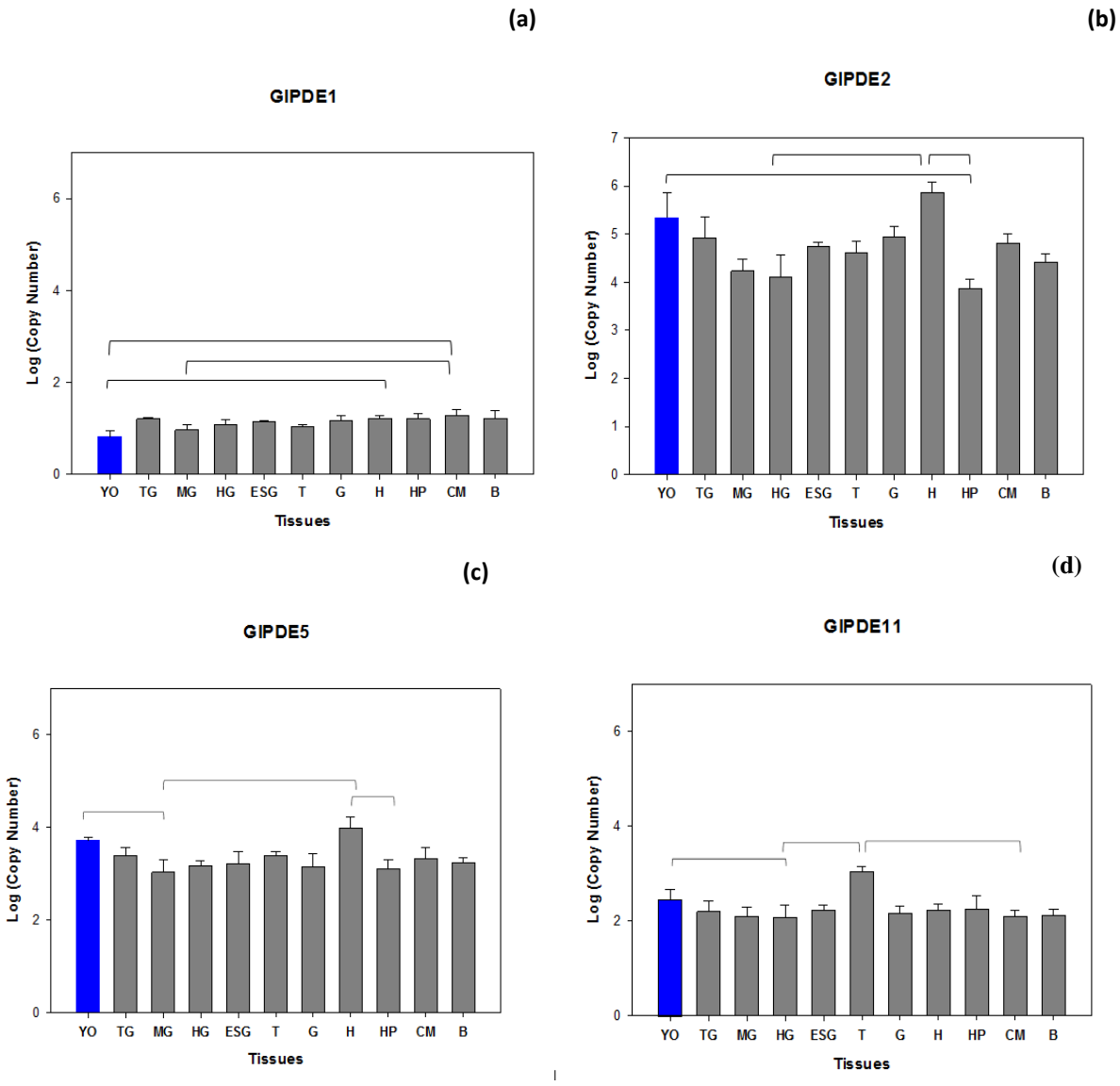


Figure 2.23. Tissue distribution panels show the expression of PDE1 (a), PDE2 (b), PDE5 (c), PDE11(d) in eleven tissues in *Gecarcinus lateralis*. qPCR was used to detect the expression of the different PDEs in eleven tissues; Y-Organ (YO), Thoracic Ganglion (TG), Mid Gut (MG), Hind Gut (HG), Eye Stalk Ganglion (ESG), Testis (T), Gill (G), Heart (H), Hepatopancrease (HP), Claw Muscle (CM), Brain (B). Means that were significantly different are represented with a bracket. Data proposed as mean ±1 SE. (n=6)

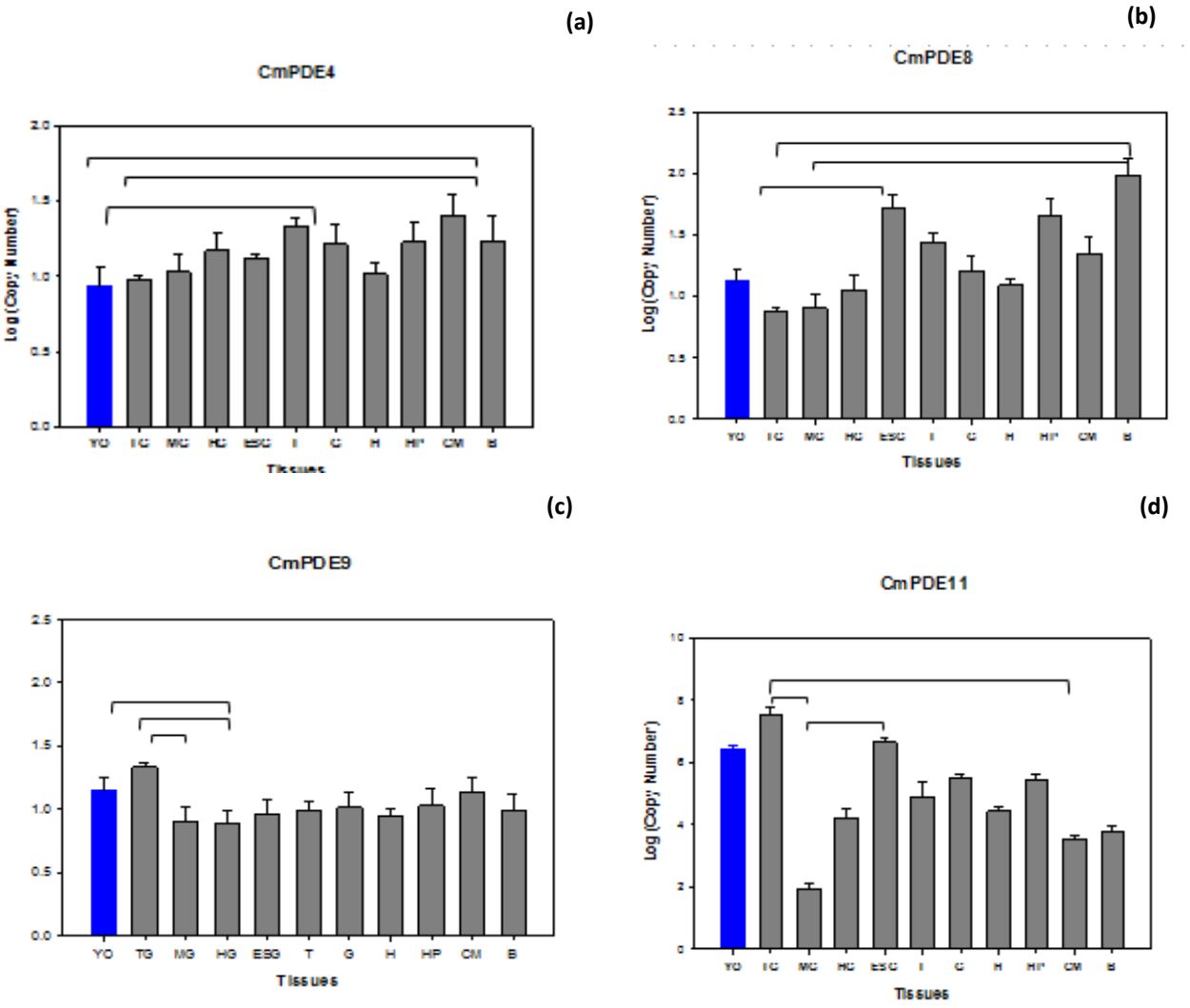


Figure 2.24. Tissue distribution panels show the expression of PDE4 (a), PDE8 (b), PDE9 (c), PDE11(d) in eleven tissues in *Carcinus maenas*. qPCR was used to detect the expression of the different PDEs in eleven tissues; Y-Organ (YO), Thoracic Ganglion (TG), Mid Gut (MG), Hind Gut (HG), Eye Stalk Ganglion (ESG), Testis (T), Gill (G), Heart (H), Hepatopancrease (HP), Claw Muscle (CM), Brain (B). Means that were significantly different are represented with a bracket. Data proposed as mean ±1 SE. (n=6)

CHAPTER THREE

EFFECTS OF MOLT INDUCTION METHODS ON CYCLIC NUCLEOTIDE PHOSPHODIESTERASE EXPRESSION IN THE DECAPOD CRUSTACEAN MOLTING GLAND

Summary

cAMP and cGMP, as second messengers, mediate the suppression of the crustacean molting gland (Y-organ or YO) by molt-inhibiting hormone (MIH). When MIH levels decrease, the YO transitions from the basal to the activated state and the animal enters premolt; such a transition requires mTOR. During mid-premolt, the YO transitions to the committed state, in which the YO becomes insensitive to MIH. Cyclic nucleotide phosphodiesterases (PDEs) convert cAMP and cGMP to AMP and GMP, respectively, and therefore can modify the response of the YO to MIH. Seven *PDE* contigs were extracted from the YO transcriptome. qPCR was used to quantify the effects of molt induction by multiple limb autotomy (MLA) or eyestalk ablation (ESA) ± mTOR inhibitor rapamycin on the mRNA levels of *PDE* 1, 2, 4, 5,7,9, and 11 in *Gecarcinus lateralis* YO. In response to MLA, all PDEs, except for *Gl-PDE5* and *Gl-PDE11*, were expressed at their highest levels in the intermolt YO. mRNA levels declined during premolt and reached their lowest levels in postmolt. qPCR results from the MLA experiment showed that both *Gl-PDE5* and *Gl-PDE11* reached high expression levels in mid premolt and late premolt, respectively. MLA transcriptomics revealed that only *PDE11* expression maximized at mid premolt. In response to ESA, the mRNA levels of *PDE*4, 5, 7, 9, and 11 showed no significant change by 7- and 14-days post-ESA. Rapamycin had no significant effect, as PDE mRNA levels were comparable to those

of controls at all time points, indicating that PDE expression is not regulated by mTOR. The qPCR results were consistent with RNA-Seq data, showing similar trends of PDE expression in both MLA and ESA ± rapamycin. The data suggest that transcriptional regulation does not contribute the reduced sensitivity of the committed YO to MIH; the increased PDE activity during mid and late premolt is likely regulated post-transcriptionally in most PDEs. Our data suggest that *Gl-PDE11* is the controlling PDE in the YO and shows mRNA level changes depending on the molt stage. This is consistent with the responsiveness of YO cells to MIH during mid/late premolt.

Introduction

Decapod crustaceans must shed their exoskeleton periodically for order to them to grow and increase in size and this mainly happens in terms of molting, ecdysis. Molting in crustaceans represents a significant event in life, and this complex physiological process requires precise coordination and regulation between the action of two hormones. Ecdysteroids are steroid hormones which are synthesized and secreted from a pair of Y-organs (molting glands) located in the anterior cephalothorax of the animal. The molt inhibiting hormone, (MIH) is a neuropeptide hormone, which is produced and secreted from the X-organ/complex gland found in the eyestalks. Molting is induced when the titers of MIH in the blood (hemolymph) decline, leading to the stimulation of the YOs to produce and secrete molting hormones, ecdysteroids. The molt cycle can be divided into the following stages: intermolt, the longest stage in the molt cycle during which the animal practices routine activities such as foraging and mating. Premolt (proecdysis) the stage preceding molting, is subdivided into 3 substages (early premolt, mid premolt, late premolt). During premolt the hemolymph ecdysteroid titers increase until they reach a peak then drop dramatically just before the actual shedding of the exoskeleton (ecdysis). The last stage is postmolt

(postecdysis); the animal is vulnerable in this stage as it is recovering from this energy consuming process. It also hardens the soft new shell, an essential hallmark observed during this stage.

The molting gland (Y-organ) is a dynamic organ that proceeds through four distinctive physiological phases throughout the molt cycle: basal phase (during intermolt) when hemolymph ecdysteroid levels are inhibited by pulses of MIH. Activated phase (early premolt), the YO shows signs of hypertrophy to elevate ecdysteroidogenesis in response to the declined circulating MIH. The activated YO is still sensitive to MIH since the animal can postpone molting if eyestalk extracts are injected at this period. During mid/late premolt, the YO transitions to the committed phase and it becomes insensitive to MIH and CHH, as ecdysteroid titers reach their maximum. MIH and CHH levels drop dramatically to initiate the actual molting before ecdysis. A repressed YO is observed in postmolt animals, during which calcification and hardening the exoskeleton, as well as claw muscle growth, occur (Nakatsuji et al., 2009; Chung et al., 2010; Chang & Mykles 2011; Covi et al., 2012; Webster et al., 2012; Shyamal et al., 2014).

Exogenous and endogenous cues contribute to the precise timing for the animal to undergo ecdysis; external factors, such as photoperiod, temperature, stress, and crowding (Skinner & Graham 1972; Weis 1976) Internal factors, such as the urge to provide extra space for the growing organs and tissues, as well as the action of the two opposing predominant hormones, MIH and ecdysteroids (Skinner 1985). In crustaceans, several physiological events take place upon reaching molting (ecdysis), including new exoskeleton synthesis, old exoskeleton degradation, claw muscle atrophy, and lost limb regeneration (Skinner 1985; Mykles 1997; Chang & Mykles 2011).

Upon binding of MIH to its receptor on the YO plasma membrane, a transient increase in cAMP, followed by a larger increase in cGMP, is involved. The delayed increase in cGMP suggests that MIH activates a soluble NO-sensitive guanylyl cyclase (GC-I), since activation of a

membrane GC would result in an immediate increase in cGMP. Both cAMP and cGMP inhibit YO ecdysteroidogenesis. Phosphodiesterases (PDEs), such as PDE1 and PDE5, hydrolyze cAMP and cGMP and thus control the responsiveness of YO organs to MIH (Covi *et al*. 2012).

G. lateralis can be easily manipulated to trigger the process of molting and as in other decapod crustaceans It can be induced by two methods: multiple leg autotomy (MLA) or eyestalk ablation (ESA) (Skinner 1985; Mykles 2001; Chang & Mykles 2011). The autotomy or voluntary loss of 5 or more of the walking legs mimics the natural way of releasing (autotomizing) an appendage when encountering a predator; this will stimulate molting, so the animal will grow a full set of limbs at the next molt. Extirpation of the eyestalks will remove the main source of MIH, which in turn will induce shedding of the old exoskeleton. ESA is a more intense and precise method to induce molting as one can monitor the different molt stages by the increase in hemolymph titers which occurs upon the activation of the YO organs (Lee and Mykles, 2006; Lee et al., 2007b; Covi et al., 2010; Knope & Larson 2014).

mTOR (The mechanistic Target of Rapamycin) is a protein kinase that mediates a variety of cellular functions from cell growth, metabolism, protein and lipid synthesis, to autophagy and cell survival. The mTOR signaling pathway is highly conserved across metazoans (Laplante & Sabatini 2009; Zonko et al., 2010). mTOR is important to increase ecdysteroid synthesis in the insect prothoracic gland (Layalle et al., 2008; Hietakangas and Cohen, 2009). mTOR is crucial for tissue growth in *G. lateralis* and *C. maenas* (Abuhagr et al., 2014). mTOR is believed to be upregulated in the activated YO, so injecting the mTOR inhibitor, rapamycin, *in-vivo* or incubating YOs with this inhibitor *in-vitro* inhibits YO ecdysteroidogenesis, thus molting (Abuhagr et al., 2016).

The committed YO becomes insensitive to MIH in mid/late premolt, a phenomenon that putatively is due to the large increase in the glandular activity of PDEs. Our hypothesis is that increased PDE activity contributes to reduced sensitivity in mid/ and late premolt YO organs. Moreover, PDE expression requires mTOR activity. The effects of MLA and ESA± rapamycin was determined on the expression of PDEs using both transcriptomics and qPCR.

Materials and Methods

Animals and experimental treatments:

Gecarcinus lateralis Adult male (blackback land crabs) were collected from their natural habitat in the Dominican Republic and then shipped to Colorado, USA by commercial air cargo. The animals were acclimated to the new conditions by maintaining them at 27 °C and a humidity of 75-90%. Intermolt crabs were kept in aerated plastic cages lined with moistened aspen bedding by using 5 p.p.t. Instant Ocean (Aquarium Systems, Mentor, OH). Crabs were maintained in an environmental chamber in a 12 hrs light:12 hrs dark cycle and were fed iceberg lettuce, carrots, and raisins twice a week (Covi et al., 2010). Blackback land crabs molt about once a year and larger crabs molt less frequently.

Two molting induction methods were used in these experiments: multiple leg autotomy (MLA) and eyestalk ablation (ESA). MLA mimics the timing of a natural progression toward molting: limb bud regenerate formation takes about 3-6 weeks before the crab enters premolt and eventually molts (Skinner 1985). ESA, however, is an intense and precise method to induce molting. ESA is effective because the X-Organ/Sinus Gland (XO/SG) (located in the eyestalks) are the primary source of MIH. As a result, ESA has an advantage over MLA in providing a precise time point of YO activation; activation can be verified since ecdysteroid hemolymph titers increase

by day-1 post-ESA (Lee et al., 2007). Limb bud regenerates were used to estimate what molt stage MLA animals are in using a measurement called the Regeneration-index (R-index). Such a measurement is calculated as the length of limb regenerate/100 *carapace width. This value ranges from 0 to ~ 24 upon reaching molting (Skinner and Graham 1972; Yu et al., 2002). Limb bud regeneration can be divided into 2 stages: (1) basal growth involves the formation of a small differentiated limb bud (R-value 8-10).This happens throughout intermolt and low ecdysteroid titers are necessisary; (2) proecdysial growth takes place during premolt and high ecdysteroid titers are required (Yu et al., 2002). Three factors can determine the molt stage: ecdysteroid titers, R-value, and the presence/absence of the membranous layer (Moriyasu and Mallet, 1986). A digital caliper was utilized to measure both the limb buds and carapace widths of the MLA animals.

G. lateralis crabs were induced to molt by MLA via autotomizing the eight walking legs (Fig. 3.1). Hemolymph was withdrawn prior to harvesting the YOs from the experimental animals at 5 different molt stages; intermolt (IM), early premolt (EP), mid premolt (MP), late premolt (LP), postmolt (PM). 100 µl of hemolymph was added to 300 µl of methanol (MeOH 100%) then stored at -20C°. Competitive ELISA (Abuhagr et al., 2014b) was used to quantify the hemolymph ecdysteroid titers to determine the accurate molt stage for each animal in addition to the R-value. Harvested YOs were reserved in RNA-later (Ambion®, California) and stored at 20C° until the time of RNA isolation. MLA animals were kept in separate moistened sand cages to provide privacy and mimic their natural habitat (Skinner 1985).

Intermolt animals were ES-ablated using a pair of sharp scissors and then cauterized immediately to minimize the amount of bleeding (Fig. 3.2) To determine the effects of rapamycin (mTOR inhibitor) on YO ecdysteroidogenesis, *in-vivo* injections were performed. The control group (ESA-rapamycin) crabs were injected by the vehicle (~1% DMSO final concentration) on

Day 0. The experimental group (ESA+ rapamycin) crabs were injected by rapamycin (~10 µM final concentration) on Day 0 (mass of the animal ×0.3µl= amount to inject). Hemolymph samples were collected and YOs were dissected at Day 0 for intermolt (intact) crabs and Days 1, 3, 5, 7, 14 post-injection for ESA ± rapamycin. Competitive ELISA (Abuhagr et al., 2014b) was used to quantify the hemolymph ecdysteroid titers. Harvested YOs were reserved in RNA-later (Ambion®, California) and stored at -20C° until the time of RNA isolation.

RNA isolation, cDNA synthesis and quantitative real-time PCR:

Y-organs from both MLA and ESA± rapamycin animals were harvested from land crabs. YOs were placed immediately in RNA-later after cleaning the tissues with crab saline. YOs were kept overnight in 4C°, then transferred to -20 C° until the time of RNA purification. Total RNA was isolated from crab YOs using TRIzol reagent (Life Technologies, Carlsbad, CA) as described by (Covi et al., 2010). YO tissues (50-200 mg) were homogenized by using a micro-tube homogenizer system. 1 ml of TRIzol was added to samples, then centrifuged at 12,000 g for 15 min at 4 ºC. Supernatants were phenol-chloroform extracted and RNA in the aqueous phase was precipitated using isopropanol (0.75 ml per 1 ml TRIzol reagent). RNA was treated with DNase I (Life Technologies), extracted twice with phenol: chloroform: isoamyl alcohol (25:24:1), precipitated with isopropanol, washed twice with 70% ethanol in DEPC water, and resuspended in nuclease-free water. cDNA was synthesized using 2 µl total RNA in a 20 µl total reaction with SuperScript III reverse transcriptase (Life Technologies) and oligo-dT (20) VN primer (50 µmol/l; IDT, Coralville, IA) as described (Covi et al., 2010). RNA was treated with RNase H (Fisher Scientific, Pittsburgh, PA) and stored at -80 ºC.

End-point PCR was used to amplify the desired product and to increase the yield of each PDE gene, as well as for making external standards of the different genes to be used later in qPCR.

Primers (Table 3.1) were utilized to detect the different PDE products in land crab. Each PCR reaction contained; 3 µl of DI H_2O, 5 µl Master Mix, 1 µl cDNA template, and 0.5 µl of each forward and reverse primers. The concentration of the primers was 20µM. cDNA was amplified in a thermocycler where denaturation occurred at 94C° for 3 minutes to initiate the process, followed by 30-35 cycles of 30 seconds at 94C°, and 30 seconds at the lowest annealing temperature (see table 3.1.) 30 seconds at 72 °C. PCR products were then separated on 1% agarose gel that contained TAE buffer (composed of 40 mM Tris acetate and 2 mM EDTA with an 8.5 pH). Ethidium bromide was applied to stain the gel and a UV light was used to visualize the gel.

Real-time PCR (RT-PCR) was used to quantify the expression of *Gl-PDE*s 1,2,4,5,7,9,11 in each point molt stage of the MLA animals, and *Gl-PDE*4,5,7,11in ESA ± rapamycin animals.

cDNA was synthesized as indicated previously, and a LightCycler 480 thermocycler (Roche Applied Science, Indianapolis, IN) was used to quantify the mRNA transcripts of *Gl-PDE1*, *PDE2, PDE4, PDE5, PDE7, PDE9,* and *PDE11* for MLA *G. lateralis* animals and *PDE4, PDE5, PDE7,* and *PDE11* for ESA ± rapamycin *G. lateralis* animals. Each reaction consisted of 1 µl cDNA or standard, 5 µl SYBR Green I Master mix (Roche Applied Science), 3 µl nuclease-free water, and 0.5 µl each of 10 mM forward and reverse primers (Table 3.1). PCR conditions were as follows: an initial denaturation at 95 ºC for 5 min, followed by 45 cycles of denaturation at 95 ºC for 10 s, annealing at 62 ºC for 20 s, and extensions at 72 ºC for 20 s, followed by melting curve analysis of the PCR product. Concentrations of mRNA transcripts were determined by the LightCycler 480 software (Roche, version 1.5) using a serial dilution of standards of the PCR product for each gene of interest. The amounts of mRNA transcript in copy numbers per µg of total RNA in the cDNA synthesis reaction were calculated based on the standard curve and the calculated molecular weight of dsDNA products.

Bioinformatics:

The expression of different *Gl-PDE*s in the *G. lateralis* YO was assessed using two databases: the MLA transcriptome and the ESA± rapamycin transcriptome (Das et al., 2016; Shyamal et al., 2018). Differential expression (DE) of *Gl-PDEs* 1,2,4,5,7,9, and 11 were determined at each molt stage (IM, EP, MP, LP, and PM) in MLA animals. Moreover, DE of *Gl-PDEs*; 4,5,7, and 11 were assessed at Day 0 for intermolt (intact) crabs; and Days 1, 3, and 7 postmolt for the ESA ± rapamycin animals.

Statistical analysis and software:

(https://www.idtdna.com/Primerquest/Home/Index). Primers were designed by IDT software. Means for mRNA transcript abundance were compared using an analysis of variance (ANOVA) for molt stages versus log copy number in the MLA experiment or days post molt versus log copy number in the ESA ± rapamycin experiment. Sigma plot 12.5 software (Systat Software, Inc., Chicago, IL, USA) was used to produce and build up the graphs and figures. Tukey test was used to determine significance among the means.

Results

Effects of Multiple Leg Autotomy (MLA) on Gl-PDEs expression in the molting gland:

G. lateralis animals were induced to molt by automatizing all 8 walking legs. Several weeks later, these crabs entered early premolt. R-values were measured weekly to estimate the molt stage of each crab. To further ensure the accuracy of the crab's molt stage, hemolymph samples were collected just before dissection, so a competitive ELISA could be performed. Ecdysteroid levels showed that the molting hormone was low in early premolt (EP), elevated during mid premolt (MP), then increased to reach its maximum at late premolt (LP) (Fig. 3.3a).

qPCR results showed that *Gl-PDE4*, *Gl-PDE7*, and *Gl-PDE9* were expressed in very low levels at all molt stages, especially *Gl-PDE9* (Fig. 3.3 b). Unexpectedly, *Gl-PDE1* was also expressed in low levels, scoring its minimum at early premolt (Fig3.3 c) *Gl-PDE2* mRNA level was high in intermolt and decreased gradually through to postmolt. Significant differences were observed between IM/LP and IM/PM, and between EP and PM (Fig3.3c). *Gl-PDE5* displayed a different pattern, as a slight increase was seen from IM to EP and was at its highest expression level in MP, then declined to its lowest level in PM. Statistical differences were noticed between IM/ LP and EP/PM in *Gl-PDE5* (Fig3.3c). *Gl-PDE11* demonstrated a similar pattern to ecdysteroid titers in different molt stages. *Gl-PDE11* showed the most robust expresson among the rest of *Gl-PDE*s; as it increased progressively to reach its maximum expression in LP, then decreased in PM. Expression levels of *Gl-PDE*11 were not identical to the hypothesized trend but was the closest to our expectations. Significance differences were detected between IM/PM and IM/MP (Fig.3.3 c).

MLA transcriptomics exhibited different expression patterns of *Gl-PDE*s. *Gl-PDE*2, *Gl-PDE*7, *Gl-PDE*8 and *Gl-PDE*9 were expressed at their highest levels at IM and lowest levels at PM (Fig.3.4 a, b). *Gl-PDE*4 showed levels of similar expression levels in IM, EP, MP; then dropped upon reaching PM (Fig.3.4 b). *Gl-PDE*5 expression was high in IM, gradually decreased until reaching LP, then slightly increased in PM (Fig.3.4 b). Statistical significance was seen between IM/PM in *Gl-PDE*2, 4, 7, 8, and 9; whereas there was a significant difference between IM/LP in *Gl-PDE*5. Once again, *Gl-PDE*11 displayed a unique pattern; it was the highest gene to be expressed to reach a peak in MP, then drop dramatically when approaching PM and a significant decrease was seen between these two molt stages (Fig.3.4 c).

Effects of Eyestalk Ablation (ESA)± rapamycin on Gl-PDEs expression in the molting gland:

G. lateralis crabs were induced to molt ESA. For the qPCR results, YOs were harvested from 3 different groups and intact animals were dissected on Day 0, control (DMSO) group (ESA- rapamycin) animals were dissected on Days 1,3,5,7, and 14, and experimental (rapamycin) group (ESA+ rapamycin) animals were also dissected on Days 1,3,5,7, and 14. ESA ±rapamycin animals were either injected with DMSO (control group) or rapamycin (experimental group) on Day 0.

A significant increase from Day 0 to Day 1 was seen in the expression of *Gl-PDE*4, *Gl-PDE*7, *Gl-PDE*11 in both control and experimental YOs. Expression then levels off with no difference between the two groups (Fig. 3.5 a, c, d). *Gl-PDE*5 showed no noticeable elevation in its expression from Day 0 to Day1 and displayed a similar trend with the other PDEs. But there was a slight increase in *Gl-PDE5* expression from Day 5 to Day 7 in the control group. Also, there was a significant decrease from Day 3 to day 5 in the experimental group (Fig. 3.5 b). Expression of *Gl-PDE*4 mRNA increased significantly at day 1 post ESA + rapamycin and Day 3 post ESA – rapamycin when compared to intact animals on Day 0 (Fig. 3.5 a). There was a slight, but a significant, increase in *Gl-PDE*5 expression between day 5 and day 7 in the control group (Fig. 3.5 b). *Gl-PDE*7 mRNA levels increased drastically from Day 0 to Day 1 and a statistical significance was observed in Day 1 in the control group when compared to Day 0 (Fig.3.5 c). *Gl-PDE*11 expression levels illustrated a significant increase in Day 1 post-ESA in experimental animals and 3 days post-ESA in control animals when compared to Day 0 (Fig. 3.5 d).

Comparable to the ESA ± rapamycin qPCR experiment, ESA ± rapamycin transcriptomics showed similar trends and patterns of Gl-PDEs expression in both control and experimental animals; an increase was seen from Day 0 to Day 1 as the YO is activated upon the ablation of the eyestalks.

*Gl-PDE*4 expression levels increased significantly at 1- day post-ESA in control and experimental animals compared to Day 0 (Fig. 3.6 a). *Gl-PDE*5 mRNA levels showed a statistical difference between Day 0 and Day 1 post-ESA in control and experimental crabs (Fig. 3.6 b). Expression levels of *Gl-PDE*7 exhibited a significant increase between Day 0 and Day 3 post-ESA in control animals. *Gl-PDE*11 expression levels increased extremely from Day 0 to Day 1 which resulted in a significant increase at 1-day post-ESA in both control and experimental animals (Fig. 3.6 d).

Discussion

cAMP and cGMP signaling pathways control a plethora of intracellular proteins that vary depending on the cell type and function. Such signaling pathways are widespread from prokaryotes to eukaryotes and are involved in activating protein kinases, including PKA and PKG. The only known negative regulators of cAMP and cGMP are cyclic nucleotide phosphodiesterases (cN-PDEs), which fall under the Class I PDE superfamily. The PDE superfamily includes eleven genes designated PDE1 to PDE11. Each family shares common biochemical features, substrate specificity, cellular/sub-cellular localization, pharmacological characteristics, and regulatory mode. Although all eleven genes include a well-conserved catalytic domain located in the carboxyl terminus, each gene has its unique regulatory domains. Our hypothesis is that an increase in PDE expression will occur in *G. lateralis* Y-organs at mid/late premolt stages, thus decreasing the responsiveness of YOs to (MIH). Such a finding parallel results from studies done on crayfish, in which glandular PDE activity was detected in YOs at specific molt stages (Nakatsuji et al., 2006a; Nakatsuji et al., 2009), as well as in green crab YOs (Mattson & Spaziani, 1985b). Prior to the current study, it was unknown which of the PDE genes might be expressed in the YOs and contribute to the most critical time of the crustacean's life.

Hemolymph ecdysteroid titers increased when animals were induced to molt by MLA (Fig. 3.3 a). qPCR showed that *Gl-PDE*4, *Gl-PDE* 7, and *Gl-PDE* 9 mRNA transcripts were expressed in low levels. Moreover, *Gl-PDE*1 and *Gl-PDE*2 showed different patterns (Fig. 3.3 c). None of the above *Gl-PDE* expression profiles met our hypothesis since we did not see increased PDE expression in mid/late premolt YOs. We conclude that these enzymes might be regulated post-transcriptionally to stabilize the mRNA and to further enhance and modify the structure of the final protein product. Such observations were symmetrical to the beta-subunit in *HsPDE*5 (Lerner et al., 2006). Moreover, studies on *HsPDE3B* adipocytes revealed both transcriptional and post-transcriptional modifications (Yan et al., 2007). Another interpretation might be that MLA has no effect on the expression of *Gl-PDE*1,2 ,4, 7, and 9. It is also possible that target cells interpret nuanced changes in PDE activity and even slight changes present a potent impact on the target cell response (Sette & Conti 1996). Conversely, *Gl-PDE*5 showed its highest expression in MP, then dropped upon reaching PM. *Gl-PDE*11 reached its maximum expression in LM and decreased in PM (Fig. 3.3 c). These findings do support our first hypothesis, that PDE activity in mid/late premolt is responsible for the YO insensitivity to MIH, which in turn triggers the animal to be committed to molt (Nakatsuji et al., 2009). Consequently, *Gl-PDE*5 (a c-GMP specific PDE) and *Gl-PDE*11 (a dual PDE) might play a remarkable role in the YO's MIH signaling pathway.

Results of the MLA transcriptomics studies demonstrated the relative expression of *Gl-PDEs* (Fig. 3.4 a, b). *Gl-PDE* 2,4,7, and 9 were relatively higher in IM and then decreased gradually while reaching their lowest expression in PM. *Gl-PDE*5 was high in intermolt then dropped to its minimum expression in LM and slightly increased in PM (Fig.3.4 b). *Gl-PDE*11 expression reached a peak in MP then declined in PM accompanied with a significant difference between these two stages (Fig.3.4 c). We conclude that MLA had no impact on *Gl-PDE* 2,4,7, and

9 expression since these results were consistent with the qPCR results seen in the MLA experiment. Notably, *Gl-PDE*5 and *Gl-PDE*11 depicted different trends as was observed in the qPCR results from the MLA experiment. Our data strongly suggest that both *Gl-PDE*5 and *Gl-PDE*11 act on the intracellular levels of cAMP/cGMP and both converge to stimulate ecdysteroid synthesis in the molting gland. This finding is consistent with recent studies in humans, in which *Hs-PDE11A* exerted a regulatory effect on cortisol excretion and synthesis (Ceyhan et al., 2012; Vezzosi et al., 2012). In contrast to *Gl-PDE*5, inhibition of *PDE*5A in rat and mouse Leydig cells by sildenafil (Viagra) *in-vivo*, illustrated an activation of the NO/cGMP pathway, thus increasing testosterone synthesis (Saraiva et al., 2009; Andric et al., 2010).

mTOR is a conserved serine/threonine kinase and found from yeast to humans. It represents a central node for a variety of cellular processes, such as gene transcription, protein synthesis, cell growth, and cell metabolism (Cornu et al., 2013). mTOR is important during molting in the activated YO in crustaceans (Abuhagr et al., 2014), as such an activation in early premolt YOs upregulates ecdysteroid synthesis (Mykles 2010). In the fruit fly, *Drosophila melanogaster*, metamorphosis depends on mTOR signaling pathway in the molting gland (prothoracic gland or PG) (Layalle et al., 2008).

Our second goal was to determine whether *Gl-PDE* expression requires mTOR activity or not. For this purpose, ESA was performed to induce molting. ESA ± rapamycin qPCR/transcriptomics results revealed an activation from Day 0 to Day 1 in the expression of *Gl-PDE*4,5,7, and 11, which then leveled off on all other days post-ESA (Fig. 3.5 a, b, c, d) and (Fig. 3.6 a, b, c, d). Furthermore, no differences were observed between control and experimental groups. In contrast, previous studies showed that PDE4D5 binds Rheb (upstream regulator of mTOR) in a noncatalytic novel fashion to inhibit mTOR, and that this binding will only be

dissociated if intracellular cAMP levels are elevated (Kim et al., 2010). Furthermore, previous studies provide evidence of cross talk between mTOR and cAMP signaling pathways; an increase in cAMP concentration inhibits mTOR complexes, thus suppressing the catalytic activity of mTOR (Xie et al., 2011). So, cAMP can either activate or inhibit mTOR depending on the tissue type and cAMP distribution (Kwon et al., 2004; Rocha et al., 2008). PDEs might have an indirect regulation mechanism, but this is not fully understood. Taken together, mTOR does not contribute to PDE expression in the crustacean's *G. lateralis* molting gland, indicating a different mechanism of regulation might be controlling Gl-PDEs, which is distinct from mammalian PDEs.

Conclusions

Molting in the blackback land crab, *Gecarcinus lateralis*, can be induced by two methods: Multiple Leg Autotomy (MLA) and Eyestalk Ablation (ESA). In mid/late premolt, molting glands (YOs) become insensitive to MIH and the animal is committed to molt. Second messengers cAMP and cGMP play a crucial role in the MIH signaling pathway and their intracellular concentrations are regulated with cyclic nucleotide phosphodiesterases (PDEs). MLA and ESA ± rapamycin transcriptomes were screened for PDE expression. qPCR for the two molt induction experiments was used to validate the transcriptomics results. MLA showed no effect on *Gl-PDE1, 2, 4, 5, 7*, and *9*, suggesting these genes might be regulated in a post-transcriptional manner. Conversely, *Gl-PDE11* (a dual PDE) is the dominant PDE in the YO and shows molt-dependent changes in the mRNA levels that is consistent with a role in reducing sensitivity in mid/late premolt. ESA increased mRNA levels of *Gl-PDE4*, *5*, *7* and 11, and was associated with YO activation. Such increases do not require mTOR activity. These data were not consistent with our hypothesis that mTOR regulates PDE expression as there was no significant difference between the control and experimental groups based on both transcriptomics and qPCR results.

Table 3.1. Oligonucleotide primers used in qPCR to identify gene expression in Gl-PDEs in MLA and ESA ± rapamycin experiments. Abbreviations: Gl, Gecarcinus lateralis; F, Forward; R, Reverse, PDE, cyclic nucleotide phosphodiesterase; Numbers (1,2,4,5,7,9,11), PDE family.

Primer name	Primer sequence (5'-3')	Amplicon Product (bp)	Annealing Temp.
Gl-PDE1 -F2	GGTGGCAAAGTGGAAAGATAAAG	226	62 C°
Gl-PDE1 -R2	CCTCCTCGTCTCTCTTCTTAGT		62 C°
Gl-PDE2 -F2	GGTGGTAGTGGCACGTTTAT	301	62 C°
Gl-PDE2 -R2	TCCCTCTTTCCTTCCTCTTCT		62 C°
Gl-PDE4 -F1	AGGCTTCTGTGTGGTACATATC	260	62 C°
Gl-PDE4 -R2	CACAAACTTGCATCCCTCAATC		62 C°
Gl-PDE5 -F2	CAGACCACCGGATGCTTATT	316	62 C°
Gl-PDE5 -R1	TCCTCGACCCGATTCTATGT		62 C°
Gl-PDE7 -F1	CATGGAAGGCATTTGGCTAAG	283	62 C°
Gl-PDE7 -R1	CTTCAGTTGGAGGTGAGTCTAC		62 C°
Gl-PDE9-F1	CAGTGGACCATTCCTCACTTC	269	62 C°
Gl-PDE9-R2	TGGTCATTCATCCCTTGCAATA		62 C°
Gl-PDE11-F2	GACTCCAGACTTGGTTCTTTCC	322	62 C°
Gl-PDE11-R2	CGACTGATGTCACTTGCATATC		62 C°

Figure 3.1. Molting can be induced by Multiple Limb Autotomy (MLA). In this picture, the crab reached ecdysis and is pulling itself out of the old exoskeleton upon losing all the walking legs. Molting in this case is important, so the animal can grow with a full set of limbs.

Figure 3.2. Molting can be induced by Eyestalk Ablation (ESA). This picture depicts the removal of both eye stalks, thus eliminating the main source of MIH (molt-inhibiting hormone).

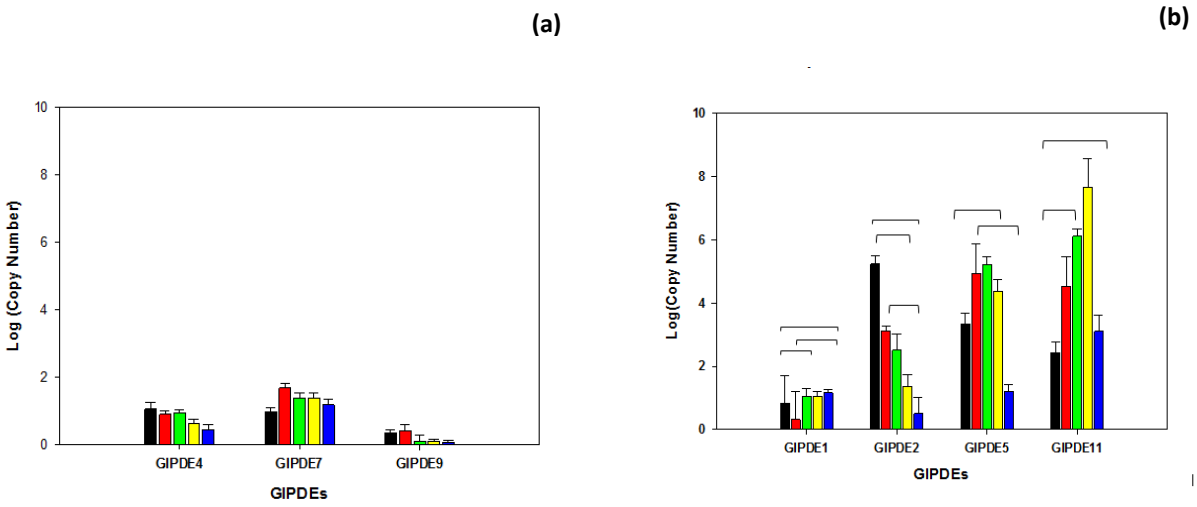
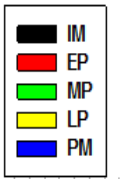


Figure 3.3. Effects of MLA on the YO expression of *Gl-PDE*4, *Gl-PDE* 7, *Gl-PDE* 9 (a) and YO expression of *Gl-PDE* 1, *Gl-PDE* 2 *Gl-PDE*,5, *Gl-PDE* 11(b). *Gl-PDE* mRNA expression for the different families was quantified by qPCR at each molt stage point; intermolt (IM), early premolt (EP), mid premolt (MP), late premolt (LP), post molt (PM). n= 10-12 for each molt stage. Data presented as mean ±1 S.E. No significant differences were observed in the means of *Gl-PDE4,7* and *9.* mRNA expression of *Gl-PDE* 1,2,5,11 appeared with different trends. Means within the same gene that were significantly different are represented with a bracket.



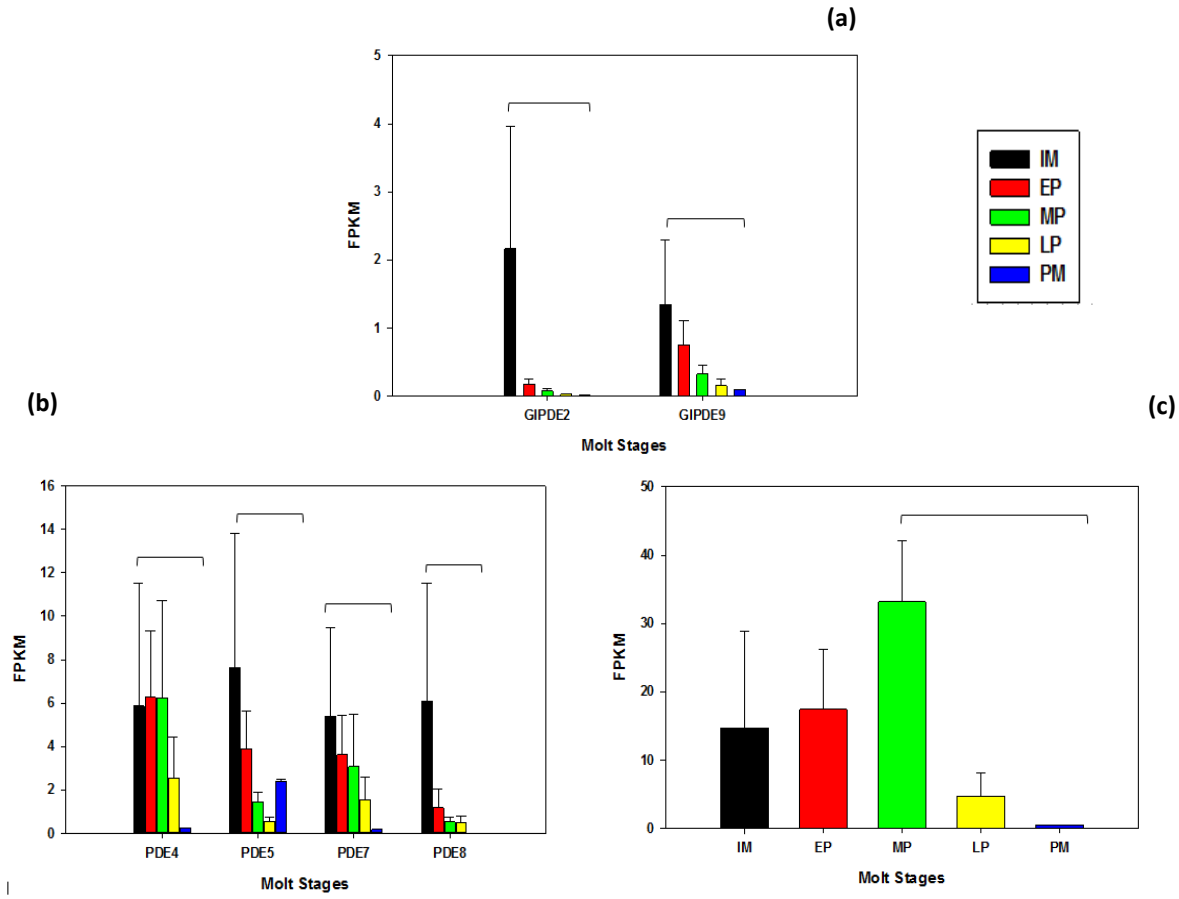


Figure 3.4. Relative expression of *Gl-PDE*2,9 (a), *Gl-PDE*4,5,7,8 (b) and *Gl-PDE*11 (c). Transcriptomics was used to assess the effects of molt stage on PDE expression. There was a significant difference between intermolt (IM) and postmolt (PM) in all Gl-PDEs, except *Gl-PDE*11 which elucidated a statistical significance between mid premolt (MP) and PM. *Gl-PDE*11 showed the highest expression among all PDEs (scales are different for each set of PDEs). Means within the same gene that were significantly different are represented with a bracket.

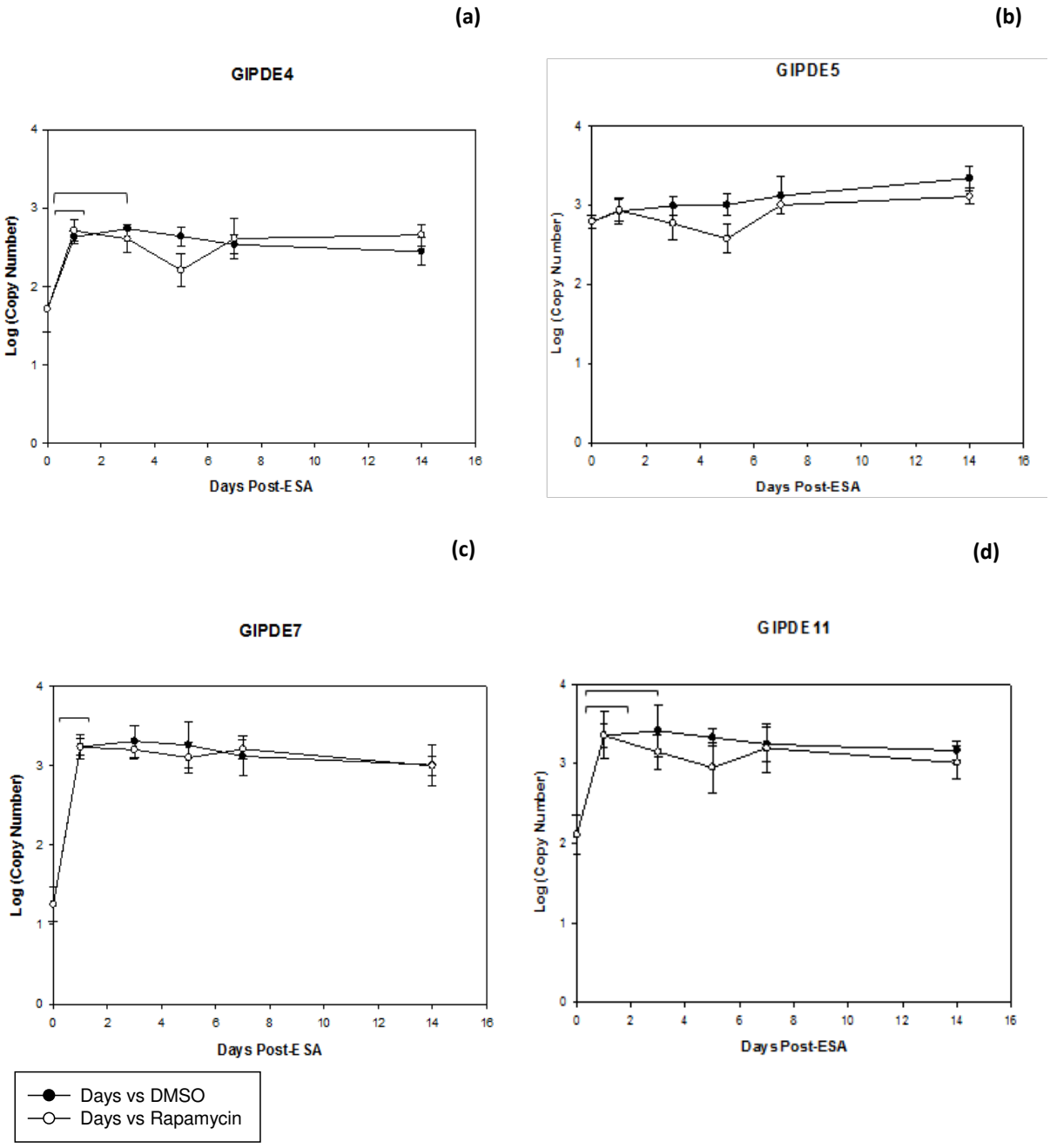


Figure 3.5. Effect of ESA± rapamycin on the expression of *Gl-PDE*4 (a), *Gl-PDE*5 (b), *Gl-PDE*7 (c), and *Gl-PDE*11 (d) in *G. lateralis* YO. qPCR was used to evaluate the expression of *Gl-PDE*4,5,7,11 at point times 1,3,5,7,14 days post ESA in control (DMSO) and experimental (rapamycin) injected animals. mRNA PDEs increased from Day 0 to Day 1, then leveled off throughout the rest time points. Means within the same gene that were significantly different are represented with a bracket. Data presented as mean± S.E (n=8-10).

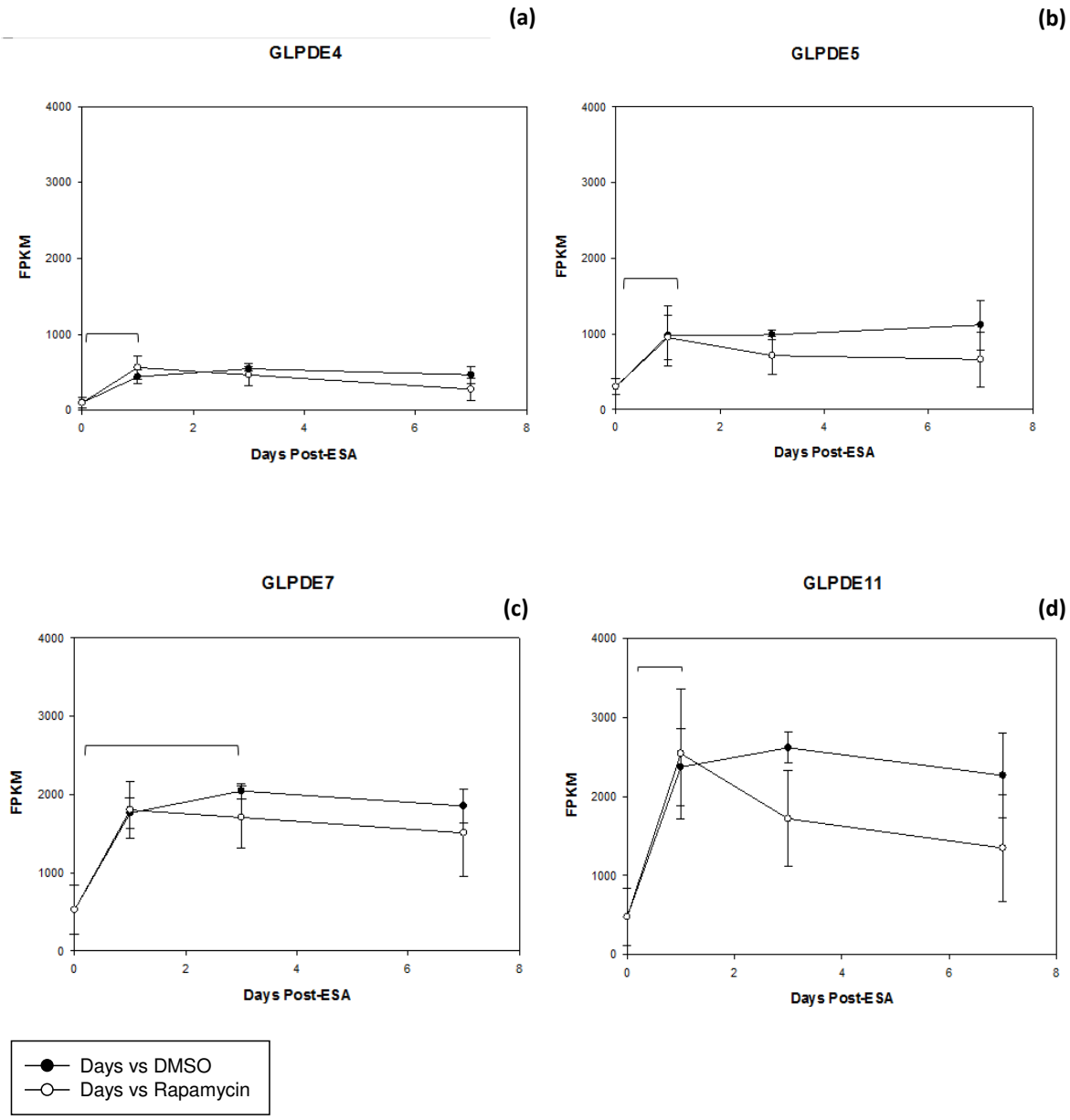


Figure 3.6. Effect of ESA± rapamycin on the expression of *Gl-PDE*4 (a), *Gl-PDE*5 (b), *Gl-PDE*7 (c), and *Gl-PDE*11 (d) in *G. lateralis* YO. Transcriptomics was used to evaluate the expression of *Gl-PDE*4,5,7,11 at point times 1,3,7 days post ESA in control (DMSO) and experimental (rapamycin) injected animals. mRNA PDEs increased from Day 0 to Day 1, then leveled off throughout the rest time points. Means within the same gene that were significantly different are represented with a bracket. Data presented as mean± S.E.

CHAPTER FOUR

SUMMARY AND FUTURE DIRECTIONS

Cyclic nucleotide signaling mediates the suppression of the crustacean molting gland (Y-organ or YO) by molt-inhibiting hormone (MIH). When MIH level drops the YO transitions from the basal to the activated state and the animal enters premolt. During mid-premolt, the YO transitions to the committed state, in which the YO becomes insensitive to MIH. Phosphodiesterases (PDEs) hydrolyze the phosphodiester bond in cAMP and cGMP to AMP and GMP, respectively, and thus can modulate the response of the YO to MIH. In some species, PDE inhibitors decrease molting hormone (ecdysteroid) biosynthesis by the YO *in-vitro*, indicating that PDE activity can keep cyclic nucleotide levels low. Increased PDE activity in the YO is correlated with a reduced sensitivity to MIH when the animal becomes committed to molt. In mammals, 21 PDE genes are organized into 11 genes, designated PDE1 to PDE11. Each PDE family has specific catalytic and biochemical properties and tissue distributions. A reference YO transcriptome from the blackback land crab (*Gecarcinus lateralis*), consisting of 3 biological replicates of intermolt animals, was analyzed for PDE sequences. Nine different contigs encoding seven full-length PDE sequences two partials were identified. Moreover, seven different contigs encoding five full-length PDE sequences two partials were identified in the *C. maenas* transcriptome. Protein alignments and ClustalX analysis of the Gl-PDE and Cm-PDE sequences with orthologs from other species in the GenBank database showed that the sequences corresponded to PDE1, 2, 3 4, 5, 7, 8, 9, and 11. General and selective inhibitors were used to characterize the PDEs regulating ecdysteroid secretion in the green crab, *Carcinus maenas* YO. IBMX, vinpocetine, EHNA and zaprinast ± rMIH significantly inhibited ecdysteroid secretion, while rolipram, dipyridamole, and BC11-38

did not. This suggests that PDE1, PDE2 and PDE5/11 are primarily responsible for regulating cAMP and cGMP levels. No effect on ecdysteroidogenesis was seen on the blackback land crab, *Gecarcinus lateralis*, YOs when exposed to the same PDE inhibitors *in-vitro*, indicating different regulatory metabolic machineries between the two species.

PDE gene expression was examined in different tissues of *G. lateralis* and *C. maenas* by qPCR. Gl-PDE2 and Gl-PDE5 showed mild levels of mRNA expression. Cm-PDE11 had the highest expression among all examined PDEs which might indicate a pivotal role in cAMP/cGMP signaling pathways.

qPCR was used to quantify the effects of molt induction by multiple limb autotomy (MLA) or eyestalk ablation (ESA) ± mTOR inhibitor rapamycin on expression of PDE 1, 2, 4, 5,7,9,11 in *Gecarcinus lateralis* YO. In response to MLA, all PDEs, except for PDE5 and PDE11, were expressed at their highest levels in the intermolt YO. mRNA levels declined during premolt and reached their lowest levels in postmolt. qPCR results from the MLA experiment showed that both Gl-PDE5 and Gl-PDE11 reached high expression levels in mid premolt and late premolt, respectively. MLA transcriptomics revealed that only PDE11 expression maximized at mid premolt. In response to ESA, the mRNA levels of PDE4, 5, 7, 9, and 11 showed no significant change by 7- and 14-days post-ESA. Rapamycin had no significant effect, as PDE mRNA levels were comparable to those of controls at all time points, indicating that PDE expression is not regulated by mTOR. The qPCR results were consistent with RNA-Seq data, showing similar trends of PDE expression in both MLA and ESA ± rapamycin. The data suggest that transcriptional regulation does not contribute to the reduced sensitivity of the committed YO to MIH; the increased PDE activity during mid and late premolt is likely regulated post-transcriptionally in most PDEs. Our data suggest that PDE11 is the controlling PDE in the YO and shows mRNA level

changes depending on the molt stage. This finding is consistent with the responsiveness of YO cells to MIH during mid/late premolt.

The number of PDE families were unknown before this project, and it was proposed that two PDE families might contribute in the MIH signaling pathway. The outcome of this project was surprising, as nine different PDEs, each with their unique properties and characteristics were found in the YO transcriptome of *G. lateralis* and the transcriptome of *C. maenas*. Moreover, our data suggest that PDE11 might be the prominent enzyme in the YO.

Future studies should investigate the protein levels of these PDEs in the YO and other tissues. Rapamycin has no effect on PDE mRNA levels, indicating mTOR activity does not control PDE gene expression in the activated YO. However, Activin/TGFβ signaling, which is required for YO commitment, may control PDE gene expression in mid and late premolt. Transcriptomics and qPCR can be used to determine whether SB431542, an inhibitor on Activin/TGFβ signaling, affects PDE mRNA levels.

REFERENCES

Abuhagr, A. M., MacLea, K. S., Chang, E. S., & Mykles, D. L. (2014). Mechanistic target of rapamycin (mTOR) signaling genes in decapod crustaceans: cloning and tissue expression of mTOR, Akt, Rheb, and p70 S6 kinase in the green crab, Carcinus maenas, and blackback land crab, Gecarcinus lateralis. *Comparative Biochemistry and Physiology A-molecular & Integrative Physiology, 168*, 25-39. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s1095643313002730

Ahmad, F., Murata, T., Shimizu, K., Degerman, E., Maurice, D., & Manganiello, V. C. (2015). Cyclic Nucleotide Phosphodiesterases: important signaling modulators and therapeutic targets. *Oral Diseases, 21*(1), 25-50. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc4275405

Ahn, H. S., Crim, W., Romano, M., Sybertz, E. J., & Pitts, B. (1989). Effects of selective inhibitors on cyclic nucleotide phosphodiesterases of rabbit aorta. *Biochemical Pharmacology, 38*(19), 3331-3339. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/000629528990631x#!

Altschul, S. F., Wootton, J. C., Gertz, E. M., Agarwala, R., Morgulis, A., Schäffer, A. A., & Yu, Y.-K. (2005). Protein Database Searches Using Compositionally Adjusted Substitution Matrices. *FEBS Journal, 272*(20), 5101-5109. Retrieved 2 17, 2019, from https://febs.onlinelibrary.wiley.com/doi/full/10.1111/j.1742-4658.2005.04945.x

Altschul, S., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*(17), 3389–3402. Retrieved 2 17, 2019

Andric, S. A., Janjic, M. M., Stojkov, N. J., & Kostic, T. S. (2010). Sildenafil treatment in-vivo stimulates Leydig cell steroidogenesis via the cAMP/cGMP signaling pathway. *American Journal of Physiology-endocrinology and Metabolism, 299*(4). Retrieved 2 17, 2019, from https://physiology.org/doi/abs/10.1152/ajpendo.00337.2010

Azevedo, M., Azevedo, M., Faucz, F. R., Faucz, F. R., Bimpaki, E. I., Horvath, A., . . . Stratakis, C. A. (2014). Clinical and Molecular Genetics of the Phosphodiesterases (PDEs). *Endocrine Reviews, 35*(2), 195-233. Retrieved 2 17, 2019, from https://academic.oup.com/edrv/article/35/2/195/2354677

Beavo, J. A., & Brunton, L. L. (2002). Cyclic nucleotide research — still expanding after half a century. *Nature Reviews Molecular Cell Biology, 3*(9), 710-717. Retrieved 2 17, 2019, from https://nature.com/articles/nrm911

Beavo, J. A., Rogers, N. L., Crofford, O. B., Hardman, J. G., Sutherland, E. W., & Newman, E. V. (1970). Effects of Xanthine Derivatives on Lipolysis and on Adenosine 3',5'-Monophosphate Phosphodiesterase Activity. *Molecular Pharmacology, 6*(6), 597-603. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/4322367

Bellen, H. J., Gregory, B. K., Olsson, C. L., & Kiger, J. A. (1987). Two Drosophila learning mutants, dunce and rutabaga, provide evidence of a maternal role for cAMP on embryogenesis. *Developmental Biology, 121*(2), 432-444. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/3034702

Bender AT, B. J. (2006). Cyclic Nucleotide Phosphodiesterases: Molecular Regulation to Clinical Use. *Pharmacological Reviews,58 (3)*, 488-520.

Bessodes, M., Bastian, G., Abushanab, E., Panzica, R. P., Berman, S. F., Marcaccio, E. J., . . . Parks, R. E. (1982). Effect of chirality in erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) on adenosine deaminase inhibition. *Biochemical Pharmacology, 31*(5), 879-882. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/0006295282904798

Bolger G, M. T. (n.d.). A family of human phosphodiesterases homologous to the dunce learning and memory gene product of Drosophila melanogaster are potential targets for antidepressant drugs. *Molecular and Cellular Biology, 13*(10), 6558–71. Retrieved 2 17, 2019, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC364715/pdf/molcellb00022-0642.pdf

Bolger G, M. T. (n.d.). A family of human phosphodiesterases homologous to the dunce learning and memory gene product of Drosophila melanogaster are potential targets for antidepressant drugs. *Molecular and Cellular Biology, 13*(10), 6558–71. Retrieved 2 18, 2019

Broderick, K. E., MacPherson, M. R., Regulski, M., Tully, T., Dow, J. A., & Davies, S. A. (2003). Interactions between epithelial nitric oxide signaling and phosphodiesterase activity in Drosophila. *American Journal of Physiology-cell Physiology, 285*(5). Retrieved 2 17, 2019, from https://physiology.org/doi/10.1152/ajpcell.00123.2003

Ceyhan, O., Birsoy, K., & Hoffman, C. S. (2012). Identification of Biologically Active PDE11-Selective Inhibitors Using a Yeast-Based High-Throughput Screen. *Chemistry & Biology, 19*(1), 155-163. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s1074552111004625

Chang, E. S., & Mykles, D. L. (2011). Regulation of crustacean molting: a review and our perspectives. *General and Comparative Endocrinology, 172*(3), 323-330. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0016648011001444

Chang, E. S., Bruce, M. J., & Tamone, S. L. (1993). Regulation of Crustacean Molting: A Multi-Hormonal System. *Integrative and Comparative Biology, 33*(3), 324-329. Retrieved 2 18, 2019, from https://academic.oup.com/icb/article/33/3/324/165907

Charbonneau, H., Beier, N., Walsh, K. A., & Beavo, J. A. (1986). Identification of a conserved domain among cyclic nucleotide phosphodiesterases from diverse species. *Proceedings of the National Academy of Sciences of the United States of America, 83*(24), 9308-9312. Retrieved 2 16, 2019, from https://ncbi.nlm.nih.gov/pubmed/3025833

Choi, C. H., Choi, C. H., Choi, C. H., Schoenfeld, B. P., Schoenfeld, B. P., Weisz, E. D., . . . McBride, S. M. (2015). PDE-4 inhibition rescues aberrant synaptic plasticity in Drosophila and mouse models of fragile X syndrome. *The Journal of Neuroscience, 35*(1), 396-408. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/25568131

Chung, J. S., Zmora, N., Katayama, H., & Tsutsui, N. (2010). Crustacean hyperglycemic hormone (CHH) neuropeptidesfamily: Functions, titer, and binding to target tissues. *General and Comparative Endocrinology, 166*(3), 447-454. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0016648009004389

Conti, M. (2000). Phosphodiesterases and Cyclic Nucleotide Signaling in Endocrine Cells. *Molecular Endocrinology, 14*(9), 1317-1327. Retrieved 2 17, 2019, from http://press.endocrine.org/doi/10.1210/mend.14.9.0534

Cornu, M., Albert, V., & Hall, M. N. (2013). mTOR in aging, metabolism, and cancer. *Current Opinion in Genetics & Development, 23*(1), 53-62. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0959437x12001499

Covi, J. A., Bader, B. D., Chang, E. S., & Mykles, D. L. (2010). Molt cycle regulation of protein synthesis in skeletal muscle of the blackback land crab, Gecarcinus lateralis, and the differential expression of a myostatin-like factor during atrophy induced by molting or unweighting. *The Journal of Experimental Biology, 213*(1), 172-183. Retrieved 2 17, 2019, from http://jeb.biologists.org/content/213/1/172

Covi, J. A., Chang, E. S., & Mykles, D. L. (2009). Conserved role of cyclic nucleotides in the regulation of ecdysteroidogenesis by the crustacean molting gland. *Comparative Biochemistry and Physiology A-molecular & Integrative Physiology, 152*(4), 470-477. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s1095643308012361#!

Covi, J. A., Chang, E. S., & Mykles, D. L. (2012). Neuropeptide signaling mechanisms in crustacean and insect molting glands. *Invertebrate Reproduction & Development, 56*(1), 33-49. Retrieved 2 17, 2019, from http://tandfonline.com/doi/abs/10.1080/07924259.2011.588009

Das, S., Pitts, N. L., Mudron, M. R., Durica, D. S., & Mykles, D. L. (2016). Transcriptome analysis of the molting gland (Y-organ) from the blackback land crab, Gecarcinus lateralis. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 17*, 26-40. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s1744117x15000672

Dauphin-Villemant, C., Böcking, D., & Sedlmeier, D. (1995). Regulation of steroidogenesis in crayfish molting glands: involvement of protein synthesis. *Molecular and Cellular Endocrinology, 109*(1), 97-103. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/030372079503489t#!

Day, J. P., Dow, J. A., Houslay, M. D., & Davies, S. A. (2005). Cyclic nucleotide phosphodiesterases in Drosophila melanogaster. *Biochemical Journal, 388*(1), 333-342. Retrieved 2 17, 2019, from http://biochemj.org/content/388/1/333

Demirbas, D., Wyman, A. R., Shimizu-Albergine, M., Cakici, O., Beavo, J. A., & Hoffman, C. S. (2013). A yeast-based chemical screen identifies a PDE inhibitor that elevates steroidogenesis in mouse Leydig cells via PDE8 and PDE4 inhibition. *PLOS ONE, 8*(8). Retrieved 2 24, 2019, from http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0071279

Deshmukh, R., Sharma, V., Mehan, S., Sharma, N., & Bedi, K. (2009). Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine -- a PDE1 inhibitor. *European Journal of Pharmacology, 620*(1), 49-56. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0014299909007146

Drach, P. (1939). Mue et cycle d'intermue chez les Crustaces Decapodes. Annls. Inst. Oceanogr. 19: 103-391.

Fajardo, A. M., Piazza, G. A., & Tinsley, H. N. (2014). The Role of Cyclic Nucleotide Signaling Pathways in Cancer: Targets for Prevention and Treatment. *Cancers, 6*(1), 436-458. Retrieved 2 17, 2019, from http://mdpi.com/2072-6694/6/1/436

Fisher DA, S. J. (n.d.). Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. *J Biol Chem, 273*(25), 15559–64. Retrieved 2 17, 2019

Francis, S. H. (2005). Phosphodiesterase 11 (PDE11): is it a player in human testicular function? *International Journal of Impotence Research, 17*(5), 467-468. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/16079899

Francis, S. H., Blount, M. A., & Corbin, J. D. (2011). Mammalian Cyclic Nucleotide Phosphodiesterases: Molecular Mechanisms and Physiological Functions. *Physiological Reviews, 91*(2), 651-690. Retrieved 2 18, 2019, from https://ncbi.nlm.nih.gov/pubmed/21527734

G.J., S., B.W., A., S.K., F., R.W., A., M.D., U., & yes. (1999). *Basic neurochemistry: molecular, cellular, and medical aspects.* American Society for Neurochemistry. Retrieved 2 17, 2019, from https://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=bnchm.section.1181

Gomez, L., & Breitenbucher, J. G. (2013). PDE2 inhibition: potential for the treatment of cognitive disorders. *Bioorganic & Medicinal Chemistry Letters, 23*(24), 6522-6527. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0960894x13012110

Goraya, T. A., & Cooper, D. M. (2005). Ca2+-calmodulin-dependent phosphodiesterase (PDE1): current perspectives. *Cellular Signalling, 17*(7), 789-797. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0898656804002931

Gresele, P., Momi, S., & Falcinelli, E. (2011). Anti-platelet therapy: phosphodiesterase inhibitors. *British Journal of Clinical Pharmacology, 72*(4), 634-646. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc3195739

Halpin, D. M. (2008). ABCD of the phosphodiesterase family: interaction and differential activity in COPD. *International Journal of Chronic Obstructive Pulmonary Disease, 3*(4), 543-561. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc2650605

Heikaus, C. C., Pandit, J., & Klevit, R. E. (2009). Cyclic Nucleotide Binding GAF Domains from Phosphodiesterases: Structural and Mechanistic Insights. *Structure, 17*(12), 1551-1557. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/20004158

Hietakangas, V., & Cohen, S. M. (2009). Regulation of tissue growth through nutrient sensing. *Annual Review of Genetics, 43*(1), 389-410. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/19694515

Ho, Y.-S. J., Burden, L. M., & Hurley, J. H. (2000). Structure of the GAF domain, a ubiquitous signaling motif and a new class of cyclic GMP receptor. *The EMBO Journal, 19*(20), 5288-5299. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc314001

Houslay, M. D., & Adams, D. R. (2003). PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. *Biochemical Journal, 370*(1), 1-18. Retrieved 2 17, 2019, from http://biochemj.org/content/370/1/1

Ke, H., Wang, H., & Ye, M. (2011). Structural Insight into the Substrate Specificity of Phosphodiesterases. *Handbook of experimental pharmacology*(204), 121-134. Retrieved 2 18, 2019, from https://ncbi.nlm.nih.gov/pubmed/21695637

Kelly, M. P. (2017). *A Role for Phosphodiesterase 11A (PDE11A) in the Formation of Social Memories and the Stabilization of Mood*. Retrieved 2 17, 2019, from https://link.springer.com/chapter/10.1007/978-3-319-58811-7_8

Kim, H. W., Ha, S. H., Lee, M. N., Huston, E., Kim, D.-H., Jang, S. K., . . . Ryu, S. H. (2010). cAMP controls mTOR through regulation of the dynamic interaction between Rheb and Phosphodiesterase 4D. *Molecular and Cellular Biochemistry, 30*, 5406-5420. Retrieved 2 17, 2019, from https://kclpure.kcl.ac.uk/portal/en/publications/camp-controls-mtor-through-regulation-of-the-dynamic-interaction-between-rheb-and-phosphodiesterase-4d(cf31d25d-ef9f-4a0c-9c27-d39117b3f954).html

Knope, M. L., & Larson, R. J. (2014). Autotomy in porcelain crabs is an effective escape mechanism from rockfish predation. *Marine Ecology, 35*(4), 471-477. Retrieved 2 17, 2019, from http://onlinelibrary.wiley.com/doi/10.1111/maec.12103/abstract

Kwon, G., Marshall, C. A., Pappan, K. L., Remedi, M. S., & McDaniel, M. L. (2004). Signaling elements involved in the metabolic regulation of mTOR by nutrients, incretins, and

growth factors in islets. *Diabetes, 53*. Retrieved 2 17, 2019, from http://diabetes.diabetesjournals.org/content/53/suppl_3/s225

Laplante, M., & Sabatini, D. M. (2009). mTOR signaling at a glance. *Journal of Cell Science, 122*(20), 3589-3594. Retrieved 2 17, 2019, from http://jcs.biologists.org/content/joces/122/20/3589.full.pdf

Layalle, S., Arquier, N., & Léopold, P. (2008). The TOR Pathway Couples Nutrition and Developmental Timing in Drosophila. *Developmental Cell, 15*(4), 568-577. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s1534580708003274

Lee, S. G., & Mykles, D. L. (2006). Proteomics and signal transduction in the crustacean molting gland. *Integrative and Comparative Biology, 46*(6), 965-977. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/21672800

Lee, S. G., Bader, B. D., Chang, E. S., & Mykles, D. L. (2007). Effects of elevated ecdysteroid on tissue expression of three guanylyl cyclases in the tropical land crab Gecarcinus lateralis: possible roles of neuropeptide signaling in the molting gland. *The Journal of Experimental Biology, 210*(18), 3245-3254. Retrieved 2 17, 2019, from http://jeb.biologists.org/content/210/18/3245

Levy, I., Horvath, A., Azevedo, M., Alexandre, R. B., Alexandre, R. B., & Stratakis, C. A. (2011). Phosphodiesterase function and endocrine cells: links to human disease and roles in tumor development and treatment. *Current Opinion in Pharmacology, 11*(6), 689-697. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s1471489211001858

Libé, R., Fratticci, A., Coste, J., Tissier, F., Horvath, A., Ragazzon, B., . . . Bertherat, J. (2008). Phosphodiesterase 11A (PDE11A) and genetic predisposition to adrenocortical tumors. *Clinical Cancer Research, 14*(12), 4016-4024. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/18559625

Lin C S, L. R. (2002). Human PDE5 gene encodes three PDE5 isoforms from two alternate promoters. *International Journal of Impotence Research volume 14*, 15-24.

Liu, S., Mansour, M. N., Dillman, K. S., Perez, J. R., Danley, D. E., Aeed, P. A., . . . Menniti, F. S. (2008). Structural basis for the catalytic mechanism of human phosphodiesterase 9. *Proceedings of the National Academy of Sciences of the United States of America, 105*(36), 13309-13314. Retrieved 2 17, 2019, from http://pnas.org/content/105/36/13309.full.pdf

Makhlouf, A., Kshirsagar, A., & Niederberger, C. (2006). Phosphodiesterase 11: a brief review of structure, expression and function. *International Journal of Impotence Research, 18*(6), 501-509. Retrieved 2 17, 2019, from http://nature.com/ijir/journal/v18/n6/full/3901441a.html

Manallack, D. T., Hughes, R. A., & Thompson, P. E. (2005). The next generation of phosphodiesterase inhibitors: structural clues to ligand and substrate selectivity of

phosphodiesterases. *Journal of Medicinal Chemistry, 48*(10), 3449-3462. Retrieved 2 17, 2019, from http://onlinelibrary.wiley.com/doi/10.1002/chin.200534323/full

Martins, T. J., Mumby, M. C., & Beavo, J. A. (1982). Purification and characterization of a cyclic GMP-stimulated cyclic nucleotide phosphodiesterase from bovine tissues. *Journal of Biological Chemistry, 257*(4), 1973-1979. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/6276403

Matoulkova, E., Michalová, E., Vojtesek, B., & Hrstka, R. (2012). The role of the 3' untranslated region in post-transcriptional regulation of protein expression in mammalian cells. *RNA Biology, 9*(5), 563-576. Retrieved 2 17, 2019, from https://tandfonline.com/doi/full/10.4161/rna.20231

Mattson, M. P., & Spaziani, E. (1985). Cyclic AMP mediates the negative regulation of Y-organ ecdysteroid production ☆. *Molecular and Cellular Endocrinology, 42*(2), 185-189. Retrieved 2 18, 2019, from https://sciencedirect.com/science/article/pii/0303720785901066#!

Medina, A. E. (2011). Therapeutic utility of phosphodiesterase type I inhibitors in neurological conditions. *Frontiers in Neuroscience, 5*, 21-21. Retrieved 2 18, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc3044262

Méhats, C., Andersen, C. B., Filopanti, M., Jin, S.-L. C., & Conti, M. (2002). Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends in Endocrinology and Metabolism, 13*(1), 29-35. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/11750860

Miller, C. L., Oikawa, M., Cai, Y.-J., Wojtovich, A. P., Nagel, D. J., Xu, X., . . . Yan, C. (2009). Role of Ca2+/Calmodulin-Stimulated Cyclic Nucleotide Phosphodiesterase 1 in Mediating Cardiomyocyte Hypertrophy. *Circulation Research, 105*(10), 956-964. Retrieved 2 17, 2019, from https://ahajournals.org/doi/10.1161/circresaha.109.198515

Mittal, R., Bencie, N., Shaikh, N., Mittal, J., Liu, X. Z., & Eshraghi, A. A. (2017). Role of Cyclic Nucleotide Phosphodiesterases in Inner Ear and Hearing. *Frontiers in Physiology, 8*, 908. Retrieved 2 17, 2019, from https://frontiersin.org/articles/10.3389/fphys.2017.00908/full

Murthy, V. S., & Mangot, A. G. (2015). Psychiatric aspects of phosphodiesterases: An overview. *Indian Journal of Pharmacology, 47*(6), 594. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/26729948

Nakatsuji, T., Lee, C.-Y., & Watson, R. D. (2009). Crustacean molt-inhibiting hormone: Structure, function, and cellular mode of action. *Comparative Biochemistry and Physiology A-molecular & Integrative Physiology, 152*(2), 139-148. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s1095643308011823

Nakatsuji, T., Sonobe, H., & Watson, R. D. (2006). Molt-inhibiting hormone-mediated regulation of ecdysteroid synthesis in Y-organs of the crayfish (Procambarus clarkii): involvement of cyclic GMP and cyclic nucleotide phosphodiesterase. *Molecular and*

Cellular Endocrinology, 253(1), 76-82. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0303720706002383

NiKulina E, T. J. (2004). The phosphodiesterase inhibitor rolipram delivered after a spinal cord lesion promotes axonal regeneration and functional recovery. *Proc. Natl. Aca. Sci.USA 101*, 8786-8790. doi: 10.1073/pnas.0402595101

P, W., P, W., RW, E., & MM, B. (2003). Identification and characterization of a new human type 9 cGMP-specific phosphodiesterase splice variant (PDE9A5). Differential tissue distribution and subcellular localization of PDE9A variants. *Gene 314*, 15-27.

Puzzo, D., Sapienza, S., Arancio, O., & Palmeri, A. (2008). Role of phosphodiesterase 5 in synaptic plasticity and memory. *Neuropsychiatric Disease and Treatment, 4*(2), 371-387. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc2518390

Qiu, Y., Chen, C.-N., Malone, T., Richter, L., Beckendorf, S. K., & Davis, R. L. (1991). Characterization of the memory gene dunce of Drosophila melanogaster. *Journal of Molecular Biology, 222*(3), 553-565. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/002228369190496s

Richter, W., Unciuleac, L., Hermsdorf, T., Kronbach, T., & Dettmer, D. (2001). Identification of inhibitor binding sites of the cAMP-specific phosphodiesterase 4. *Cellular Signalling, 13*(4), 287-297. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0898656801001504

Rocha, A. S., Rocha, A. S., Paternot, S., Coulonval, K., Dumont, J. E., Soares, P., & Roger, P. P. (2008). Cyclic AMP Inhibits the Proliferation of Thyroid Carcinoma Cell Lines through Regulation of CDK4 Phosphorylation. *Molecular Biology of the Cell, 19*(11), 4814-4825. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/18799615

Sandeep, G., Bhasker, S., & Ranganath, Y. S. (2008). Phosphodiesterase as a novel target in Cancer Chemotherapy. *The Internet Journal of Pharmacology, 7*(1). Retrieved 2 17, 2019, from http://ispub.com/ijpharm/7/1/3739

Saraiva, K. L., Silva, A. K., Wanderley, M. I., Araújo, A. A., Souza, J. R., & Peixoto, C. A. (2009). Chronic treatment with sildenafil stimulates Leydig cell and testosterone secretion. *International Journal of Experimental Pathology, 90*(4), 454-462. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc2741156

Sette, C., & Conti, M. (1996). Phosphorylation and Activation of a cAMP-specific Phosphodiesterase by the cAMP-dependent Protein Kinase INVOLVEMENT OF SERINE 54 IN THE ENZYME ACTIVATION. *Journal of Biological Chemistry, 271*(28), 16526-16534. Retrieved 2 17, 2019, from http://jbc.org/content/271/28/16526.long

Shyamal, S., Sudha, K., Gayathri, N., & Anilkumar, G. (2014). The Y-organ secretory activity fluctuates in relation to seasons of molt and reproduction in the brachyuran crab, Metopograpsus messor (Grapsidae): Ultrastructural and immunohistochemical study.

General and Comparative Endocrinology, 196, 81-90. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0016648013004577

Siegel GJ, A. B. (1999). *Basic Neurochemistiry: Molecular, Cellular and Medical Aspects. 6th edition.* Philadephia: lippincott-raven.

Skinner, D. M. (1962). THE STRUCTURE AND METABOLISM OF A CRUSTACEAN INTEGUMENTARY TISSUE DURING A MOLT CYCLE. *The Biological Bulletin, 123*(3), 635-647. Retrieved 2 18, 2019, from https://journals.uchicago.edu/doi/abs/10.2307/1539584

Skinner, D. M. (1985). Interacting Factors in the Control of the Crustacean Molt Cycle. *Integrative and Comparative Biology, 25*(1), 275-284. Retrieved 2 17, 2019, from https://academic.oup.com/icb/article-pdf/25/1/275/5940264/25-1-275.pdf

Skinner, D. M., & Graham, D. E. (1972). LOSS OF LIMBS AS A STIMULUS TO ECDYSIS IN BRACHYURA (TRUE CRABS). *The Biological Bulletin, 143*(1), 222-233. Retrieved 2 17, 2019, from https://journals.uchicago.edu/doi/abs/10.2307/1540342

Smith, W. A. (1993). Second Messengers and the Action of Prothoracicotropic Hormone in Manduca sexta. *Integrative and Comparative Biology, 33*(3), 330-339. Retrieved 2 18, 2019, from https://academic.oup.com/icb/article/33/3/330/165920

Soderling, S. H., & Beavo, J. A. (2000). Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Current Opinion in Cell Biology, 12*(2), 174-179. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0955067499000733

Sonnenburg, W. K., Seger, D., Kwak, K. S., Huang, J., Charbonneau, H., & Beavo, J. A. (1995). Identification of Inhibitory and Calmodulin-binding Domains of the PDE1A1 and PDE1A2 Calmodulin-stimulated Cyclic Nucleotide Phosphodiesterases. *Journal of Biological Chemistry, 270*(52), 30989-31000. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/8537356

Tepolt, C. K., & Palumbi, S. R. (2015). Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. *Molecular Ecology, 24*(16), 4145-4158. Retrieved 2 24, 2019, from http://palumbi.stanford.edu/manuscripts/tepolt_et_al-2015-molecular_ecology.pdf

Thompson, W. J., & Appleman, M. M. (1971). Characterization of Cyclic Nucleotide Phosphodiesterases of Rat Tissues. *Journal of Biological Chemistry, 246*(10), 3145-3150. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/4324892

Tomes, C., Rossi, S., & Silvia, M. (1993). Isobutylmethylxanthine and other classical cyclic nucleotide phosphodiesterase inhibitors affect cAMP-dependent protein kinase activity. *Cellular Signalling, 5*(5), 615-621. Retrieved 2 18, 2019, from https://sciencedirect.com/science/article/pii/089865689390056r

Tsai, L. C., & Beavo, J. A. (2011). The roles of cyclic nucleotide phosphodiesterases (PDEs) in steroidogenesis. *Current Opinion in Pharmacology, 11*(6), 670-675. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc4034742

Tsai, L.-C. L., & Beavo, J. A. (2012). Regulation of adrenal steroidogenesis by the high-affinity phosphodiesterase 8 family. *Hormone and Metabolic Research, 44*(10), 790-794. Retrieved 2 24, 2019, from https://ncbi.nlm.nih.gov/pubmed/22903278

Verbruggen, B., Bickley, L. K., Santos, E. M., Tyler, C. R., Stentiford, G. D., Bateman, K. S., & Aerle, R. v. (2015). De novo assembly of the Carcinus maenas transcriptome and characterization of innate immune system pathways. *BMC Genomics, 16*(1), 458-458. Retrieved 2 24, 2019, from https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-015-1667-1

Vezzosi, D., Vezzosi, D., Libé, R., Libé, R., Baudry, C., Baudry, C., . . . Bertherat, J. (2012). Phosphodiesterase 11A (PDE11A) Gene Defects in Patients with ACTH-Independent Macronodular Adrenal Hyperplasia (AIMAH): Functional Variants May Contribute to Genetic Susceptibility of Bilateral Adrenal Tumors. *The Journal of Clinical Endocrinology and Metabolism, 97*(11). Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc3485605

Walter, M., & Kiger, J. (1984). The Dunce gene of Drosophila: roles of Ca2+ and calmodulin in adenosine 3':5'-cyclic monophosphate-specific phosphodiesterase activity. *The Journal of Neuroscience, 4*(2), 495-501. Retrieved 2 17, 2019, from http://jneurosci.org/content/jneuro/4/2/495.full.pdf

Wang, P., Wu, P., Egan, R. W., & Billah, M. M. (2003). Identification and characterization of a new human type 9 cGMP-specific phosphodiesterase splice variant (PDE9A5): Differential tissue distribution and subcellular localization of PDE9A variants. *Gene, 314*, 15-27. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0378111903007339

Weber, S., Zeller, M., Guan, K., Wunder, F., Wagner, M., & El-Armouche, A. (2017). PDE2 at the crossway between cAMP and cGMP signalling in the heart. *Cellular Signalling, 38*, 76-84. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s089865681730178x

Webster, S. G., Keller, R., & Dircksen, H. (2012). The CHH-superfamily of multifunctional peptide hormones controlling crustacean metabolism, osmoregulation, moulting, and reproduction. *General and Comparative Endocrinology, 175*(2), 217-233. Retrieved 2 17, 2019, from http://crustacean-biology.co.uk/wp-content/uploads/2014/02/simon-ref-4_2014-update_webster-et-al-2013_chh-review.pdf

Weeks, J. L., Corbin, J. D., & Francis, S. H. (2009). Interactions between Cyclic Nucleotide Phosphodiesterase 11 Catalytic Site and Substrates or Tadalafil and Role of a Critical Gln-869 Hydrogen Bond. *Journal of Pharmacology and Experimental Therapeutics,*

331(1), 133-141. Retrieved 2 17, 2019, from http://jpet.aspetjournals.org/content/331/1/133

Weis, J. S. (1976). EFFECTS OF ENVIRONMENTAL FACTORS ON REGENERATION AND MOLTING IN FIDDLER CRABS. *The Biological Bulletin, 150*(1), 152-162. Retrieved 2 17, 2019, from https://journals.uchicago.edu/doi/abs/10.2307/1540596

Xie, J., Ponuwei, G. A., Moore, C. E., Willars, G. B., Tee, A. R., & Herbert, T. P. (2011). cAMP inhibits mammalian target of rapamycin complex-1 and -2 (mTORC1 and 2) by promoting complex dissociation and inhibiting mTOR kinase activity. *Cellular Signalling, 23*(12), 1927-1935. Retrieved 2 17, 2019, from https://sciencedirect.com/science/article/pii/s0898656811001975

Xu, R. X., Hassell, A. M., Vanderwall, D., Lambert, M. H., Holmes, W. D., Luther, M. A., . . . Nolte, R. T. (2000). Atomic structure of PDE4: insights into phosphodiesterase mechanism and specificity. *Science, 288*(5472), 1822-1825. Retrieved 2 17, 2019, from https://rcsb.org/structure/1f0j

Xu, R. X., Rocque, W. J., Lambert, M. H., Vanderwall, D. E., Luther, M. A., & Nolte, R. T. (2004). Crystal structures of the catalytic domain of phosphodiesterase 4B complexed with AMP, 8-Br-AMP, and rolipram. *Journal of Molecular Biology, 337*(2), 355-365. Retrieved 2 17, 2019, from https://rcsb.org/structure/1ro6

Yan, K., Gao, L.-N., Cui, Y. L., Zhang, Y., & Zhou, X. (2016). The cyclic AMP signaling pathway: Exploring targets for successful drug discovery (Review). *Molecular Medicine Reports, 13*(5), 3715-3723. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc4838136

Yu, X., Chang, E. S., & Mykles, D. L. (2002). Characterization of Limb Autotomy Factor-Proecdysis (LAFpro), Isolated From Limb Regenerates, That Suspends Molting in the Land Crab Gecarcinus lateralis. *The Biological Bulletin, 202*(3), 204-212. Retrieved 2 18, 2019, from https://journals.uchicago.edu/doi/full/10.2307/1543470

Yuasa, K., Kanoh, Y., Okumura, K., & Omori, K. (2001). Genomic organization of the human phosphodiesterase PDE11A gene. *FEBS Journal, 268*(1), 168-178. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/11121118

Zoncu, R., Efeyan, A., & Sabatini, D. M. (2011). mTOR: from growth signal integration to cancer, diabetes and ageing. *Nature Reviews Molecular Cell Biology, 12*(1), 21-35. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pmc/articles/pmc3390257

Zoncu, R., Sabatini, D. M., & Efeyan, A. (2010). *mTOR: from growth signal integration to cancer, diabetes and ageing*. Retrieved 2 18, 2019, from http://dspace.mit.edu/handle/1721.1/74551?show=full

Zoraghi, R., & Seebeck, T. (2002). The cAMP-specific phosphodiesterase TbPDE2C is an essential enzyme in bloodstream form Trypanosoma brucei. *Proceedings of the National*

Academy of Sciences of the United States of America, 99(7), 4343-4348. Retrieved 2 17, 2019, from https://ncbi.nlm.nih.gov/pubmed/11930001