0026-895X/04/6502-267–278$20.00
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 65:267–278, 2004

Vol. 65, No. 2
2925/1122751
Printed in U.S.A.

MINIREVIEW

Properties and Functions of GAF Domains in Cyclic Nucleotide Phosphodiesterases and Other Proteins

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Received August 27, 2003; accepted September 15, 2003

This article is available online at http://molpharm.aspetjournals.org

This review focuses primarily on our current understanding of the structure and function of GAF domains in cyclic nucleotide phosphodiesterases (PDEs). The GAF domain was originally identified by Aravind and Ponting (1997) using the position-specific iterative BLAST method (Altschul et al., 1997). The consensus sequence defining GAF domains is taken from a characteristic primary sequence of chemically conserved amino acids (AAs) that is associated with a particular pattern of predicted secondary structures. GAF domains occur in a variety of proteins and in association with a diverse collection of other functional domains.

There are no invariant AAs in the predicted motifs that characterize these domains. The predicted GAF domains in closely related GAF-containing proteins contain sequence similarities; even among these, however, there are few invariant AAs. Among the 14 predicted GAF domains in human PDEs, a Phe is the only invariant amino acid. Twelve of the 14 predicted GAF domains contain the NK/RX_nFX_3DE signature sequence that we first described derived from the sequences in PDEs 2, 5, and 6 (McAllister-Lucas et al., 1995; Turko et al., 1996). Using site-directed mutagenesis of these AAs in PDE5, our laboratory demonstrated that each contributes importantly to the structural requirements for the allosteric cGMP-binding function in PDE5 (McAllister-Lucas et al., 1995; Turko et al., 1996). However, this Asn, Lys, and Asp arrangement occurs in GAF domains for which no ligand-binding function has been described, and its function is not understood.

The GAF acronym is derived from the names of the first three classes of proteins recognized to contain this domain: mammalian cGMP-binding PDEs, *Anabaena* adenylyl cyclases, and *Escherichia coli* FhlA (Aravind and Ponting, 1997). There are now more than 1400 proteins in the nonredundant database that are predicted to contain a GAF domain (Schultz et al., 1998; Letunic et al., 2002; see http://smart.embl-heidelberg.de). GAFs have been shown to be associated with gene regulation in bacteria (Aravind and Ponting, 1997), light-detection and signaling pathways in plant and cyanobacterial phytochromes (Sharrock and Quail, 1989; Montgomery and Lagarias, 2002), ethylene detection and signaling in plants (Sato-Nara et al., 1999), nitrogen fixation in bacteria (Joerger et al., 1989), feedback control of a cyanobacterial adenylyl cyclase by cAMP-binding (Kanacher et al., 2002), and the two-component sensor histidine kinase in viruses, bacteria, and plants (Table 1) (Kaneko et al., 2001; Urao et al., 2001). Notably, sequences predicted to form GAF domains are found in PDEs from diverse organisms including trypanosomatids, nematodes, sponges, insects, and mammals (Koyanagi et al., 1998; Schultz et al., 1998; Letunic et al., 2002; Rascon et al., 2002; Zoraghi and Seebeck, 2002; see http://smart.embl-heidelberg.de). GAF domains are described as one of the largest families of small-molecule-binding regulatory (R) domains, but direct evidence of ligand binding has only been demonstrated for a very few GAFs. The first demonstration of GAF domain binding of ligand was binding of cGMP to mammalian PDE5 (Lincoln et al., 1976; Francis et al., 1980). Increasingly, roles other than ligand binding are being documented, and it seems likely that all of the functions provided by GAF domains have not yet been fully appreciated.

Distribution of GAF Domains in PDEs. GAF motifs are composed of ~110 AA and are present in one to four (and even partial) copies in all living organisms from archaea to mammals; they are particularly abundant in plants and bacteria (Table 1). To date, the predicted GAF domains are distributed as follows: archaea (45), bacteria (463), viruses (1), fungi (11), plants (432), arthropods (9), nematodes (2),

This work was supported by National Institutes of Health research grants DK40299 and DK58277 and American Heart Association Postdoctoral Fellowship 032525B.

ABBREVIATIONS: GAF, a conserved domain found in mammalian cGMP-binding PDEs *Anabaena* adenylyl cyclases and *Escherichia coli* FhlA; PDE, phosphodiesterase; AA, amino acid; cN, cyclic nucleotide; CAP, catabolite gene-activating protein; PKA, cAMP-dependent protein kinases; PKG, cGMP-dependent protein kinases; C, catalytic; R, regulatory; TbPDE2, *Trypanosoma brucei* phosphodiesterase 2.

and chordates (99) (http://smart.embl-heidelberg.de). The first AA sequences associated with GAF domains in mammalian proteins were discovered by Charbonneau and colleagues long before the GAF motif was recognized (Charbonneau et al., 1990). These workers reported that the AA sequences of the R domains of several cGMP-binding PDEs (PDE2 and PDE6) contain two segments of homologous sequences composed of approximately 110 AAs. These segments are arranged in tandem and are separated by approximately 70 intervening AAs. At the time of this discovery, these homologous repeats had no obvious structural similarities with sequences in other known proteins. Charbonneau and colleagues (1990) suggested that these sequence repeats within the R domains of PDE2 and PDE6 provide for important functional features of these enzymes, including the allosteric cGMP binding that was known to be associated with this region. The first validation of this prediction was provided by the results of site-directed mutagenesis of conserved AAs in GAFs *a* and *b* of PDE5 (McAllister-Lucas et al., 1995; Turko et al., 1996). Many subsequent reports have also validated Charbonneau's prediction regarding the functional role of GAFs in PDEs (Thomas et al., 1990a; McAllister-Lucas et al., 1995; Aravind and Ponting, 1997; Anantharaman et al., 2001; Galperin et al., 2001; Francis et al., 2002; Liu et al., 2002; Martinez et al., 2002b).

The prominent role of GAF domains in PDE functions is now evident. The R domains of five of the 11 known class I mammalian PDE families (PDEs 2, 5, 6, 10, and 11) contain either one, two, or partial predicted GAF domain sequences (Fig. 1). The PDE superfamily is the only known group of mammalian proteins to have such an abundance of GAF domains. In the current database, GAF motifs occur in only two other proteins in chordates: 1) the latent transforming growth factor-β binding protein 4 from *Homo sapiens*, which has recently been cloned, contains one GAF motif and belongs to the family of extracellular microfibrillar proteins that may bind transforming growth factor-β (Giltay et al., 1997); and 2) the hypothetical EF-hand containing protein in *Mus muscularis*, which has only one GAF motif, although this protein has not been found, and its function is unknown (Okazaki et al., 2002).

Evolutionary Considerations of Predicted GAF Domains. The sequence similarity, domain organization, and branching pattern in the phylogenic tree indicate that all GAF subtypes apparently diverged from a common ancestral gene by gene duplication very early in the evolution of life when bacterial and eukaryotic lineages (>2 billion years ago) were established. However, over the long time span of animal evolution since the parazoan-eumetazoan split, there is no apparent subtype duplication (Kanacher et al., 2002). Furthermore, the GAF domain structure seems to have given rise to other domains with very different functions, including a domain in the eukaryote-specific actin-binding protein profilin, which has a protein-protein interaction function (Schluter et al., 1997) and the Cache domain, which has recently been shown to participate in extracellular ligand binding (Anantharaman et al., 2001). In PDEs, individual GAF domains have taken on multiple functional roles. Studies derived from genomic organization analysis of the catalytic domain (C domain) of PDEs reveal that PDE5A, PDE6s, and PDE11A are apparently derived from a common ancestral gene, and PDE2A and PDE10A are on a different branch of this ancestry (Yuasa et al., 2001). The sequence identity between the predicted GAF domains of human PDEs is shown in Fig. 2A. The phylogenic tree analysis of the AA sequences of these GAF domains demonstrates that GAF *a* domains of PDE5 and PDE11 are ~52% identical and seem to have a common origin (Figs. 2, A and B). From AA sequence

TABLE 1
GAF motif-containing proteins

Protein	Number of GAF Motifs	Functions	Organisms
Adenylyl cyclase	1–2	Signal transduction	Bacteria
Ethylene receptor	1	Ethylene metabolism	Plant (*Tracheophyta*), bacteria
Sensory histidine kinase	1–2	Signal transduction	Archea, bacteria, fungi, yeast
Two-component sensor histidine kinase	1–2–3–4	Signal transduction	Bacteria, viruses
Ser/Thr protein kinase	1–2	Signal transduction	Bacteria
Autolysin sensor kinase	1	Cell autolysis	Bacteria
LytS and LytR	1	Cell autolysis	Bacteria
cN phosphodiesterase	1–2	Signal transduction	Protobacteria, bacteria, trypanosomatids, sponges, insects, nematodes, mammals
Diguanylate cyclase/phosphodiesterase	1–2	Signal transduction	Bacteria
Methyl-accepting chemotaxis protein	1–3–4	Chemotaxis	Bacteria
Phosphoglucose isomerase	1	Glucose metabolism	Plant (*Tracheophyta*)
Phosphoenolpyruvate protein phosphotransferase	1	Glucose metabolism	Bacteria
Nitrate/nitrile sensor protein	1	Nitrogen fixation	Bacteria
Formate hydrogenlyase transcriptional activator	2	Gene regulation	Bacteria
NifA-specific transcriptional regulator	1	Gene regulation, nitrogen fixation	Bacteria, plasmodium
NtrC-specific transcriptional regulator	1	Gene regulation	Dictyostelium, bacteria
Sigma factor regulator of σ-B activity	1–2	Gene regulation	Bacteria
Transcriptional regulator AcoR	1	Gene regulation	Bacteria
Glycerol metabolism operon regulatory protein	1	Gene regulation	Bacteria
Bacterio-opsin activator	1	Phototransduction	Archea
Circadian protein kinase	1	Phototransduction	Archea
Phytochromes (A, B, C, D, E, F, N, O, P)	1	Phototransduction	Plant (*Tracheophyta*)
Photoreceptor	1	Phototransduction	Plant (*Bryophyta*)
Bacteriophytochrome	1	Phototransduction	Bacteria

NifA, specific activator of Nif genes; NtrC, nitrogen-regulatory protein C.

analysis of GAF domains of human PDEs (Figs. 2A and 3), one cannot conclude that GAF *a* domains have a significantly stronger relationship with each other than with GAF *b* and vice versa. This contrasts with the pattern of distribution of the cyclic nucleotide (cN)-binding sites in the cAMP-dependent protein kinases (PKA) and the cGMP-dependent protein kinases (PKG) (Shabb and Corbin, 1992). These enzymes contain two homologous cN-binding sites composed of ~120 AAs each that are evolutionarily distinct from GAF motifs, and that are also arranged in tandem in the protein sequences. The degree of similarity among cN-binding sites *a* in the various isoforms of PKG and PKA with other cN-binding sites *a* is greater than with the cN-binding site *b* either in the same protein or in other PKAs and PKGs. The same is true for the cN-binding site *b*. Remarkably, the cN-binding site *b* is the higher affinity site in PKA and PKGII, whereas in PKGI, cN-binding site *a* is the higher affinity site. This indicates that gene duplication of the sites occurred before the divergence of PKA and PKG, and functional differences evolved subsequently. In the GAF domains of PDEs, there is no such algorithm, suggesting that the evolutionary development of these GAF motifs and the cN-binding sites in the kinases differs.

There is apparently no relationship between the number of GAF motifs in a particular protein and the evolutionary status of the organism. In some mammalian PDE families (PDEs 2, 5, 6, and 10), all known isoforms contain two predicted GAF domains arranged in tandem. In contrast, the isoforms of PDE11 and a trypanosomatid PDE family (TbPDE2) contain either a single GAF motif, two GAF motifs arranged in tandem, or partial GAF motifs either alone or in combination with a complete GAF motif (Fig. 1) (Fujishige et al., 1999; Kotera et al., 1999; Yuasa et al., 2000; Fawcett et al., 2000; Seebeck et al., 2001). The only known PDE in nematodes has a single GAF motif. In all cases, the GAF motifs in PDE isoforms with only one GAF motif are more similar to the GAF *b* sites in PDEs containing two motifs. The origin of this pattern provokes a very interesting evolutionary question: did some PDEs acquire a single GAF that was then replicated to generate two GAFs per PDE, or did some PDEs originate with two GAFs followed by deletion of one? In trypanosomatids, the different PDE isoenzymes are

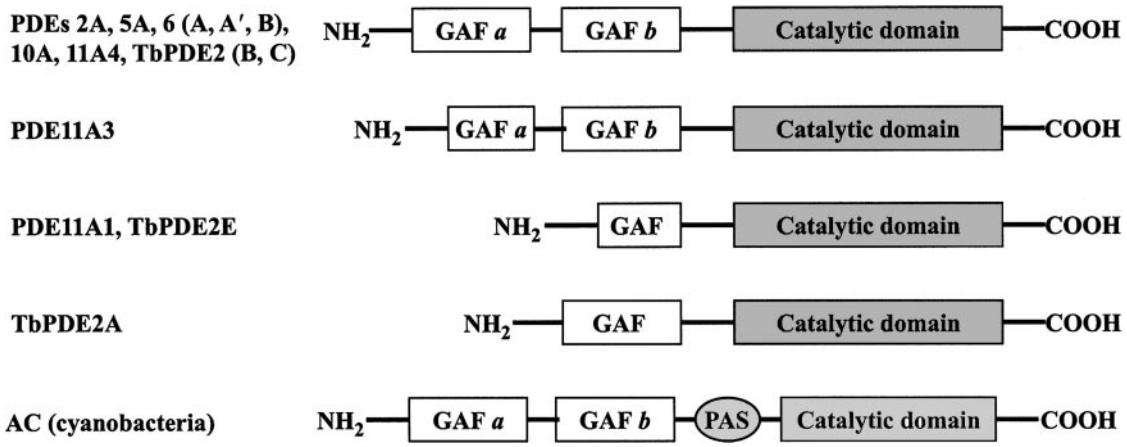

Fig. 1. The domain organization in mammalian and trypanosomatid PDEs and cyanobacterial adenylyl cyclase (AC). In all GAF-containing PDEs identified to date, the GAF domains are located N-terminal to a catalytic domain. A similar pattern is found in *Anabaena* AC, although here a PAS domain, which is evolutionarily related to GAF domains, is located between GAF domains and catalytic domain. Isoforms of the mammalian PDE11 family and a TbPDE2 family contain a complex pattern of GAF motifs, including truncated versions of GAF domains.

A

	2-*a*	2-*b*	5-*a*	5-*b*	6A-*a*	6A-*b*	6A′-*a*	6A′-*b*	6B-*a*	6B-*b*	10-*a*	10-*b*	11-*a*
2-*b*	22.8												
5-*a*	31.0	42.8											
5-*b*	20.1	30.4	34.9										
6A-*a*	28.3	32.4	33.7	18.8									
6A-*b*	18.3	28.8	29.0	28.5	29.0								
6A′-*a*	27.7	32.1	30.9	22.6	53.0	26.6							
6A′-*b*	18.8	33.8	29.6	28.5	31.2	77.4	28.6						
6B-*a*	28.9	31.5	35.7	21.4	65.2	31.8	55.7	28.6					
6B-*b*	17.2	31.0	25.5	27.6	32.4	82.8	19.1	78.5	24.1				
10-*a*	17.6	24.1	21.6	21.6	19.3	25.5	18.7	27.8	18.1	23.8			
10-*b*	24.1	46.9	44.7	31.8	30.5	25.9	30.3	27.2	28.0	21.9	27.8		
11-*a*	30.3	36.7	51.6	24.5	30.6	31.8	31.6	25.9	32.5	27.7	22.9	42.2	
11-*b*	22.3	38.0	43.7	41.9	18.8	33.7	17.3	30.8	25.0	34.6	20.9	39.0	41.2

B

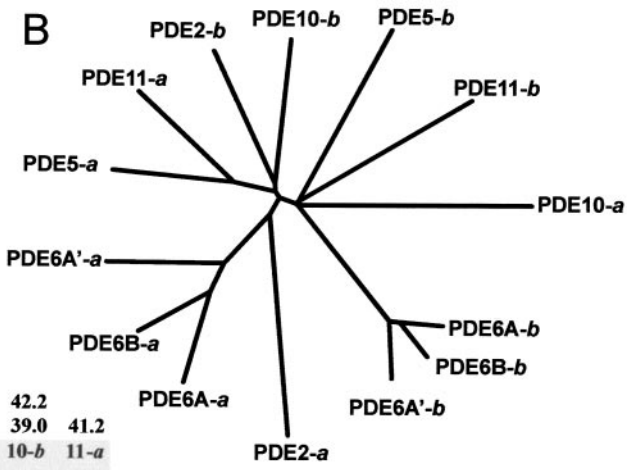

Fig. 2. The relatedness of human PDE GAF domains. A, the AA sequence conservation (percentage of identity) among GAF domains (*a* and *b*) of human PDE families calculated by GAP from Genetics computer group's (GCG) Wisconsin package. B, the phylogenic tree of the GAF domains of human PDE families. The tree is generated using the NJ algorithm of PHYLIP on the basis of a multiple alignment of the PDE GAF domains analyzed with Clustal W.

coded by distinct genes, whereas the mammalian PDE11 isoenzymes are splice variants of the same gene. Furthermore, in isoenzymes of mammalian PDE11s and the trypanosomatid PDEs containing a single GAF motif, sequences of ~40 to 50 AAs located in the N-terminal region of the GAF motif have no similarity with any segment of the R domain or the N-terminal region of the GAF *b* motif in isoforms with two GAF motifs.

Domain Structure of PDEs. The C domain of PDEs catalyzes the hydrolysis of cAMP and cGMP; the balance between synthesis of cNs by adenylyl or guanylyl cyclases and breakdown by PDEs largely determines cellular cN levels. PDEs are key regulators of cAMP- and cGMP-signaling pathways by controlling the spatial and temporal components of cN signals as well as the steady-state levels of intracellular cAMP and cGMP (Boekhoff et al., 1994). The regulatory features provided by GAF domains in certain PDE families contribute importantly to the modulation of cN levels (MacFarland et al., 1991; Wyatt et al., 1998; Corbin et al., 2003; Mullershausen et al., 2003; Rybalkin et al., 2003).

PDEs have been subdivided into three evolutionarily distinct classes, i.e., classes I, II, and III (Francis et al., 2001; Richter, 2002). All known mammalian PDEs are class I PDEs, and it seems that only this class contains the predicted GAF domains. There are 11 known families of mammalian PDEs that are products of more than 20 genes; they have been classified by DNA sequence analysis and by biochemical and pharmacological characteristics. Almost all class I PDEs are dimeric, but the contribution of dimerization to function is poorly understood. Each PDE monomer is a chimeric protein that contains a highly conserved C domain of ~270 AAs. Twenty AA in the C domain are invariant among mammalian PDEs, whereas if all mammalian and non-mammalian class I PDEs are included, i.e., regA of *Dictyostelium discoideum*, PDE2 of *Saccharomyces cerevisiae*, TbPDE2 of *Trypanosoma brucei*, and dunce of *Drosophila melanogaster*, only 14 AAs are invariant. The conserved C domain of each class I PDE is complexed with an R domain that has an N-terminal location to the C domain (Fig. 1). The R domains contain functional subdomains that contribute to regulation; these include phosphorylation sites, binding site(s) for protein inhibitors or activators, autoinhibitory sequences, subcellular localization signals, dimerization motifs, and allosteric cGMP-binding sites that are provided by GAF domains (Burns et al., 1996; Rybalkin and Beavo, 1996; Francis et al., 2001; Muradov et al., 2003b). To date, GAF domains in some PDEs have been shown to provide for dimerization, to interact with regulatory proteins, and to interact with small ligands including cGMP and cAMP (Francis et al., 2002; Martinez et al., 2002b).

Whereas functions of the C domains such as catalytic rate and affinity for substrate and inhibitors can be modulated by the respective R domains, ligand binding to the C domain can also impact functions of the respective R domains. This is as predicted from the principle of reciprocity (Weber, 1975). For example, interaction of cGMP or an analog (e.g., sildenafil) with the catalytic site of PDE5 profoundly increases cGMP binding to the allosteric sites (GAF domains) of its R domain, and cGMP binding to the GAF domains increases the affinity for sildenafil binding and cGMP interaction with the C domain (Okada and Asakawa, 2002; Corbin et al., 2003; Mullershausen et al., 2003; Rybalkin et al., 2003).

GAF Domain Structure and Functions in PDEs. Given the diverse evolutionary and functional contexts within which GAF domains occur, it is not surprising that in PDEs these domains possess a variety of functions (Fig. 4). Some functions may require the combined contributions of two GAFs because the presence of a duo of GAF domains is common in PDEs (Fig. 1) as well as in some other proteins. In PDEs, GAFs have been demonstrated to provide for the following: 1) cGMP binding to one or more GAFs in the R domains of PDEs 2 and 5; 2) dimerization and specificity of dimerization of the monomers in PDEs 2, 5, 6$\alpha\beta$, and 6$\alpha'\alpha'$; 3) interaction of the inhibitory subunit Pγ with PDE6 R domain (D'Amours and Cote, 1999; Muradov et al., 2002); 4) relief of autoinhibition of enzyme functions through direct effects in PDEs 2 and 5; and 5) relief of PDE autoinhibition by an indirect effect of phosphorylation through conformational changes in PDE5 (Fig. 4). The pathophysiological importance of GAF domains and cGMP-induced allostery in PDEs is illustrated by the fact that mutations in the GAF domains of human PDE6β subunit apparently cause autosomal-dominant congenital stationary night blindness and autosomal-recessive inheritance of retinitis pigmentosa (Gal et al., 1994; Danciger et al., 1995).

The precise boundaries required to form each of the GAF domains, to provide for structural stability, and to effect the regulation of PDEs have not been clearly defined. Three X-ray crystal structures of GAF domains have been reported; these include the two GAF domains (*a* and *b*) of PDE2 (Fig. 5A) (Martinez et al., 2002b) and the single GAF motif (YKG9) of unknown function from *S. cerevisiae* (Ho et al., 2000). Both are dimers. YKG9 has no known ligand-binding function. YKG9 is only distantly related to the GAFs of PDE2, but it contains the NK/RX_nFX_3DE sequence referred to earlier.

Structural Features of GAF Domains in PDEs. The X-ray crystal structure determined for the GAF domains of PDE2 provides a major advance in our knowledge of GAF domain structure and its relationship to function. First, it provides the first structures of two GAF domains from a mammalian protein. Second, in at least one conformation, it reveals the molecular basis for the relationship between the two GAFs that are arranged in tandem. Third, it reveals the molecular contacts that provide for distinct functions fulfilled by the GAFs in PDE2, i.e., cGMP-binding and dimerization. Fourth, it provides the first structure of a physiological cGMP-binding receptor containing bound cGMP; furthermore, this structure is novel and is evolutionarily unrelated to the cN-binding domains that occur in the bacterial catabolite gene-activating protein (CAP), PKG, PKA, and cN-gated cation channels (McKay and Steitz, 1981; Su et al., 1995; Biel et al., 1999; Pfeifer et al., 1999).

The X-ray crystal structure of the GAFs (*a* and *b*) in PDE2 reveals that cGMP is bound deeply in GAF *b* pocket; GAF *a* provides for dimerization (Fig. 5A). In contrast, cGMP binds with high affinity to the isolated GAF *a* in PDE5 ((Liu et al., 2002; R. Zoraghi, unpublished results). The GAF domains in PDE2 are rich in β sheets (β1–β6), which are packed on the back side with 2 to 4 α helices and on the other side with a mixture of short α helices and loops which in GAF *b* forms the sides of the ligand-binding pocket. The structures of the GAF domains in PDE2 and YKG9 are very similar except that YKG9 has an additional N-terminal helix compared with the PDE2 GAF *a* (Fig. 5A).

Functions of GAF Domains in PDEs

Ligand Binding. GAF domains are commonly described as small ligand-binding domains, but this has been documented for only a few of the predicted GAF domains. GAFs have been shown to bind formate, 2-oxoglutarate, aromatic compounds (e.g., tetrapyrroles and photopigments), and cNs (Hopper et al., 1996; Korsa and Bock, 1997; Anantharaman et al., 2001; Reyes-Ramirez et al., 2002; Little and Dixon, 2003). Cyclic GMP is the only ligand that has been directly demonstrated to bind to GAFs in PDEs; this binding can result in marked changes in PDE structure and function. Cyclic GMP binding to the GAF(s) in PDE5 or to its isolated R domain causes an apparent elongation (Thomas et al., 1990b; Francis et al., 1998, 2002). In PDEs 2 and 5, cGMP binding to the GAF domains activates catalysis (Erneux et al., 1981; Martins et al., 1982; Okada and Asakawa, 2002; Corbin, 2003; Mullershausen et al., 2003; Rybalkin et al., 2003).

The allosteric cGMP-binding function provided by the GAF domains in PDEs 2, 5, and 6 strongly prefers cGMP over cAMP, and these sites are intolerant of modifications at almost any group on cGMP. In PDEs 5 and 6, cAMP shows

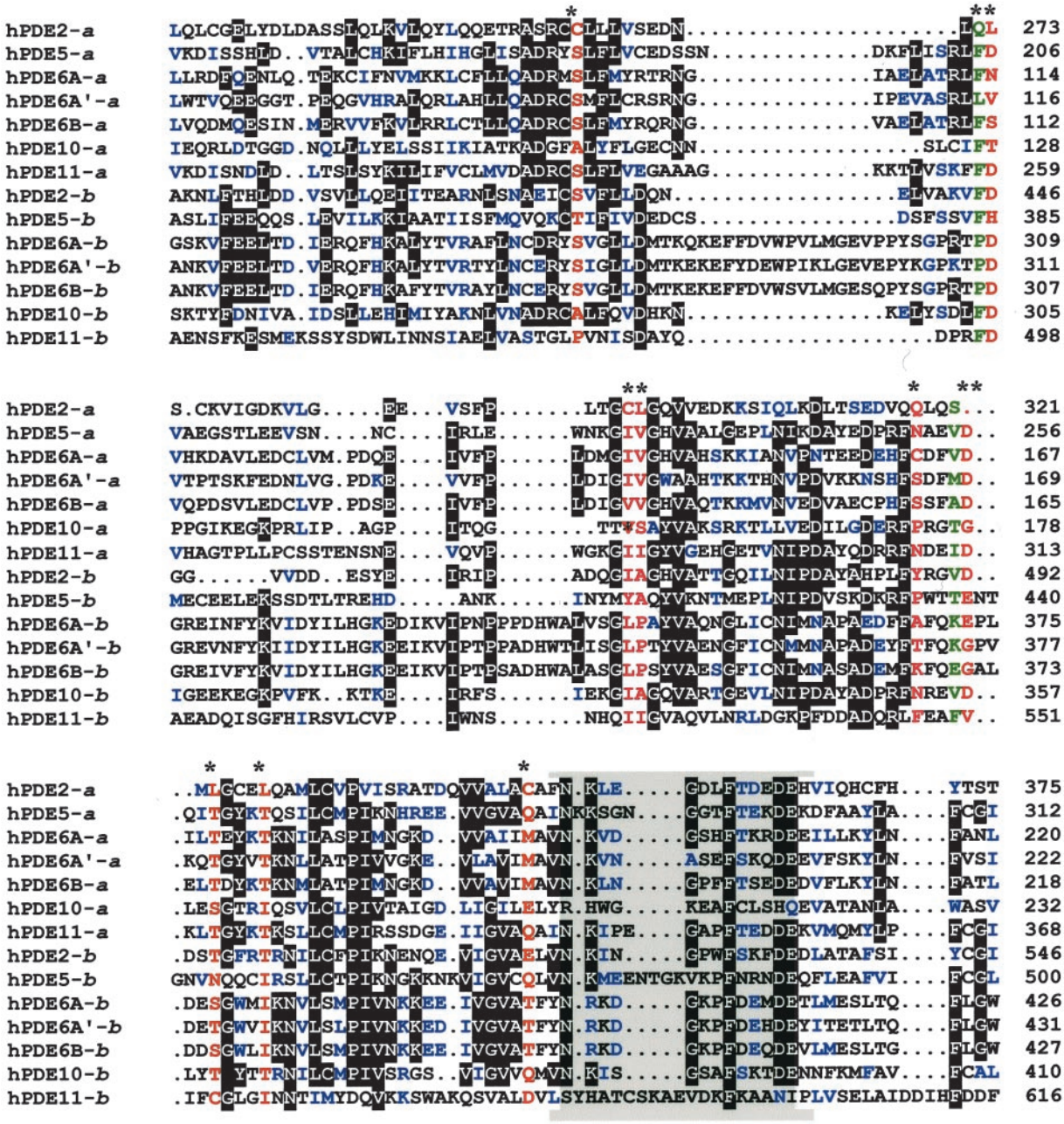

Fig. 3. Clustal W sequence alignment of GAF domains of mammalian PDEs. hPDE2, human PDE2A (AAC51320); hPDE5, human PDE5 (CAA06170); hPDE6A, human PDE6A (NP_000431); hPDE6A′, human PDE6C (NP_006195); hPDE6B, human PDE6B (NP_000274); hPDE10, human PDE10A (NP_006652); hPDE11, human PDE11A (BAB16371); *a,* GAF *a*; *b,* GAF *b*. The region containing the sequence NKX_nFX_3DE originally identified as a PDE GAF domain signature is indicated in the grey box. Highly conserved AAs are indicated in white, and conserved residues are shown in blue. Colored columns indicate the following contacts to cGMP as shown in crystal structure of mouse PDE2A GAF *b*: red, polar side chains; green, hydrophobic side chains; pink, backbone amides. ⋆, positions of the 11 AAs that contact cGMP.

little competition with cGMP. In PDE2, the cGMP-versus-cAMP selectivity of the allosteric cN-binding sites is considerably lower, and other molecules may also interact with these sites to stimulate catalytic activity (Erneux et al., 1985; Manganiello et al., 1990; Martinez et al., 2002b). The specificity requirements for cGMP binding to the GAF(s) in PDEs 5 and 6 are the most restrictive of any known cN-binding protein, and these sites tolerate very few cN analogs (Francis et al., 1990; Hebert et al., 1998).

Although cGMP is bound in GAF *b* of PDE2 (Martinez et al., 2002b), studies using truncation mutagenesis have established that the isolated GAF *a* in PDE5 is sufficient for high-affinity cGMP binding (Liu et al., 2002; R. Zoraghi, unpublished results). The region in the R domain of PDE6 that provides for cGMP-binding in PDE6 has not been determined experimentally. In PDE2, cGMP is bound in the *anti*-conformation of the *N*-glycosidic linkage, and the cyclic phosphate moiety is in the energetically unfavorable boat conformation. Earlier studies using cGMP analogs with PDE5 and PDE6 had predicted that cGMP is bound in the *anti*-conformation (Francis et al., 1990; Hebert et al., 1998).

In PDE2 GAF *b*, cGMP is coordinated through 11 contacts. The large number of contacts may be required to stabilize the energetically unfavorable boat conformation of the cyclic phosphate moiety and the *anti*-conformation of cGMP (Fig. 5B) (Martinez et al., 2002b). The ribose and cyclic phosphate moieties of cGMP, along with three bound waters, are completely buried in the binding pocket, whereas N-1 and C-6, which are located on the specificity-determining edge of the guanine, are buried to a lesser extent. Based on the extent to which cGMP is buried, Martinez et al. (2002b) predicted that before binding ligand, GAF *b* must be in a more open conformation. The exocyclic oxygens of the 3′,5′-cyclic phosphate group of cGMP make two hydrogen bonds with backbone amides. The negatively charged phosphate group of cGMP is stabilized by the positive end of the adjacent α helix. However, the guanine and ribose of the cGMP make a total of six polar and two hydrophobic contacts with AAs of the PDE2 GAF *b* domain. The N-1 hydrogen interacts with the side-chain carboxyl group of an Asp, and the C-6 carbonyl group of the guanine interacts with the main-chain amide group of that same AA (shown as Asp439 in Fig. 5B, corresponding to Asp447 in bovine PDE2A, accession number AAA87353, and Asp446 in human PDE2A, accession number: NP_002590); this Asp is the only AA in the binding site with an obvious role in discriminating between guanine and adenine. Cyclic AMP has an amino group at C-6, which is incompatible with contact to the backbone amide bond at the above-mentioned Asp. In addition, N-1 in cAMP is not protonated and cannot engage in a hydrogen-bond with the negatively charged side chain of the Asp. This Asp is conserved in other GAFs that bind cGMP, e.g., PDE5 GAF *a*, as well as some GAFs that have not yet been shown to bind a cN including GAFs *b* in PDEs 6, 10, 11 and GAF *a* in PDE11 (Fig. 3), but it is not conserved in the cAMP-binding GAF *b* of *Anabaena* adenylyl cyclase. In addition, the C-2 amide has a water-mediated interaction with the side chain of a conserved Thr that is located close to the protein surface (shown as Thr488 in Fig.

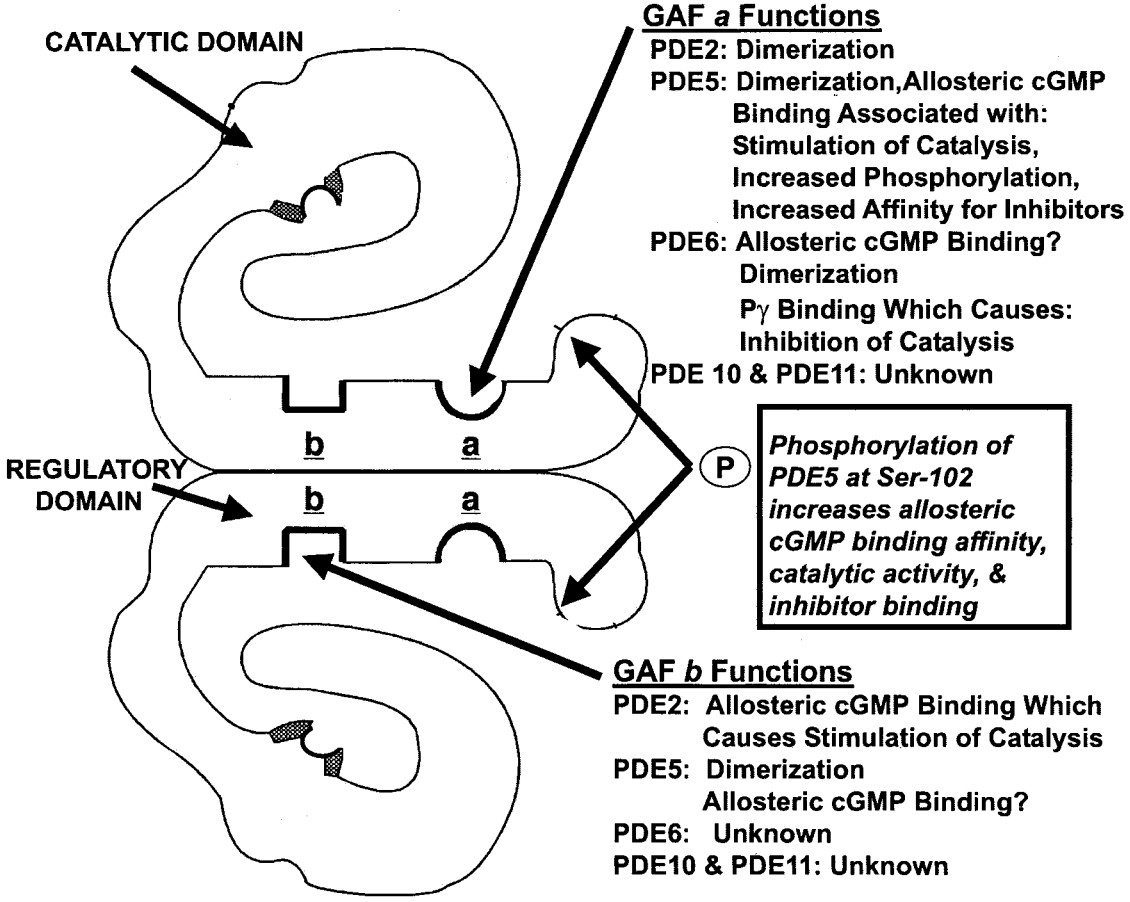

Fig. 4. Summary of known GAF domain functions in PDEs.

5B, corresponding to Thr496 in bovine PDE2A accession number:AAA87353 and Thr495 in human PDE2A, accession number: NP_002590).

Based on the crystal structure of mouse PDE2A GAF domains and the sequence similarities among PDE2 GAF *b*, PDE5 GAF *a,* and PDE6 GAF *a* domains, Beavo and coworkers proposed an 11-residue fingerprint sequence for cGMP-binding (also see Figs. 3 and 5) that spans approximately 90 residues (Scheme 1). The Phe-Asp dyad is potentially involved in specific purine recognition. The Asp in this dyad is the one that binds to N-1 hydrogen and C-6 carbonyl of cGMP. Mutation of the Phe in the Phe-Asp dyad of PDE5 GAF *a* domain abolishes cGMP binding (Sopory et al., 2003). In PDEs 10 and 11, the entire motif is conserved only in GAF *a* domain of PDE11 (Fig. 3), but careful cN-binding studies have not been reported for either family.

The pattern of contacts revealed in the X-ray crystal structure of the PDE2 GAF *b* in complex with cGMP makes it difficult to identify a cN-binding signature sequence in GAF domains, because some contacts in the crystal structure of the PDE2A GAF *b* domain involve the protein backbone, and the binding motif is not completely conserved in all known cGMP-binding PDE GAF domains. In PDEs 5 and 6, the importance of the 2′-OH interaction in allosteric cGMP binding has been demonstrated by analog studies (Francis et al., 1990; Hebert et al., 1998), but the crystal structure of PDE2 GAF *b* domain does not reveal a bond with this position. In addition, the allosteric cN-binding sites in PDE5 and PDE6 have much higher specificity for cGMP versus cAMP compared with PDE2, and PDE6 binds cGMP with higher affinity than does the PDE2 or PDE5 (Erneux et al., 1985; Gillespie and Beavo, 1988, 1989; Thomas et al., 1990a; Cote et al., 1994). These characteristics must reflect significant differences in contacts between cGMP and the respective sites in PDEs 2, 5, and 6.

As noted above, the GAF domains are evolutionarily, structurally, and biochemically distinct from the CAP-related cN-binding sites found in the R domains of cN-regulated protein kinases, ion channels, and guanine nucleotide exchange factors (Passner et al., 2000; Akamine et al., 2003). Six invariant AAs provide for the binding of cN by sites in CAP-related proteins. These include three Gly, one Arg, one Ala, and one Glu. Three of these directly contact the cN. In the regulatory subunit of PKA, the Arg and Ala interact with the equatorial oxygen of cAMP, and the Glu interacts with the 2′-OH of the ribose. In CAP-related proteins, the 2′-OH is necessary for high-affinity cN binding. Stacking interactions between the adenine of cAMP and the aromatic ring of Trp or Tyr in each of the cN-binding sites in PKA contribute importantly to the high-affinity cAMP binding (Francis and Corbin, 1999). The cyclic phosphate and ribose moieties of cAMP are bound by hydrogen bonds between two β strands connected by a short α helix in these sites. Specificity for cGMP versus cAMP in these sites is largely provided by a single Thr/Ala substitution (Shabb and Corbin, 1992).

Cyclic AMP binds only ~11-fold more weakly to the PDE2 GAF *b* domain than does cGMP (Martinez et al., 2002b), and like cGMP, cAMP binding to the PDE2 R domain stimulates catalytic breakdown of either cGMP or cAMP in vitro (Moss et al., 1977; Manganiello et al., 1990). Because the cellular cAMP level is 10- to 100-fold higher than that of cGMP, cAMP could interact with the GAF domain in PDE2 to in-

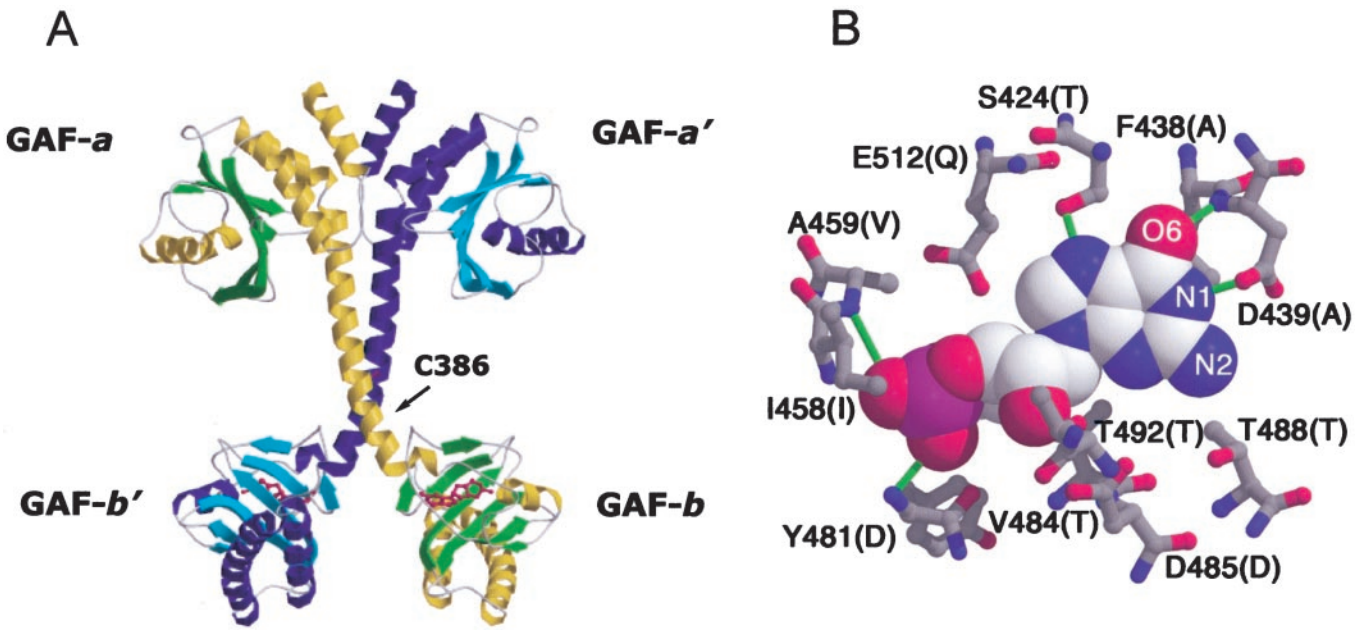

Fig. 5. The X-ray crystallographic structure of the mouse PDE2A regulatory domain. A, the 2.9 Å crystal structure of the mouse PDE2A regulatory domain (GAFs *a* and *b*) in complex with cGMP has revealed that GAF *a* domain is a dimer, whereas GAF *b* domain is distant and contains a cGMP molecule buried deeply in a cGMP-binding site. [Reprinted from Martinez SE, Wu AY, Glavas NA, Tang XB, Turley S, Hol WG, and Beavo JA (2002b) The two GAF domains in phosphodiesterase 2A have distinct roles in dimerization and in cGMP binding. *Proc Natl Acad Sci USA* **99:**13260–13265. Copyright © 2002 National Academy of Sciences, U.S.A. Used with permission.] B, close-up view of cGMP bound to the PDE2A GAF *b* pocket, taken from the Protein Data Bank (PDB) coordinates 1MC0. The cGMP is shown in a space-filling representation. Colors are blue for nitrogen, red for oxygen, magenta for phosphorous, white or gray for carbon, and green for hydrogen bonds. The 11 fingerprint residues for cGMP-binding are shown in a ball-and-stick representation. The residue positions in cGMP-binding GAF *b* of PDE2 are identified, and the residues that occupy these positions in the cAMP-binding GAF *b* domain of *Anabaena* adenylyl cyclase are shown in parentheses. [Reproduced from Hurley JH (2003) GAF domains: cyclic nucleotides come full circle. *Sci STKE* **164:**PE1. Copyright © 2003 American Association for the Advancement of Science. Used with permission.]

crease catalytic activity and provide a negative feedback mechanism for lowering cAMP. GAF domains in PDEs 5 and 6 have more stringent specificity toward cGMP and are less likely to interact with cAMP under physiological conditions.

Cyclic GMP binds to the photoreceptor PDE family (PDE6A-C) with higher intrinsic affinity than to either PDE2 or PDE5 (Erneux et al., 1985; Gillespie and Beavo, 1988, 1989; Thomas et al., 1990a; Cote et al., 1994; Hebert et al., 1998). Comparison of the cGMP-binding contacts derived from the PDE2 GAF *b* with sequences in the GAFs of PDEs 5 and 6 led Beavo and colleagues to predict that, like PDE5, cGMP-binding in PDE6 most likely occurs in GAF *a* (Martinez et al., 2002b). Analog studies demonstrate that numerous interactions between the purine ring and the ribose of cGMP and the GAFs of rod photoreceptor PDE6 account for both the high affinity for cGMP and the high level of discrimination for cGMP. In PDE6, analog studies indicate that a hydrogen bond at C-8 in cGMP is necessary for high-affinity binding; the major determinant for discrimination of cGMP over cAMP involves interactions between the protein and the N-1/C-6 region of cGMP, where hydrogen bonding possibly fosters the cGMP selectivity. The ribose 2′-OH also contributes to stabilizing cGMP binding in PDE6.

The isolated dimeric R domains of PDEs 2 and 5 bind cGMP with characteristics similar to those of the holoenzymes (Thomas et al., 1990a; Francis et al., 2002; Liu et al., 2002; Martinez et al., 2002b). Whether both GAF domains in these PDEs can bind cGMP is not known. By stoichiometric determinations, only one cGMP is bound per monomer of PDEs 2, 5, and 6, but cGMP binding to these PDEs is kinetically heterogeneous. This could result from differences in the structure/function of a single GAF population (either GAF *a* or *b*), from different kinetics associated with cGMP-binding to both GAF *a* and *b* within a single monomer or dimer, or from PDE molecules in different conformations. We have recently shown that phosphorylation of the isolated PDE5 R domain converts the biphasic kinetics of cGMP dissociation/exchange into a single high-affinity species (Francis et al., 2002). This suggests that in the PDE5 R domain, cGMP binds to a single type of site that exists in interconvertible kinetic states. However, results of site-directed mutagenesis, kinetic analysis, and other findings suggest that PDE5 may bind cGMP in both GAF domains. Substitution of an Ala for an invariant Asp in either of the GAF domains of bovine PDE5 (Asp289 in GAF *a* or Asp478 in GAF *b*) markedly decreases the affinity for cGMP in the high- and low-affinity sites, respectively. The physiological significance and the mechanism that accounts for the kinetic heterogeneity of cGMP-binding in PDEs 2, 5, and 6 must await further experimentation (McAllister-Lucas et al., 1995; Francis et al., 1998; Turko et al., 1998).

Many of the predicted PDE GAF domains contain a conserved NK/RX_nFX_3DE sequence. Site-directed mutagenesis of this motif in PDE5 established that the Asn, Lys, and Asp contribute importantly to allosteric cGMP binding; we suggested that this is a signature motif for cN-binding in GAF domains (McAllister-Lucas et al., 1995; Turko et al., 1996). However, in the structure of PDE2 GAF *b*, these three AAs are not near the bound cGMP, suggesting that they play a function in the overall structure of some GAFs. The AAs that were mutated in the GAF domains of PDE5 were selected because of their high degree of conservation in other PDE GAF domains and the resemblance of the NK/RX_nFX_3DE motif to the canonical NKXD motif in GTP binding proteins (Pai et al., 1989). In the GTP-binding proteins, the carboxylate of Asp interacts with both the C-2 amino and the N-1 hydrogen of the guanine of GTP. From the results of studies of the binding of cN analogs, we proposed a working model for cGMP binding and suggested that an Asp interacts with the N-1 hydrogen of cGMP (Francis et al., 1990; Thomas et al., 1992; McAllister-Lucas et al., 1995; Turko et al., 1996). However, the mechanisms by which substitution of Asn279, Lys277, or Asp289 affect cGMP binding in PDE5 GAF *a* remain to be determined.

$$\underline{\mathbf{S}}X_{(13\text{-}18)}\underline{\mathbf{FD}}X_{(18\text{-}22)}\underline{\mathit{IA}}X_{(21)}[\underline{\mathit{Y/N}}]X_{(2)}\underline{\mathbf{VD}}X_{(2)}\underline{\mathbf{T}}X_{(3)}\underline{\mathbf{T}}X_{(19)}[\underline{\mathbf{E/Q}}]$$

(* * *)

Scheme 1. The AAs in bold are those in PDE2 GAF *b* that are in contact with the bound cGMP. Eight of these interact with cGMP through their side chains (underscored), three (A, Y, and T) make contact through waters (marked with ⋆), and three (I, A, and Y) make contact through their backbone amides (italicized). Five of these are conserved in the cAMP-binding GAF *b* domain of *Anabaena* (shown in gray boxes), which suggests their involvement in the recognition of common moieties in cAMP and cGMP (Kanacher et al., 2002) (Fig. 5B).

Protein-Protein Interactions. GAF domains in PDEs participate in protein-protein interactions that provide for homologous and heterologous dimerization of PDE catalytic monomers as well as for binding of PDE6 catalytic monomers with specific members of the family of inhibitory proteins known as Pγ. With one exception, all class I PDEs that have been studied are dimers. The isolated C domains from a number of PDEs retain the salient features of the holoenzymes (Cheung et al., 1996; Jacobitz et al., 1996; Fink et al., 1999; Francis et al., 2001; Richter and Conti, 2002), but dimerization may be important for regulation, enzyme stability, subcellular localization, or other features. If so, the role of GAFs to provide for dimerization in some PDEs takes on a very important role.

Interactions between the GAF *a* domains mediate dimerization of PDE2A (Fig. 5A) (Martinez et al., 2002b). Hydrophobic AAs in the α1 and α1′ helices are involved in forming the dimer interface, including Leu223 (of helix α1), which inserts into a hydrophobic pocket formed by Ile222′, Leu223′, Cys226′ (all from helix α1′), and Tyr365′ (from the kink between α5′ and connecting helices). This pocket is sealed by Asp219 on α1′. Residues located on the first seven turns of the connecting helix (Val369, Ser372, Phe376, Glu379, Lys383, Cys386, and Leu390) also form critical contacts (Martinez et al., 2002b). GAFs *a* and *b* in PDE2A are linked through short sequences to a long connecting helix of nine turns. The first five turns of the connecting helix also provide intersubunit contacts, but there are no additional contacts after a Cys (shown as Cys386 in Fig. 5A, corresponding to Cys394 of bovine PDE2A, accession number AAA87353, and Cys393 in human PDE2A, accession number NP_002590) that forms a disulfide bond (Fig. 5A). Mutagenesis studies suggest that this disulfide has little effect on conformation (Martinez et al., 2002b).

Based on studies generated by N- and C-terminal truncation mutagenesis, PDE5 is dimerized by contacts between the two GAF *b* domains and contacts between the two GAF *a* domains (R. Zoraghi, unpublished results). Whether both sets of contacts are involved in dimerization of the full-length PDE5 remains to be determined. Only a few of the AA in-

volved in the dimer interface of PDE2A GAF *a* are conserved in other PDE GAF domains: Leu223, which is also conserved in PDE2 GAF *b*, PDE5 GAFs (*a* and *b*), PDE6A′ GAFs (*a* and *b*), PDE6B GAF *a,* and PDE6A GAF *b*, and Glu379, which is also conserved in PDE2 GAF *b*. The lack of conservation of dimerization contacts and the fact that YKG9 has an entirely different dimer interface (Ho et al., 2000) suggest that GAFs may use different mechanisms for dimerization. This may account for the specificity in forming PDE homodimers even in the presence of other GAF-containing PDEs.

PDE6$\alpha\beta$ from rod photoreceptor cells is the only PDE that is known to form heterodimers of PDE catalytic subunits. As in PDE2, the GAF *a* domains of PDE6$\alpha\beta$ provide for the affinity and selectivity of dimerization; the α and β chains will not heterodimerize with either PDE6α' subunits or PDE5 subunits. The key dimerization selectivity module of the PDE6 α and β subunits is localized to a short segment within the N-terminal part of GAF *a* domains: PDE6α-59-74/PDE6β-57-72. PDE6α-59-74 and PDE6β-57-72 correspond to a region of PDE2A GAF *a* α1 helix-α1/α2 loop that is involved in forming the PDE2 dimer interface. PDE6α-59-74 and PDE6β-57-72 may also interact with residues at the start of the helix connecting GAFs *a* and *b* (Muradov et al., 2003a).

In PDE6, the GAF *a* domains are involved in protein-protein interactions with the Pγ inhibitory subunit, and GAF domain function is modulated by these contacts. The interactions are quite specific because rod and cone PDEs have high affinity for different isoforms of Pγ (Pγ-rod and Pγ-cone, respectively). There are two regions of direct contact between Pγ and the PDE6 catalytic subunits: 1) the region within the C-terminal tail of the Pγ (75–87) that interacts with the C domain induces conformational changes in the GAF domains and is the key inhibitory domain (Skiba et al., 1995; Mou et al., 1999; Mou and Cote, 2001); and 2) the central polycationic region of Pγ (24–45) which binds to the GAF *a* domain of PDE6 and enhances cGMP-binding affinity (Granovsky et al., 1998; Kajimura et al., 2002; Muradov et al., 2002). The polycationic region of Pγ may provide for the reciprocal relationship between Pγ binding to the C domain and cGMP binding to high-affinity sites in the GAF domains (Mou and Cote, 2001). Pγ binding is influenced by the GAF *b* domain in PDE6β, because mutation of His257 located in the N-terminal portion of GAF *b* impairs interaction with Pγ and is linked to congenital stationary night blindness (Muradov et al., 2003b).

Interaction of the GAF domains of PDE6 with Pγ and with cGMP may contribute to the structural stability of the enzyme. This is supported by studies showing that mutations in the GAF domains of PDE6 (Gal et al., 1994) or in a Pγ gene (Tsang et al., 1996) affect the levels of expression and/or activation of PDE6 (Muradov et al., 2003b). The potential for large conformational changes in GAF domain-containing PDEs is supported by the conformational effects of cGMP binding and/or phosphorylation of PDE5 (Martins et al., 1982; Francis et al., 1998; Corbin et al., 2000). Remarkably, the GAF *a* in the PDE6 isoforms may have three roles: 1) cGMP-binding as predicted by Martinez et al. (2002b); 2) dimerization and specificity of dimerization (Muradov et al., 2003a); and 3) interaction with Pγ as demonstrated in the protein three-dimensional structure using electron microscopy/image analysis and by photoaffinity labeling/mass spectrometry (Kajimura et al., 2002; Muradov et al., 2002). Because no direct allosteric communication between the R and C domains of PDE6 has been detected, the Pγ subunit may play a critical role in this process. In addition to the inhibition of cGMP hydrolysis at the C domain, Pγ enhances the affinity of the regulatory GAF domains for cGMP. Reciprocally, binding of cGMP to GAF domain(s) enhances the affinity of the interaction between Pγ and the C domain, leading to enzyme inhibition (Cote et al., 1994; Mou and Cote, 2001).

The mammalian GAF-containing PDEs 10 and 11 families are not yet well characterized, and the role of the GAFs in these proteins is unknown. One study suggested that PDE10A may contain a low-affinity cGMP-binding GAF domain (Soderling et al., 1999), but no allosteric effect on the catalytic site has been documented. Detection of binding of cAMP or cGMP to GAF(s) of PDE11 has not been reported.

Relief of Autoinhibition and/or Activation of Enzyme Functions. GAF domain function(s) are sometimes associated with the activation/inactivation of enzyme functions. In PDE2, cGMP binding to GAF domain(s) produces as much as a 10-fold increase in the hydrolysis of cGMP and/or cAMP at the catalytic site (Martins et al., 1982; Manganiello et al., 1990; Stroop and Beavo, 1992). This occurs both in vitro and in intact cells. In adrenal cortex cells, elevation of cGMP by atrial natriuretic peptide increases PDE2 catalytic activity, resulting in lowering of cAMP and reduced aldosterone production (MacFarland et al., 1991). Cyclic GMP binding to PDE2 may also play a role in olfactory sensory function (Juilfs et al., 1997). Regulation of the autoinhibition/activation of PDE5 involves cGMP interaction with the GAF domains of the R domain. Cyclic GMP-binding to GAF(s) in the R domain of PDE5 produces a number of changes, including the following: 1) a conformational change that exposes a serine (Ser102 in human PDE5) for phosphorylation by either PKG or the catalytic subunit of PKA, which in turn activates both catalytic and allosteric cGMP-binding activities; 2) increased affinity for cGMP at the catalytic site; 3) increased catalytic activity through a direct effect on the conformational state of the enzyme; and 4) increased catalytic-site affinity for inhibitors such as sildenafil (Viagra; Pfizer, New York, NY) (Thomas et al., 1990b; Turko et al., 1998; Okada and Asakawa, 2002; Corbin et al., 2003; Mullershausen et al., 2003; Rybalkin et al., 2003). The existing data suggest that PDE5 may possess only a low intrinsic catalytic activity in the absence of the stimulatory effect of cGMP binding to the GAF domains. The similarity of the electron microscopic structure of PDEs 5 and 6 (Kameni Tcheudji et al., 2001) led Beavo and coworkers to suggest that cGMP binding to PDE6 GAF domains may have regulatory effects on catalytic activity, but this has not yet been documented (Rybalkin et al., 2003).

Insight into the mechanism of the GAF domain effects on catalytic activity of PDEs may also be derived from studies with other GAF domain-containing proteins. A supporting example comes from a recent study which found that cAMP binding to the GAF *b* domain of cyanobacterial adenylyl cyclase exponentially activates the enzyme (Kanacher et al., 2002). By replacing the cyanobacterial GAF domain in the cyclase with the cGMP-binding GAF(s) from the rat PDE2, the cyclase was converted to a cGMP-stimulated adenylyl cyclase. This demonstrates the functional conservation of the GAF domain since the divergence of bacterial and eukaryotic lineages >2 billion years ago and shows that the GAFs can

somehow activate very different proteins using the same basic mechanism. It raises the question as to whether the GAF structures contain a common inhibitory interface that is displaced by ligand (cGMP or cAMP) binding.

Unlike the GAFs in PDE2 or PDE5, the GAF domains in the PDE6 catalytic subunit are uncoupled from the catalytic domain unless Pγ is bound to bridge the two domains. Cyclic GMP binding to the GAF domains of the PDE6 may indirectly regulate the enzyme by affecting the strength of interaction of the Pγ subunit with the catalytic subunit (see above) (Mou et al., 1999).

Regulation of GAF Domain Functions in PDEs. GAF domain functions are regulated by features within the region of the defined GAF domain(s) and by elements external to the GAF domains. In PDE5, there is very little allosteric cGMP binding, to the GAF domain(s) when the catalytic site is unoccupied. This suggests that the function of the allosteric cGMP binding by the GAF domain(s) is somehow suppressed (i.e., autoinhibited) in the complex between the R and C domains. In addition, phosphorylation of Ser102 that is well outside the sequence of the GAF domains in PDE5 increases the cGMP-binding affinity of the GAF domain(s), i.e., relieves autoinhibition of function. More recent results have determined that autoinhibition/activation of cGMP-binding to PDE5 R domain is modulated by oxidation/reduction of cysteines. The majority of the effects of either phosphorylation or oxidation/reduction are mediated within the region of PDE5 containing the GAF domain(s) (Francis et al., 2002; S. H. Francis, unpublished results). Furthermore, the cGMP-binding affinity of the isolated R domain of PDE2A is 4-fold higher than that reported for PDE2A holoenzyme, and the affinity of the isolated GAF *a* of PDE5 for cGMP is also much greater than that of the holoenzyme or R domain (Martinez et al., 2002b; R. Zoraghi, unpublished results). This indicates that the intrinsic affinity of these GAF(s) for cGMP undergoes autoinhibition.

In PDE6, the affinity of cGMP-binding in GAF domain(s) is also regulated (Cote et al., 1994; Mou and Cote, 2001). Occupancy of the PDE6 GAF domains by cGMP enhances the interaction of Pγ with the catalytic dimer. Conversely, binding of the central polycationic region of Pγ subunit (AAs 24–45) to the catalytic core enhances cGMP-binding affinity for the GAF domains, and upon cGMP-binding, Pγ acts to restore high-affinity binding of a low-affinity class of GAF domains to the catalytic subunits (Mou and Cote, 2001).

Concluding Remarks. The importance of GAF domains in protein function is only beginning to unfold. Despite structural similarities, these proteins must contain novel features that provide for differences in cN selectivity and binding affinity among the cGMP-binding PDEs. Furthermore, GAF domains in PDEs provide multiple functional roles, and all GAFs may not bind small ligands. PDEs that are stimulated by cN binding are not necessarily GAF-containing proteins. In *Dictyostelium discoideum,* a class II cGMP-stimulated PDE contains two cN-binding domains that belong to the CAP family of cN-binding proteins. The enzyme is activated upon binding of either cAMP or cGMP to these cN-binding domains (Bosgraaf et al., 2002). It is intriguing that a class II PDE biochemically resembles mammalian cGMP-stimulated PDEs, although the AA sequences, including those of the cN-binding domains, and the arrangement of the R and C domains are quite different.

PDEs containing GAF motifs are found in the genome of a number of organisms that lack cGMP. This emphasizes the likelihood that these GAF domains have other functions. For example, members of a PDE family (TbPDE2) in *T. brucei* have either one or two GAF motifs, although the presence of cGMP in this organism has not been documented. The variety of ligands bound by GAF domains raises the intriguing possibility of unknown small ligands that might regulate the enzymatic activities of GAF-containing PDEs. Such ligands could alter GAF-containing PDEs to cross-talk with other signaling pathways.

GAF motifs in mammalian proteins are almost completely limited to cN PDEs and have a prominent role in the regulation of the activity of a number of these PDEs. This makes them particularly attractive targets for pharmacological intervention of cAMP and cGMP signaling. It is likely that both agonists and antagonists could be designed to bind to PDE GAF domains and to select for different PDE GAFs. Thus, PDEs could be activated or inactivated by causing the relevant conformational changes.

PDE2A, PDE5A, and both photoreceptor PDE6s are attractive targets for GAF-binding agonists and antagonists. Atrial natriuretic peptide released from cardiac tissue activates guanylyl cyclase, thereby increasing cGMP in adrenal cortex; this activates PDE2A resulting in lowering of cAMP and reduced production of aldosterone, which in turn leads to a reduction in blood volume and blood pressure (MacFarland et al., 1991). Therefore, agonists for the GAF domains of PDE2 might be useful for the treatment of hypertension. Among other things, the use of agonists and antagonists for interaction with PDE5A GAF domains may also prove to be useful. PDE5 GAF domain antagonists may block activation of PDE5 catalytic activity and could be an alternative to PDE5 catalytic site inhibitors in the treatment of male erectile dysfunction. They might also be useful in mediating the elevation of cGMP in peripheral vascular smooth muscle, which might have clinical value in the treatment of systemic or pulmonary hypertension, chest pain, and recovery from stroke (Zhang et al., 2002; Sebkhi et al., 2003). On the other hand, PDE5 GAF domain agonists could also be considered valuable chemical interventions to activate the enzyme at low concentrations of cGMP, a process which could blunt a large increase in cGMP levels in response to pathologic stimuli such as enterotoxins. Such activation might have clinical use for the treatment of vascular problems associated with pathologies such as ischemic injury after stroke, which is associated with high levels of cGMP (Kader et al., 1993). Because mutations in GAF domains of PDE6 α and β genes are responsible for 3 to 4% of cases of recessive retinitis pigmentosa (McLaughlin et al., 1995; Dryja et al., 1999), and the elevated intracellular level of cGMP is believed to be a general cause of photoreceptor deterioration (Lolley et al., 1977; Aquirre et al., 1978), PDE6 GAF *a* antagonists might also be used for activation of the enzyme. On the other hand, it has been shown that constitutive activity of rod PDE6 leads to desensitization of dark-adapted photoreceptors in congenital stationary night blindness (Gal et al., 1994). PDE6 GAF(s) agonists might alleviate this condition by inactivating the enzyme. To summarize clinical relevance, GAF domains could be considered potential targets of pharmacological intervention for a wide variety of medical problems.

References

Akamine P, Madhusudan WJ, Xuoung NH, Ten Eyck LF, and Taylor SS (2003) Dynamic features of cAMP-dependent protein kinase revealed by apoenzyme crystal structure. *J Mol Biol* **327:**159–171.

Anantharaman V, Koonin EV, and Aravind L (2001) Regulatory potential, phyletic distribution and evolution of ancient, intracellular small-molecule-binding domains. *J Mol Biol* **307:**1271–1292.

Aquirre G, Farber D, Lolley R, Fletcher RT, and Chader GJ (1978) Rod-cone dysplasia in Irish setters: a defect in cyclic GMP metabolism in visual cells. *Science (Wash DC)* **201:**1133–1134.

Aravind L and Ponting CP (1997) The GAF domain: an evolutionary link between diverse phototransducing proteins. *Trends Biochem Sci* **22:**458–459.

Biel M, Zong X, Ludwig A, Sautter A, and Hofmann F (1999) Structure and function of cyclic nucleotide-gated channels. *Rev Physiol Biochem Pharmacol* **135:**151–171.

Boekhoff I, Michel WC, Breer H, and Ache BW (1994) Single odors differentially stimulate dual second messenger pathways in lobster olfactory receptor cells. *J Neurosci* **14:**3304–3309.

Bosgraaf L, Russcher H, Snippe H, Bader S, Wind J, and Van Hasstert PJ (2002) Identification and characterization of two unusual cGMP-stimulated phoshodiesterases in *Dictyostelium*. *Mol Biol Cell* **13:**3878–3889.

Burns F, Zhao AZ, and Beavo JA (1996) Cyclic nucleotide phosphodiesterases: gene complexity, regulation by phosphorylation and physiological implications. *Adv Pharmacol* **36:**29–48.

Charbonneau H, Prusti RK, LeTrong H, Sonnenburg WK, Mullaney PJ, Walsh KA, and Beavo JA (1990) Identification of a noncatalytic cGMP-binding domain conserved in both the cGMP-stimulated and photoreceptor cyclic nucleotide phosphodiesterases. *Proc Natl Acad Sci USA* **87:**288–292.

Cheung PP, Xu H, McLaughlin MM, Ghazaleh FA, Livi GP, and Colman RW (1996) Human platelet cGI-PDE: expression in yeast and localization of the catalytic domain by deletion mutagenesis. *Blood* **88:**1321–1329.

Corbin JD, Blount MA, Weeks JL 2nd, Beasley A, Kuhn KP, Ho YS, Saidi LF, Hurley JH, Kotera J, and Francis SH (2003) [3H]Sildenafil binding to phosphodiesterase-5 is specific, kinetically heterogeneous and stimulated by cGMP. *Mol Pharmacol* **63:**1364–1372.

Corbin JD, Turko IV, Beasley A, and Francis SH (2000) Phosphorylation of phosphodiesterase-5 by cyclic nucleotide-dependent protein kinase alters its catalytic and allosteric cGMP-binding activities. *Eur J Biochem* **267:**2760–2767.

Cote RH, Bownds MD, and Arshavsky VY (1994) cGMP binding sites on photoreceptor phosphodiesterase: role in feedback regulation of visual transduction. *Proc Natl Acad Sci USA* **91:**4845–4849.

D'Amours MR and Cote RH (1999) Regulation of photoreceptor phosphodiesterase catalysis by its non-catalytic cGMP-binding sites. *Biochem J* **340:**863–869.

Danciger M, Blaney J, Gao YQ, Zhao DY, Heckenlively JR, Jacobson SG, and Farber DB (1995) Mutations in the PDE6B gene in autosomal recessive retinitis pigmentosa. *Genomics* **30:**1–7.

Dryja TP, Rucinski DE, Chen SH, and Berson EL (1999) Frequency of mutations in the gene encoding the alpha subunit of rod cGMP-phosphodiesterase in autosomal recessive retinitis pigmentosa. *Investig Ophthalmol Vis Sci* **40:**1859–1865.

Erneux C, Couchie D, Dumont JE, Baraniak J, Stec WJ, Abbad EG, Petridis G, and Jastorff B (1981) Specificity of cyclic GMP activation of a multi-substrate cyclic nucleotide phosphodiesterase from rat liver. *Eur J Biochem* **115:**503–510.

Erneux C, Miot F, Van Haastert PJ, and Jastorff B (1985) The binding of cyclic nucleotide analogs to a purified cyclic GMP-stimulated phosphodiesterase from bovine adrenal tissue. *J Cyclic Nucleotide Protein Phosphor Res* **10:**463–472.

Fawcett L, Baxendale R, Stacey P, McGrouther C, Harrow I, Soderling S, Hetman J, Beavo JA, and Phillips SC (2000) Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. *Proc Natl Acad Sci USA* **97:**3702–3707.

Fink TL, Francis SH, Beasley A, Grimes KA, and Corbin JD (1999) Expression of an active, monomeric catalytic domain of the cGMP-binding cGMP-specific phosphodiesterase (PDE5). *J Biol Chem* **274:**34613–34620.

Francis SH, Bessay EP, Kotera J, Grimes KA, Liu L, Thompson WJ, and Corbin JD (2002) Phosphorylation of isolated human phosphodiesterase-5 regulatory domain induces an apparent conformational change and increases cGMP binding affinity. *J Biol Chem* **277:**47581–47587.

Francis SH, Chu DM, Thomas MK, Beasley A, Grimes K, Busch JL, Turko IV, Haik TL, and Corbin JD (1998) Ligand-induced conformational changes in cyclic nucleotide phosphodiesterases and cyclic nucleotide-dependent protein kinases. *Methods* **14:**81–92.

Francis SH and Corbin JD (1999) Cyclic nucleotide-dependent protein kinases: intracellular receptors for cAMP and cGMP action. *Crit Rev Clin Lab Sci* **36:**275–328.

Francis SH, Lincoln TM, Corbin JD (1980) Characterization of a novel cGMP binding protein from rat lung. *J Biol Chem* **255:**620–626.

Francis SH, Thomas MK, and Corbin JD (1990) Cyclic GMP-specific phosphodiesterase from lung, in *Cyclic Nucleotide Phosphodiesterases: Structure Regulation and Drug Action* (Beavo J and Houslay MD eds) pp 117–140, Wiley, Chichester, New York.

Francis SH, Turko IV, and Corbin JD (2001) Cyclic nucleotide phosphodiesterases: relating structure and function. *Progr Nucleic Acid Res Mol Biol* **65:**1–52.

Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, and Omori K (1999) Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). *J Biol Chem* **274:**18438–18445.

Gal A, Orth U, Baehr W, Schwinger E, and Rosenberg T (1994) Heterozygous missense mutation in the rod cGMP phosphodiesterase beta-subunit gene in autosomal dominant stationary night blindness [erratum appears in *Nat Genet* 1994;7:551]. *Nat Genet* **7:**64–68.

Galperin MY, Nikolskaya AN, and Koonin EV (2001) Novel domains of the prokaryotic two-component signal transduction systems [erratum appears in *FEMS Microbiol Lett* 2001;204:213–214]. *FEMS Microbiol Lett* **203:**11–21.

Gillespie PG and Beavo JA (1988) Characterization of a bovine cone photoreceptor phosphodiesterase purified by cyclic GMP-sepharose chromatography. *J Biol Chem* **263:**8133–8141.

Gillespie PG and Beavo JA (1989) cGMP is tightly bound to bovine retinal rod phosphodiesterase. *Proc Natl Acad Sci USA* **86:**4311–4315.

Giltay R, Kostka G, and Timpl R (1997) Sequence and expression of a novel member (LTBP-4) of the family of latent transforming growth factor-beta binding proteins. *FEBS Lett* **411:**164–168.

Granovsky AE, Natochin M, McEntaffer RL, Haik TL, Francis SH, Corbin JD, and Artemyev NO (1998) Probing domain functions of chimeric PDE6alpha′/PDE5 cGMP-phosphodiesterase. *J Biol Chem* **273:**24485–24490.

Hebert MC, Schwede F, Jastorff B, and Cote RH (1998) Structural features of the noncatalytic cGMP binding sites of frog photoreceptor phosphodiesterase using cGMP analogs. *J Biol Chem* **273:**5557–5565.

Ho YS, Burden LM, and Hurley JH (2000) Structure of the GAF domain, a ubiquitous signaling motif and a new class of cyclic GMP receptor [erratum appears in *EMBO (Eur Mol Biol Organ) J* 2001;20:1483]. *EMBO (Eur Mol Biol Organ) J* **19:**5288–5299.

Hopper S, Korsa I, and Bock A (1996) The nucleotide concentration determines the specificity of in vitro transcription activation by the sigma 54-dependent activator FhlA. *J Bacteriol* **178:**199–203.

Hurley JH (2003) GAF domains: cyclic nucleotides come full circle. *Sci STKE* **164:** PE1.

Jacobitz S, McLaughlin MM, Livi GP, Burman M, and Torphy TJ (1996) Mapping the functional domains of human recombinant phosphodiesterase 4A: structural requirements for catalytic activity and rolipram binding. *Mol Pharmacol* **50:**891–899.

Joerger RD, Jacobson MR, and Bishop PE (1989) Two nifA-like genes required for expression of alternative nitrogenases by *Azotobacter vinelandii*. *J Bacteriol* **171:** 3258–3267.

Juilfs DM, Fulle HJ, Zhao AZ, Houslay MD, Garbers DL, and Beavo JA (1997) A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. *Proc Natl Acad Sci USA* **94:**3388–3395.

Kader A, Frazzini VI, Solomon RA, and Trifiletti RR (1993) Nitric oxide production during focal cerebral ischemia in rats. *Stroke* **24:**1709–1716.

Kajimura N, Yamazaki M, Morikawa K, Yamazaki A, and Mayanagi K (2002) Three-dimensional structure of non-activated cGMP phosphodiesterase 6 and comparison of its image with those of activated forms. *J Struct Biol* **139:**27–38.

Kameni Tcheudji JF, Lebeau L, Virmaux N, Maftei CG, Cote RH, Lugnier C, and Schultz P (2001) Molecular organization of bovine rod cGMP-phosphodiesterase 6. *J Mol Biol* **310:**781–791.

Kanacher T, Schultz A, Linder JU, and Schultz JE (2002) A GAF-domain-regulated adenylyl cyclase from *Anabaena* is a self-activating cAMP switch. *EMBO (Eur Mol Biol Organ) J* **21:**3672–3680.

Kaneko T, Nakamura Y, Wolk CP, Kuritz T, Sasamoto S, Watanabe A, Iriguchi M, Ishikawa A, Kawashima K, Kimura T, et al. (2001) Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. *DNA Res* **8:**205–213, 227–253.

Korsa I and Bock A (1997) Characterization of fhlA mutations resulting in ligand-independent transcriptional activation and ATP hydrolysis. *J Bacteriol* **179:**41–45.

Kotera J, Fujishige K, Yuasa K, and Omori K (1999) Characterization and phosphorylation of PDE10A2, a novel alternative splice variant of human phosphodiesterase that hydrolyzes cAMP and cGMP. *Biochem Biophys Res Commun* **261:**551–557.

Koyanagi M, Suga H, Hoshiyama D, Ono K, Iwabe N, Kuma K, and Miyata T (1998) Ancient gene duplication and domain shuffling in the animal cyclic nucleotide phosphodiesterase family. *FEBS Lett* **436:**323–328.

Letunic I, Goodstadt L, Dickens NJ, Doerks T, Schultz J, Mott R, Ciccarelli F, Copley RR, Ponting CP, and Bork P (2002) Recent improvements to the SMART domain-based sequence annotation resource. *Nucleic Acids Res* **30:**242–244.

Little R and Dixon R (2003) The amino-terminal GAF domain of azotobacter vinelandii NifA binds 2-oxoglutarate to resist inhibition by NifL under nitrogen-limiting conditions. *J Biol Chem* **278:**28711–28718.

Lincoln TM, Hall CL, Park CR, Corbin JD (1976) Guanosine 3′:5′-cyclic monophosphate binding proteins in rat tissues. *Proc Natl Acad Sci USA* **73:**2559–2563.

Liu L, Underwood T, Li H, Pamukcu R, and Thompson WJ (2002) Specific cGMP binding by the cGMP binding domains of cGMP-binding cGMP specific phosphodiesterase. *Cell Signal* **14:**45–51.

Lolley RN, Farber DB, Rayborn ME, and Hollyfield JG (1977) Cyclic GMP accumulation causes degeneration of photoreceptor cells: simulation of an inherited disease. *Science (Wash DC)* **196:**664–666.

MacFarland RT, Zelus BD, and Beavo JA (1991) High concentrations of a cGMP-stimulated phosphodiesterase mediate ANP-induced decreases in cAMP and steroidogenesis in adrenal glomerulosa cells. *J Biol Chem* **266:**136–142.

Manganiello VC, Tanaka T, and Murashima S (1990) Cyclic GMP-stimulated cyclic nucleotide phosphodiesterases, in *Cyclic Nucleotide Phosphodiesterases: Structure Regulation and Drug Action* (Beavo J and Houslay MD eds) pp 61–85, Wiley, Chichester, New York.

Martinez SE, Beavo JA, and Hol WG (2002a) GAF domains: two billion year old molecular switches that bind cyclic nucleotides. *Mol Intervent* **2:**317–323.

Martinez SE, Wu AY, Glavas NA, Tang XB, Turley S, Hol WG, and Beavo JA (2002b) The two GAF domains in phosphodiesterase 2A have distinct roles in dimerization and in cGMP binding. *Proc Natl Acad Sci USA* **99:**13260–13265.

Martins TJ, Mumby MC, and Beavo JA (1982) Purification and characterization of a cyclic GMP-stimulated cyclic nucleotide phosphodiesterase from bovine tissues. *J Biol Chem* **257:**1973–1979.

McAllister-Lucas LM, Haik TL, Colbran JL, Sonnenburg WK, Seger D, Turko IV, Beavo JA, Francis SH, and Corbin JD (1995) An essential aspartic acid at each of

two allosteric cGMP-binding sites of a cGMP-specific phosphodiesterase. *J Biol Chem* **270:**30671–30679.
McKay DB and Steitz TA (1981) Structure of catabolite gene activator protein at 2.9 A resolution suggests binding to left-handed B-DNA. *Nature (Lond)* **290:**744–749.
McLaughlin ME, Ehrhart TL, Berson EL, and Dryja TP (1995) Mutation spectrum of the gene encoding the beta subunit of rod phosphodiesterase among patients with autosomal recessive retinitis pigmentosa. *Proc Natl Acad Sci USA* **92:**3249–3253.
Montgomery BL and Lagarias JC (2002) Phytochrome ancestry: sensors of bilins and light. *Trends Plant Sci* **7:**357–366.
Moss J, Manganiello VC, and Vaughan M (1977) Substrate and effector specificity of a guanosine 3′:5′-monophosphate phosphodiesterase from rat liver. *J Biol Chem* **252:**5211–5215.
Mou H and Cote RH (2001) The catalytic and GAF domains of the rod cGMP phosphodiesterase (PDE6) heterodimer are regulated by distinct regions of its inhibitory gamma subunit. *J Biol Chem* **276:**27527–27534.
Mou H, Grazio HJ 3rd, Cook TA, Beavo JA, and Cote RH (1999) cGMP binding to noncatalytic sites on mammalian rod photoreceptor phosphodiesterase is regulated by binding of its gamma and delta subunits. *J Biol Chem* **274:**18813–18820.
Mullershausen F, Friebe A, Feil R, Thompson WJ, Hofmann F, and Koesling D (2003) Direct activation of PDE5 by cGMP: long-term effects within NO/cGMP signaling. *J Cell Biol* **160:**719–727.
Muradov KG, Boyd KK, Martinez SE, Beavo JA, and Artemyev NO (2003a) The GAFa domains of rod cGMP-phosphodiesterase 6 determine the selectivity of the enzyme dimerization. *J Biol Chem* **278:**10594–601.
Muradov KG, Granovsky AE, and Artemyev NO (2003b) Mutation in rod PDE6 linked to congenital stationary night blindness impairs the enzyme inhibition by its gamma-subunit. *Biochemistry* **42:**3305–3310.
Muradov KG, Granovsky AE, Schey KL, and Artemyev NO (2002) Direct interaction of the inhibitory gamma-subunit of rod cGMP phosphodiesterase (PDE6) with the PDE6 GAFa domains. *Biochemistry* **41:**3884–3890.
Okada D and Asakawa S (2002) Allosteric activation of cGMP-specific, cGMP-binding phosphodiesterase (PDE5) by cGMP. *Biochemistry* **41:**9672–9679.
Okazaki Y, Furuno M, Kasukawa T, Adachi J, Bono H, Kondo S, Nikaido I, Osato N, Saito R, Suzuki H, et al. (2002) Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature (Lond)* **420:**563–573.
Pai EF, Kabsch W, Krengel U, Holmes KC, John J, and Wittinghofer A (1989) Structure of the guanine-nucleotide-binding domain of the Ha-ras oncogene product p21 in the triphosphate conformation. *Nature (Lond)* **341:**209–214.
Passner JM, Schultz SC, and Steitz TA (2000) Modeling the cAMP-induced allosteric transition using the crystal structure of CAP-cAMP at 2.1 A resolution. *J Mol Biol* **304:**847–859.
Pfeifer A, Ruth P, Dostmann W, Sausbier M, Klatt P, and Hofmann F (1999) Structure and function of cGMP-dependent protein kinases. *Rev Physiol Biochem Pharmacol* **135:**105–149.
Rascon A, Soderling SH, Schaefer JB, and Beavo JA (2002) Cloning and characterization of a cAMP-specific phosphodiesterase (TbPDE2B) from *Trypanosoma brucei*. *Proc Natl Acad Sci USA* **99:**4714–4719.
Reyes-Ramirez F, Little R, and Dixon R (2002) Mutant forms of the *Azotobacter vinelandii* transcriptional activator NifA resistant to inhibition by the NifL regulatory protein. *J Bacteriol* **184:**6777–6785.
Richter W (2002) 3′,5′Cyclic nucleotide phosphodiesterases class III: members, structure and catalytic mechanism. *Proteins* **46:**278–286.
Richter W and Conti M (2002) Dimerization of the type 4 cAMP-specific phosphodiesterases is mediated by the upstream conserved regions (UCRs). *J Biol Chem* **277:**40212–40221.
Rybalkin SD and Beavo JA (1996) Multiplicity within cyclic nucleotide phosphodiesterases. *Biochem Soc Trans* **24:**1005–1009.
Rybalkin SD, Rybalkina IG, Shimizu-Albergine M, Tang XB, and Beavo JA (2003) PDE5 is converted to an activated state upon cGMP binding to the GAF A domain. *EMBO (Eur Mol Biol Organ) J* **22:**469–478.
Sato-Nara K, Yuhashi KI, Higashi K, Hosoya K, Kubota M, and Ezura H (1999) Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiol* **120:**321–330.
Schluter K, Jockusch BM, and Rothkegel M (1997) Profilins as regulators of actin dynamics. *Biochim Biophys Acta* **1359:**97–109.
Schultz J, Milpetz F, Bork P, and Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. *Proc Natl Acad Sci USA* **95:**5857–5864.
Sebkhi A, Strange JW, Phillips SC, Wharton J, and Wilkins MR (2003) Phosphodiesterase type 5 as a target for the treatment of hypoxia-induced pulmonary hypertension. *Circulation* **107:**3230–3235.
Seebeck T, Gong K, Kunz S, Schaub R, Shalaby T, and Zoraghi R (2001) cAMP signalling in *Trypanosoma brucei*. *Int J Parasitol* **31:**491–498.
Shabb JB and Corbin JD (1992) Cyclic nucleotide-binding domains in proteins having diverse functions. *J Biol Chem* **267:**5723–5726.
Sharrock RA and Quail PH (1989) Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution and differential expression of a plant regulatory photoreceptor family. *Genes Dev* **3:**1745–1757.
Skiba NP, Artemyev NO, and Hamm HE (1995) The carboxyl terminus of the gamma-subunit of rod cGMP phosphodiesterase contains distinct sites of interaction with the enzyme catalytic subunits and the alpha-subunit of transducin. *J Biol Chem* **270:**13210–13215.
Soderling SH, Bayuga SJ, and Beavo JA (1999) Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. *Proc Natl Acad Sci USA* **96:**7071–7076.
Sopory S, Balaji S, Srinivasan N, and Visweswariah SS (2003) Modeling and mutational analysis of the GAF domain of the cGMP-binding, cGMP-specific phosphodiesterase, PDE5. *FEBS Lett* **539:**161–166.
Stroop SD and Beavo JA (1992) Sequence homology and structure-function studies of the bovine cyclic-GMP-stimulated and retinal phosphodiesterases. *Adv Second Messenger Phosphoprotein Res* **25:**55–71.
Su Y, Dostmann WR, Herberg FW, Durick K, Xuong NH, Ten Eyck L, Taylor SS, and Varughese KI (1995) Regulatory subunit of protein kinase A: structure of deletion mutant with cAMP binding domains. *Science (Wash DC)* **269:**807–813.
Thomas MK, Francis SH, Beebe SJ, Gettys TW, and Corbin JD (1992) Partial mapping of cyclic nucleotide sites and studies of regulatory mechanisms of phosphodiesterases using cyclic nucleotide analogues. *Adv Second Messenger Phosphoprotein Res* **25:**45–53.
Thomas MK, Francis SH, and Corbin JD (1990a) Characterization of a purified bovine lung cGMP-binding cGMP phosphodiesterase. *J Biol Chem* **265:**14964–14970.
Thomas MK, Francis SH, and Corbin JD (1990b) Substrate- and kinase-directed regulation of phosphorylation of a cGMP-binding phosphodiesterase by cGMP. *J Biol Chem* **265:**14971–14978.
Tsang SH, Gouras P, Yamashita CK, Kjeldbye H, Fisher J, Farber DB, and Goff SP (1996) Retinal degeneration in mice lacking the gamma subunit of the rod cGMP phosphodiesterase. *Science (Wash DC)* **272:**1026–1029.
Turko IV, Francis SH, and Corbin JD (1998) Binding of cGMP to both allosteric sites of cGMP-binding cGMP-specific phosphodiesterase (PDE5) is required for its phosphorylation. *Biochem J* **329:**505–510.
Turko IV, Haik TL, McAllister-Lucas LM, Burns F, Francis SH, and Corbin JD (1996) Identification of key amino acids in a conserved cGMP-binding site of cGMP-binding phosphodiesterases. A putative NKXnD motif for cGMP binding. *J Biol Chem* **271:**22240–22244.
Urao T, Yamaguchi-Shinozaki K, and Shinozaki K (2001) Plant histidine kinases: an emerging picture of two-component signal transduction in hormone and environmental responses. *Science STKE* **2001:**RE18.
Weber G (1975) Energetics of ligand binding to proteins. *Adv Protein Chem* **29:**1–83.
Wyatt TA, Naftilan AJ, Francis SH, and Corbin JD (1998) ANF elicits phosphorylation of the cGMP phosphodiesterase in vascular smooth muscle cells. *Am J Physiol* **274:**H448–H455.
Yuasa K, Kanoh Y, Okumura K, and Omori K (2001) Genomic organization of the human phosphodiesterase PDE11A gene. Evolutionary relatedness with other PDEs containing GAF domains. *Eur J Biochem* **268:**168–178.
Yuasa K, Kotera J, Fujishige K, Michibata H, Sasaki T, and Omori K (2000) Isolation and characterization of two novel phosphodiesterase PDE11A variants showing unique structure and tissue-specific expression. *J Biol Chem* **275:**31469–31479.
Zhang R, Wang Y, Zhang L, Zhang Z, Tsang W, Lu M, and Chopp M (2002) Sildenafil (Viagra) induces neurogenesis and promotes functional recovery after stroke in rats. *Stroke* **33:**2675–2680.
Zoraghi R and Seebeck T (2002) The cAMP-specific phosphodiesterase TbPDE2C is an essential enzyme in bloodstream from *Trypanosoma brucei*. *Proc Natl Acad Sci USA* **99:**4343–4348.

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