cAMP Is a Ligand for the Tandem GAF Domain of Human Phosphodiesterase 10 and cGMP for the Tandem GAF Domain of Phosphodiesterase 11*

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N-terminal tandem GAF domains are present in 5 out of 11 mammalian phosphodiesterase (PDE) families. The ligand for the GAF domains of PDEs 2, 5, and 6 is cGMP, whereas those for PDEs 10 and 11 remained enigmatic for years. Here we used the cyanobacterial cyaB1 adenylyl cyclase, which has an N-terminal tandem GAF domain closely related to those of the mammalian PDEs, as an assay system to identify the ligands for the human PDEs 10 and 11 GAF domains. We report that a chimera between the PDE10 GAF domain and the cyanobacterial cyclase was 9-fold stimulated by cAMP (EC_{50} = 19.8 μM), whereas cGMP had only low activity. cAMP increased V_{max} in a non-cooperative manner and did not affect the K_m for ATP of 27 μM. In an analogous chimeric construct with the tandem GAF domain of human PDE11A4, cGMP was identified as an allosteric activator (EC_{50} = 72.5 μM) that increased V_{max} of the cyclase non-cooperatively 4-fold. GAF-B of PDE10 and GAF-A of PDE11A4 contain an invariant NKFDE motif present in all mammalian PDE GAF ensembles. We mutated the aspartates within this motif in both regions and found that intramolecular signaling was considerably reduced or abolished. This was in line with all data concerning GAF domains with a NKFDE motif as far as they have been tested. The data appeared to define those GAF domains as a distinct subclass within the >3100 annotated GAF domains for which we propose a tentative classification scheme.

In essentially all cells, the second messengers cAMP and cGMP participate in a variety of signal transduction pathways (1). Therefore, intracellular concentrations of cAMP and cGMP are strictly regulated by the rate of biosynthesis and degradation by a set of cyclase and phosphodiesterase (PDE) isozymes, respectively (2, 3). In mammals, 11 PDE families exist that are biochemically characterized on the basis of substrate specificities, regulation by endogenous effectors, and sensitivity to inhibitors (4–7). Although all PDE catalytic domains possess characteristic similarities in their amino acid sequences, their substrate specificities differ (6). Further, different N-terminal domains of the PDEs are responsible for distinct regulatory properties (8, 9). Among those regulatory domains, the N-terminal tandem GAF domains (the acronym derives from their initial identification in cGMP-stimulated PDEs, Anabaena adenylly cyclases (ACs), and the Escherichia coli transcription factor EhIA) of PDEs 2, 5, 6, 10, and 11 are notable because GAF domains have been identified in more than 800 other proteins (3, 10, 11). They are a divergent group of domains originally proposed to bind a variety of small molecules (12), yet other functions, such as dimerization, have been documented (13, 14, 24). Inspection of the tandem GAF domains of the mammalian PDEs 2, 5, 6, 10, and 11, of a PDE from Trypanosoma brucei (TbPDE2B), and of the ACs cyaB1 and cyaB2 from the cyanobacterium Anabaena reveals that they are related (3, 15). They have an identical domain organization, i.e. an N-terminal GAF-A domain of ~160 amino acids is linked via about 30 amino acids to a GAF-B domain of about 160 amino acids. Further, they have considerable sequence similarity, e.g. the rat PDE2 GAF domain is 25% identical and 42% similar to that of the cyanobacterial cyaB1 AC. In addition, all carry an invariant NKFDE motif in GAF-A, GAF-B, or both regions as a signature sequence (Fig. 1) (3). The GAF domains of cyaB1 activate the associated AC via cAMP. The GAF domains of PDEs 2 and 5 activate their respective PDEs via cGMP (16–18). PDE6 GAF binds cGMP and that of TbPDE2B binds cAMP, although a role in PDE regulation is as yet unknown (19, 20). For the GAF domains of mammalian PDEs 10 and 11, no ligands have been identified to date.

Notwithstanding an evolutionary distance of about three billion years, the mammalian and cyanobacterial tandem GAF domains are surprisingly also functionally closely related (14, 21, 22). The cyanobacterial tandem GAF domain can be substituted by the mammalian GAF domain of PDE2, i.e. the cAMP-activated AC is converted to a cGMP-activated cyclase (23). In PDE2, cGMP is the allosteric regulator and concurrently the substrate, a situation that poses intractable kinetic problems. In the PDE-cyclase chimera, the allosteric regulator (cGMP) and the substrate (ATP) differ and do not interfere with each other. Here we asked whether we could determine the unknown ligands of the tandem GAF domains of the human PDEs 10 and 11 by constructing chimeras in which they replace the cyanobacterial GAF domains using the AC activity as a convenient assay system. We report that PDE10 is the only family of the mammalian PDEs in which a GAF domain ligand is cAMP, whereas for the PDE11 GAF domain, cGMP is a ligand as it is already known for PDEs 2, 5, and 6.

MATERIALS AND METHODS

Recombinant DNAs—The cyaB1 gene (gi 15553050) was a gift from Prof. M. Ohmori, University of Tokyo. cDNA clones of human PDEs 10 and 11 (PDE10A1, gi 4894715; PDE11A4, gi 15128482) were kindly provided by Dr. Quintini, AltanaPharma, Konstanz, Germany. Nucleotides were purchased from either Roche Diagnostics or Biolog Life Science Institute (Bremen, Germany).

PDE10A-GAF-CyaB1-Adenylyl Cyclase Chimeras—The N-terminal tandem GAF domain of PDE10A (amino acids Leu-14–His-422) was amplified by PCR. It was cloned via BamHI and SalI sites in front of the CyaB1 AC (amino acids 386–859) in Bluescript II SK(−) generating...
PDE10A1-422/cyaB1A-C386–859. The PDE10 GAF-B domain mutation D397A was introduced by site-directed mutagenesis using nearby restriction sites for cloning (BamHI and HindIII) producing PDE10A1-422/cyaB1AC386–859 D397A. Both PDE10/cyaB1 constructs were transferred into expression vector pRSETA using a 5′-SacI and 3′-MfeI sites producing PDE10A114–422/cyaB1AC386–859. The PDE10 GAF-B domain mutation D355A in GAF-A was introduced by site-directed mutagenesis using restriction sites located nearby (SacI and MfeI) generating PDE10A114–422/cyaB1AC386–859. The PDE10 GAF-B domain mutation D355A in GAF-A was introduced by site-directed mutagenesis using nearby restriction sites for cloning (BamHI and HindIII) producing PDE10A1-422/cyaB1AC386–859 D397A. Both PDE10/cyaB1 constructs were transferred into expression vector pRSETA using a 5′-SacI and 3′-MfeI site. This added an N-terminal MRGSH6GM affinity tag.

**PDE11A-GAF-CyaB1-Adenylyl Cyclase Chimeras**—The tandem GAF domain (Lys197–Lys568) of PDE11A4 was amplified by PCR. The product was inserted into the cyaB1 AC from SfuI and Sall sites and replaced the cyaB1 tandem GAF domain (Gln49 to Leu385) in Bluescript II SK(−), the tandem N terminus of this was retained in this construct (CyaB11–97/PDE11A497–568/cyaB1386–859). Secondly, the PDE11A4 tandem GAF domain including its N terminus (Met1–Lys568) was amplified by PCR and cloned in front of the CyaB1 AC (amino acids 386–859) via its BamHI and Sall sites in BlueScript II SK(−) generating PDE11A41–568/cyaB1386–859. Finally, the PDE11/cyaB1 constructs were transferred into the expression vector PET16b via a 5′-Ndel and 3′-XhoI sites. This added an N-terminal MGH10SSGHIEGRH affinity tag.

The mutation D355A in GAF-A was introduced by site-directed mutagenesis using restriction sites located nearby (SacI and MfeI) generating PDE11A41–568/cyaB1386–859 D355A. All primer sequences are available on request.

**Expression and Purification of Recombinant Proteins**—All constructs were transformed into E. coli BL21 (DE3) (pREP4). Cultures were grown in Luria-Bertani broth containing 100 mg of ampicillin and 50 mg/liter kanamycin. Usually, expression was induced with 200 μM isopropyl-β-thiogalactopyranoside at an A600 of 0.6, and the culture was continued overnight at 16–19 °C. Bacteria were harvested by centrifugation, rinsed once with 50 mM Tris-HCl pH 8.5 (PDE11) or pH 8.0, 1 mM EDTA (PDE10) at 4 °C, and stored at −20 °C.

For purification, cells were lysed at 1000 p.s.i. with a French press in lysis buffer (PDE10, 50 mM Tris-HCl pH 8.0, 50 mM NaCl, 20% glycerol, 15 mM imidazole; PDE11, 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 20% glycerol, and Complete® EDTA-free protease inhibitor mixture tablets, Roche Diagnostics) at 4 °C. After removal of debris (48,000 × g, 45 min), a Ni2+-nitrilotriacetic acid slurry (0.1 ml) was added for affinity purification. Protein binding was for 2 h at 0°C; the resin was then transferred into a column and washed (2 ml/wash). PDE10-CyaB1 chimeras were washed with 6 ml of buffer A (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 20% glycerol, 35 mM imidazole), 8 ml of buffer B (50 mM Tris-HCl, pH 8.0, 2 mM MgCl2, 400 mM NaCl, 70 mM imidazole, 20% glycerol), and 6 ml of buffer C (buffer B with 10 mM NaCl). For PDE11-CyaB1 chimeras, the columns were washed with 2 ml of buffer D (50 mM Tris-HCl, pH 8.5, 2 mM MgCl2, 400 mM NaCl, 5 mM imidazole, 20% glycerin), buffer E (buffer A + 15 mM imidazole), and buffer F (buffer D + 10 mM NaCl and 25 mM imidazole). Proteins were eluted with 0.3 ml of buffer C plus 300 mM imidazole. To remove imidazole, the fractions were dialyzed for 2 h against 50 mM Tris-HCl, pH 7.5 (PDE10) or pH 8.5 (PDE11), 2 mM MgCl2, 10 mM NaCl, and 35% glycerol and stored at −20 °C.

**Adenylyl Cyclase Assay**—The AC activity was assayed for 10 min at 37 °C in 100 μl (25). The reactions contained 22% glycerol, 50 μg of bovine serum albumin, 50 mM Tris-HCl pH 7.5, 10 mM MgCl2, and 75 μM [α-32P]ATP (25 kBq; from Hartmann Analytic, Braunschweig, Germany). 2 mM [2.8-3H]cAMP (150 Bq; from Amersham Biosciences) was added after stopping the reaction to determine yield during product isolation. The protein concentration was adjusted to limit substrate conversion to <10%. The reaction was started by the addition of the substrate. Values are given as means ± S.E. (n = 4). PDE activity was absent in all affinity-purified recombinant proteins.

**Western Blot Analysis**—Protein in sample buffer after SDS-PAGE (12.5%) was blotted onto polyvinylidene difluoride membranes and sequentially probed with either an anti-RGS-H4 or an anti-His4 antibody (Qiagen, Hilden, Germany) and with a 1:5000 dilution of a peroxidase-conjugated goat anti-mouse IgG secondary antibody (Dianova, Hamburg, Germany). Peroxidase detection was carried out with the ECL Plus kit (Amersham Biosciences).

**RESULTS**

**Determination of a Ligand for the GAF Domains of Human PDE10**—Six splice variants of human PDE10A1 have been reported that differ at their N and C termini, whereas the GAF domains are identical among all variants (26). The two major isoforms are PDE10A1 and A2 (PDE10A2, gi 5902442). We attached amino acids Leu-14 to His-422 of PDE10A1, i.e. the tandem GAF domain and an N terminus of 57 amino acids, which are identical in the PDE10A1 and A2 isoforms, to the cyanobacterial adenylyl cyclase cyaB1 (amino acid 386–859), generating PDE10A1-422/cyaB1AC386–859. The full-length PDE10A1 N terminus would include 13 amino acids, and that of PDE10A2 would contain 23 additional amino acids, respectively. The chimeric protein was expressed in E. coli, affinity-purified, and assayed for AC activity. Basal AC activity of 45 ± 3.9 nmol of cAMP/mg·min−1 was linear with protein concentration. The kcat for ATP was 27 ± 0.8 μM, and the Vmax was 84.5 ± 15 nmol of cAMP/mg·min−1 at pH 7.6. In contrast to PDEs 2, 5, and 6, cGMP did not stimulate AC activity. However, for the first time for a mammalian GAF domain, we observed activation for the first time for a mammalian GAF domain, we observed activation.

**FIGURE 1. Alignment of the conserved NKDFE motifs of the GAF-A and GAF-B regions from human phosphodiesterases types 10 and 11, the Arabocharisma cyaB1 adenylyl cyclase, trypanosomal phosphodiesterase 2B, and a tandem GAF domain coded by Paramecium gene 11075 (Paramecium Genome Browser). The amino acids of the NKDFE motif are boxed. The length variation between the conserved NK and F is also present in the cyaB2 adenylyl cyclase (28).**
exception of cIMP, which enhanced cAMP formation 1.6-fold at 100 μM (p < 0.05 when compared with the unstimulated control (n = 4)), students t test), none of these compounds had any significant stimulatory activity. The minor effect of cIMP was significantly different from the stimulation observed with 100 μM cAMP (p < 0.001; n = 4). Stimulation by cAMP was direct, and no preincubation was required to achieve full activation (data not shown). Similarly, deactivation was immediate upon removal of cAMP by a 200-fold dilution because subsequent AC activity measurements consistently yielded basal activities (data not shown). This indicated that association and dissociation of cAMP to and from the PDE10 tandem GAF domain was rapid, resulting in high on/off rates. cAMP stimulation increased V_{max} of the AC to 859 ± 146 nmol of cAMP·mg^{-1}·min^{-1} (derived from a linear Lineweaver-Burk plot) and did not affect K_{m}. A dose-response curve for cAMP gave an EC_{50} of 19.8 ± 1.6 μM cAMP (n = 4), and the Hill coefficient was 1.0, i.e. no cooperativity was detected (Fig. 2). The EC_{50} for cAMP was considerably higher when compared with 1 μM cAMP for activation of cyaB1 AC or 3 μM cGMP for activation of PDE2 in association with the cyanobacterial AC (23). In fact, a K_{d} well above 9 μM was predicted for the PDE10A GAF domains based on negative results of cAMP and cGMP binding studies (27). With a K_{d} of 22 μM cAMP, our data confirmed this prediction experimentally.

Role of the NKFDE Motif in GAF-B of Human PDE11—As mentioned above, the NKFDE signature sequence is strongly implicated in cyclic nucleotide binding, possibly via formation of the binding pocket, because a direct structural correlate is not evident from the existing x-ray structures (3, 13, 14, 22, 28). The PDE10 tandem GAF domain contains a conserved NKFDE motif only in GAF-B; in GAF-A, the corresponding positions are RHFH (Fig. 1). It was, therefore, likely that GAF-B would be involved in cAMP signaling, whereas the GAF-A site may serve some other function. Therefore, we generated a D397A mutation in GAF-B (PDE10A1_{14–422}/cyaB1AC_{386–859}D397A). The specific activity of the cyanobacterial AC was reduced by 97% (no change in K_{m}) in accordance with results concerning an analogous construct with the tandem GAF domains of PDE2 and the cyaB1 AC (23), possibly indicating a generally deleterious effect by such a mutation on enzyme activity. The minor residual stimulation observed with cAMP had an EC_{50} of 268 ± 4.2 μM (n = 4), i.e. 13.5-fold higher than the unmutated AC chimera (Fig. 2). The difference in the EC_{50} values between PDE10A1_{14–422}/cyaB1AC_{386–859} and PDE10A1_{14–422}/cyaB1AC_{386–859}D397A was highly significant (≤0.001; n = 4).

Determination of a Ligand for the GAF Domains of Human PDE11—PDE11 is the most recent PDE family identified. It is coded by a single gene, PDE11A (29). The existence of four tissue-specific splice variants that differ in the length of their N-terminal regions (7, 30, 31) has been described (PDE11A1–PDE11A4). PDE11 is a dual specificity PDE (30). PDE11A4 is the only splice variant that has a complete N-terminal tandem GAF domain. The GAF-A domain of PDE11A4 consists of about
FIGURE 3. Stimulation of the cyaB1 adenyl cyclase via the tandem GAF domain of human PDE11A4 with different N termini. A, the domain organization of the constructs is depicted schematically on top with dotted frames derived from PDE11 (see also the legend for Fig. 2). Note that in construct I, the N terminus (N-Term) is derived from cyaB1, and in construct II, it is derived from PDE11A4. B, stimulation of cAMP formation by cGMP (circles) and cAMP (squares) using construct I (means ± S.E. are shown, n = 4). Stimulations were
170 amino acids and is connected to GAF B (166 amino acids) via a 36-amino-acid linker, i.e. the domain organization is identical to that in the other PDEs and cyanobacterial ACs. In PDE11A4, the GAF ensemble is preceded by a 196-amino-acid-long N terminus of unknown function (30). Similar to the PDE10 GAF domains, all efforts to assign a function to the PDE11A4 GAF domains were unsuccessful (30). Neither cAMP nor cGMP bound to the GAF domain or affected the hydrolytic activity (30). In the first chimera, we omitted the 196-amino-acid N-terminal of PDE11A4 and replaced it with the 50-amino-acid N terminus activity (30). In the first chimera, we omitted the 196-amino-acid N-terminal of PDE11A4 and replaced it with the 50-amino-acid N-terminal of the bacterial cyaB1 to ease expression in E. coli. This yielded the chimera cyaB1–50PDE11A41–197,568cyaB1366–859 (Fig. 3A, construct I) in which the tandem GAF arrangement of PDE11A4 exactly replaced that of cyaB1. CyaB1–50PDE11A41–197,568cyaB1366–859 was expressed in E. coli as a soluble protein and affinity-purified. The basal AC activity was 5.3 ± 0.6 nmol of cAMP·mg⁻¹·min⁻¹; however, it was not significantly affected by the addition of cAMP or cGMP up to 10 μM (Fig. 3B). In view of these negative data, we suspected that the 196-amino-acid-long N terminus of PDE11A4 might have an intrinsic function in GAF domain signaling. Therefore, in the next construct, the complete PDE11A4 N terminus was linked to cyaB1 AC, generating PDE11A41–568cyaB1386–859 (Fig. 3A, construct II). Expression in E. coli yielded a soluble protein. The basal activity of the affinity-purified chimera was 5.8 ± 0.3 nmol of cAMP·mg⁻¹·min⁻¹. The Km for ATP was 19 ± 1 μM, and the Vmax was 10 ± 0.2 nmol of cAMP·mg⁻¹·min⁻¹ at pH 7.5. This construct was stimulated 4-fold by cGMP with significant stimulations beginning with 10 μM cGMP. The EC₅₀ for cGMP was 0.2 nmol of cAMP·mg⁻¹·min⁻¹ and did not affect Km. The EC₅₀ for cGMP was 72.5 ± 1 μM, and maximal activation was obtained at 3 mM cGMP, most likely not a physiologically relevant cGMP concentration. We also determined the on/off rates via activation/deactivation assays. A plot of cAMP formation over time after the addition of 3 mM cGMP was linear for at least 10 min, passing through the origin on backward extrapolation. This established that the cGMP activation was virtually instantaneous. Similarly, when the recombinant protein was preincubated with 1 mM cGMP for 15 min at 0 °C and diluted 200-fold to 5 μM cGMP immediately prior to assay, we did not observe a persisting AC activity. This means that on/off rates for cGMP were rather rapid, as we found for cAMP in PDE10 (see above). These rapid on/off rates may explain why no cGMP or cAMP binding has been observed in earlier experiments (27, 30). To determine the nucleotide specificity of the activation, we tested the following purine nucleotides and nucleosides with PDE11A41–568cyaB1366–859 at 1 mM concentrations: ADP, AMP, GTP, GDP, GMP, cXMP, cIMP, dicyclic guanosine monophosphate (100 μM), guanosine, and adenosine. We did not see any statistically significant or insignificant stimulatory or inhibitory potency. This leaves cGMP as the only purine nucleotide capable of stimulation of the PDE11A tandem GAF domain.

**Role of the NKFDE Motif in GAF-B of Human PDE10**—In PDE11A, a single NKFDE signature motif is present in GAF-A, and it is blurred (NRFDQ) in GAF-B. Because so far the conservation of the NKFDE motif has turned out to be a major requirement for cAMP (see above) and cGMP binding and activation (3), we assumed that in PDE11A4, signaling will occur via GAF-A and generated a D355A mutant (PDE11A41–568cyaB1386–859D355A). Basal activity was significantly reduced to 2.1 ± 0.2 nmol of cAMP·mg⁻¹·min⁻¹ when compared with the wild-type PDE11A GAF domain. Activation by cGMP was not completely lost but significantly reduced from a 4-fold stimulation to a 2.6-fold stimulation (statistically significant at 2p < 0.05, n = 4; Fig. 3C). This result was expected and in line with data reported for cyclic nucleotide binding and activation of other GAF domains in which a residual activity often seemed to remain (22, 28). This was reassuring in view of the rather high EC₅₀ concentration for cGMP because it established that the PDE11 tandem GAF domain belongs exactly in the same category as the other tandem GAF domains of mammalian PDEs.

**DISCUSSION**

We identified ligands for the tandem GAF domains of the human PDEs 10 and 11 as cAMP and cGMP, respectively. Thus, the GAF domain of PDE10 is the only one of the five mammalian PDE families that binds and regulates via cAMP. The EC₅₀ concentrations of 19.8 μM cAMP for PDE10 and 72.5 μM cGMP for PDE11A4 were 2–23-fold higher when compared with those for cGMP activation via the GAF domains of PDE2 or PDE5 (23, 32). Are these physiologically relevant concentrations in terms of PDEs 10 and 11 regulation, or are they artificially high due to the test system? We believe that they are not artifically high because chimeras between the cyanobacterial AC and the tandem GAF domains of PDEs 2 and 5 have yielded EC₅₀ concentrations for cGMP-stimulated cAMP formation around 1 μM, which compares favorably with those determined in binding and PDE activation assays (23). Thus, this chimeric assay system is validated with analogous constructs. The rather high EC₅₀ values explain why the ligands were not identified earlier. Too low concentrations of cAMP and cGMP were employed (27, 30), and the immediacy of the on/off rates probably caused loss of ligand upon rinsing of the filters.

The significance of the cAMP-stimulated GAF domain for the regulation of intracellular PDE10 activity remains to be established. An EC₅₀ of 19.8 μM cAMP appears to be reasonable, and it is not necessary to invoke potential cAMP and PDE10 sequestration in cellular compartments. Of course, this does not exclude the possibility for specific complexes composed of biosynthetic and hydrolytic activities together with a protein kinase for downstream signaling. This might locally facilitate and coordinate certain signal transduction processes within a cell. PDE10 has been shown to be expressed in testis and thyroid and to be particularly abundant in the putamen and caudate nucleus regions of the brain (27). Because these brain areas control major behavioral and motor functions, implying dopaminergic neurotransmission possibly involving cyclic nucleotide pathways, PDE10 as a dual specificity enzyme is currently envisaged as a potential drug target for the development of mood-affecting drugs (33). The data of a-CAMP-regulated PDE10 now allow a novel look at this strategy.

Concerning PDE11, the EC₅₀ of 72.5 μM cGMP is much higher than the generally accepted cellular cGMP concentration of around 1 μM. Therefore, a cGMP-signaling complex involving PDE11A would be expected to be spatially confined such that the local concentration of cGMP may reach levels close to the EC₅₀ concentration reported here. Another possibility might be that PDE11A4 is undergoing secondary modifications that enhance cGMP sensitivity of its N-terminal tandem GAF domain. These questions have to be addressed using the PDE11A holoenzyme.
GAF Domains of PDEs 10 and 11

Regarding the 196-amino-acid-long N terminus of PDE11A4, we accidentally noted an effect on signaling. With the cyaB1 N terminus preceding the PDE11A GAF domains as in the cyaB1–56–PDE11A4–97–568–cytB1–386–859 chimera, cGMP did not activate, whereas it did when the complete N terminus of PDE11A4 was swapped for the cyanobacterial one as in the PDE11A4–97–568–cytB1–386–859 chimera. This result could have implications for the activation process. One may argue that the N-terminal GAF domain in the cyaB1–56–PDE11A4–97–568–cytB1–386–859 construct did not fold correctly. Considering the impossibility to check cGMP binding of the PDE11A4 GAF domains, this cannot be completely ruled out. However, this would imply that of the two domains in a single protein, one folded correctly and the other did not. Obviously, the tandem GAF domain did not interfere with dimerization, which is absolutely required for AC activity. The $K_{\text{cat}}$ for this construct was 48 μM ATP, and $V_{\text{max}}$ was 15 nmol of cAMP·mg$^{-1}$·min$^{-1}$, strongly indicating that loss of activation was due to an effect of the N terminus on intramolecular signaling. Actually, this effect appears to have a counterpart in the PDE11 holohemolysin enzyme. The effects of inhibitors on the activity of PDE11A3 (gi 63253296), which has a different N terminus when compared with PDE11A4, were 2–3-fold more potent than those on the PE11A4 isoform (30, 34). This suggests that the N terminus of PDE11A affects the overall conformation of the protein, leading to changes of the enzymatic PDE profile and, as shown here, of the associated AC dimer. An interesting question will be to determine whether the N termini of other PDEs similarly affect intramolecular signaling.

A physiological function for PDE11 needs to be established. Interestingly, in humans, PDE11A4 was reported to be the only isoform that is expressed in humans, PDE11A4 was reported to be the only isoform that is expressed in the regulation of potassium channels via cGMP-dependent protein kinase intramolecular signaling. As shown here, of the associated AC dimer. An interesting question will be to determine whether the N termini of other PDEs similarly affect intramolecular signaling.

A physiological function for PDE11 needs to be established. Interestingly, in humans, PDE11A4 was reported to be the only isoform that is expressed in human prostate, whereas other human tissues contain only minor amounts (34). In the prostate, cGMP is reported to be involved in proliferation of stromal cells and in the regulation of potassium channels via cGMP-dependent protein kinase and apoptotic processes in prostate cancer cells (35, 36). Thus, PDE11 may be involved in these processes. In rat and mouse, several other PDE11A isoforms and their tissue-specific expression have been demonstrated (37, 38). However, a PDE11 knock-out mouse did not exhibit any striking suggestions. A redution in sperm motility was taken as evidence for a role in spermatogenesis and fertilization, albeit the knock-out did not affect animal fecundity (38).

Finally, based on the effects of the D/A mutation within the NKFDE motif in all GAF domains of this type, we would propose that the NKFDE motif defines a distinct subclass of GAF domains that is required, but not absolutely sufficient (e.g. in GAF-A of PDE2), for cAMP or cGMP signaling. The above prediction may be tested with a tandem GAF domain containing a canonical NKFDE motif in GAF-A, which was recently identified in an open reading frame from the prototoxin Paramaecium (Fig. 1). This subclass is set apart from a subclass proposed to be present in phytochromes. Those GAF domains that have different signature sequences either directly bind a bilin-type chromophore or are necessary for phytochrome binding by an adjacent PAS-like domain (39). A third subclass of GAF domains may comprise the one present in the transcription activator FlhA, which is regulated by formate, and the one in the Nif-L/NifA transcriptional regulatory complex, which is regulated by α-ketobutyrate as in Azotobacter vinelandii (40). A subclassification of the more than 800 annotated GAF domains would greatly aid in ligand prediction.