A novel gene encoding an adenylyl cyclase, designated cyaG, was identified in the filamentous cyanobacterium *Spirulina platensis*. The predicted amino acid sequence of the C-terminal region of cyaG was similar to the catalytic domains of Class III adenylyl and guanylyl cyclases. The N-terminal region next to the catalytic domain of CyaG was similar to the dimerization domain, which is highly conserved among guanylyl cyclases. As a whole, CyaG is more closely related to guanylyl cyclases than to adenylyl cyclases in its primary structure. The catalytic domain of CyaG was expressed in *Escherichia coli* and partially purified. CyaG showed adenylyl cyclase (but not guanylyl cyclase) activity. By site-directed mutagenesis of three amino acid residues (Lys533, Ile603, and Asp605) within the purine ring recognition site of cGMP, we found that the catalytic activity of CyaG was transformed to a guanylyl cyclase that produced cGMP instead of cAMP. Thus having properties of both cyclases, CyaG may therefore represent a critical position in the evolution of Class III adenylyl and guanylyl cyclases.

Adenylyl cyclase (AC), which synthesizes the signaling molecule cAMP, plays an important role in regulating various cell processes. So far, many genes encoding ACs have been isolated from a number of organisms. ACs can be separated into three classes according to the primary structure of the catalytic domains: Class I ACs are mainly composed of enterobacterial ACs, Class II ACs have been found in pathogenic bacteria and are activated by the eukaryotic cofactors calmodulin (2, 3) and an as yet unidentified factor (4). Class III ACs are widely distributed from bacteria to mammals and form the biggest family of ACs. Recently, novel ACs showing a unique primary structure have been found in archaebacteria. These are proposed to represent a fourth class of ACs (5). No sequence homology can be found among all four AC classes.

cGMP also functions as an important signaling molecule and is synthesized by guanylyl cyclase (GC). A large number of GC genes have been isolated from eukaryotes. However, only one GC gene has been reported in prokaryotes (6). In contrast to the divergent nature of the catalytic domains of ACs, the catalytic domains of all GCs identified are homologous to those of Class III ACs. Thus, Class III ACs and GCs are thought to have evolved from a common ancestor.

Crystal structures of the catalytic domains of mammalian Class III ACs have been solved (7, 8). Based on these structures and modeling studies, essential residues required for substrate binding (ATP or GTP) have been identified (9). The amino acid residues that form hydrogen bonds with adenine and guanine are conserved among Class III ACs and GCs (9). By exchanging these residues, GCs can be converted to ACs (10, 11). Similarly, an AC has been converted to a nonselective purine nucleotide cyclase (11).

The N-terminal regions next to the catalytic domain (~50 amino acid residues) are conserved among GCs, but not among Class III ACs. These regions are predicted to form an amphipathic α-helix and to function as a dimerization domain (12). Besides the importance for dimerization, this domain has also been shown to play an important role in stimulating human retinal GC-1 catalytic activity by GC-activating proteins (13).

We have isolated six AC genes from the cyanobacterium *Spirulina platensis* by functional screening using *Escherichia coli* defective in its AC gene (14). We previously characterized two AC genes, cyaA and cyaC (14, 15). The cyaA gene encodes a putative membrane-bound AC, whereas the cyaC gene encodes an AC that is activated in response to autophosphorylation (14, 16). In this study, we describe the characterization of a third AC gene from *S. platensis*, cyaG. The amino acid sequence of the putative catalytic domain of CyaG was found to be homologous to those of the catalytic domains of Class III ACs and GCs. Interestingly, the upstream regions next to the catalytic domain of CyaG are homologous to the dimerization domain of GCs. We investigated the catalytic activity of CyaG by generating wild-type and mutant recombinant proteins. We found that CyaG, a strict adenylyl cyclase, was transformed to a guanylyl cyclase by replacing three key amino acid residues within the substrate-binding site. Based on the results, the evolution of Class III ACs and GCs is discussed.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Growth Media—**The *E. coli* strains JM109 (recA1, endA1, gyrA96, thi, hsdR17 k+ mK+), supE44, relA1, Δlac-proAB::F′ (traD36, proA8, lacQ, lacZΔM15) and MP2339 (aroB+), cprA39, ilvC-, cyaΔ) were used for cloning and expression of recombinant proteins, respectively. Bacteria were grown in LB medium. When required, ampicillin, kanamycin, or chloramphenicol was added at 100, 25, or 30 μg/ml, respectively.

**Genetic Methods—**Plasmid preparations, restriction enzyme digests, and ligations were performed as described by Maniatis et al. (17). Cloning of the cyaG Gene from *S. platensis*—The genomic library of *S. platensis* was screened by functional complementation of the mutant.
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of *E. coli* MK1010 defective in AC activity. The positive clone pCYA68 was isolated as described in our previous report (14).

To isolate overlapping fragments, the genomic library of *S. platensis* was produced with a phage vector (15). Recombinant phages were screened for the presence of the desired sequence using the *Bam* HI fragment as a probe. Following secondary screening, positive recombinant phages were converted to pBK-CMV plasmids by *in vivo* excision using the Stratagene protocol.

**DNA Sequencing and Analysis**—A 2.6-kilobase pair DNA fragment containing the 2.0-kilobase pair DNA insert of *S. platensis* was excised from pCYA68 with *Hind* III and Sulf digestion and subcloned into the *Hind* III site of pBluescript II KS+ (Stratagene). A 2.0-kilobase pair fragment was excised from pBKG1 with *Bst* I and DraI, treated with the Klenow fragment to fill the ends, and subcloned into the EcoRI site of pBluescript SK+ (Stratagene). Deletions were constructed using exonuclease III and mung bean nuclease. The nucleotide sequence was determined by the dyeoxy chain termination method using DNA sequencer (Model 373A) and a *Tag* dyeoxy terminator cycle sequencing kit (both from Applied Biosystems). The DNA sequence and predicted amino acid sequence were analyzed with DNASTAR software. Data base searches for similarity to other proteins were performed using the GenBank BLAST server.

**Construction of the Expression Plasmid for the GST-CyaG-CD Protein**—A *Bam* HI restriction site was introduced immediately upstream of the second letter of the codon encoding Ser380, and an EcoRI restriction site was introduced 75 base pairs downstream of the stop codon using PCR. The PCR product was ligated into the pGEX-3X vector digested with *Bam* HI and EcoRI. The resulting plasmid, pGEX-CyaG-CD (where CD is catalytic and dimerization domains), contained a portion of the *cyaG* gene fused to the GST tag sequence from pGEX-6p-1 (Stratagene). A 2.0-kilobase pair fragment of pBKG1 was isolated, and restriction mapping confirmed that an EcoRI fragment (Fig. 1). An ORF that encodes a protein with the *cyaG* gene was found in the sequence, but the N terminal domain is double-underlined.

**Site-directed Mutagenesis**—The *GST-CyaG-CD-K533E* mutant was constructed with mismatch oligonucleotides and PCR using ExTaq DNA polymerase (Takara). PCR s were performed with primers P1 (5'-AAGAATTCATCGAGATGTACGAG-3') and P3 (5'-ATATATATATATACAGATGTCGAC-3') or primers P2 (5'-GTTTATCAATTTTCTGAGGTCGAC-3') and P4 (5'-GATTCGATCTCGTCACGAC-3'). The resulting PCR products were mixed, and PCR was repeated with P1 and P4. The PCR product was digested with EcoRI and *Bam* HI (partial) and ligated into pGEX-CyaG-CD digested with EcoRI and *Bam* HI (partial). The resulting construct, pGEX-CyaG-CD-E, contained the *cyaG* gene with the CyaG K533E mutation.

The *GST-CyaG-CD-K533F* mutant was constructed with mismatch oligonucleotides and PCR using ExTaq DNA polymerase. PCRs were performed with primers P1 and P5 (5'-CCAGATACGATAATGTTTATATC-3') or primers P4 and P6 (5'-ATATATATATATATACAGATGTCGAC-3'). The PCR product was ligated into the pCRX vector (Invitrogen) to make pCRX. The EcoRI-XhoI fragment of pCRX (500 base pairs) was ligated into pGEX-CyaG-CD-E digested with EcoRI and XhoI. The resulting construct, pGEX-CyaG-CD-E-D, contained the *cyaG* gene with the CyaG K533F, K533R, and D605C mutations. The portion of pGEX-CyaG-CD-E digested with *Eco* RI and *Kpn* I was ligated into pGEX-CyaG-CD-ERC digested from the PCR fragments to ensure that no other mutations had arisen.

**AC and GC Activity Assays**—Unless otherwise stated, the in vitro AC or GC reaction was performed in 0.1 ml of 50 mM Tris-HCl (pH 7.5) containing 1 mM MnCl2, 1 mM dithiothreitol, 0.1% (w/v) bovine serum albumin, and 1 mM ATP or GTP, respectively. The reactions were performed at 37 °C for 20 min and terminated by addition of 1 ml of 5% (w/v) trichloroacetic acid. After trichloroacetic acid was removed from each reaction mixture by extraction with ethyl ether, samples were lyophilized. cAMP or cGMP content was measured by an enzyme immunoassay system (EIA system, Amersham Pharmacia Biotech) according to the manufacturer's protocol.

**DNA Sequencing**—A nucleotide sequence was determined by the method of Bradford (18) as described in the instructions of the Bio-Rad protein assay kit. Bovine serum albumin was used as the standard. Gel electrophoresis on polyacrylamide gel containing 0.1% SDS was carried out following the method of Laemmli (19).

**RESULTS**

**Isolation of the *S. platensis* AC Gene**—The genomic library of *S. platensis* was screened by functional complementation of the *E. coli* strain MK1010, which is defective in AC activity and therefore lacks cAMP. Seven distinct positive clones were isolated (14). A significant level of cAMP was detected in cells transformed with pCYA68, one of the positive clones, suggesting that pCYA68 contains an AC gene of *S. platensis* (14). We determined the nucleotide sequence of the insert region of pCYA68. An ORF was found in the sequence, but the N terminus of the ORF was absent in pCYA68. To isolate an overlapping clone, we screened an *S. platensis* genomic library as described under "Experimental Procedures." A positive clone (pBRG1) was isolated, and restriction mapping confirmed that this clone contained the N terminus of the ORF (data not shown).

**DNA Sequencing**—A nucleotide sequence (2449 base pairs) was determined by sequencing the insert region of pCYA68 and a *Dra*I-*Bst*I fragment of pBRG1 (Fig. 1). An ORF that encodes a polypeptide of 671 amino acids with a predicted molecular mass of 75,365 Da was identified in this sequence.
FIG. 2. Amino acid alignment and phylogenetic analysis of the catalytic domain of CyaG. A, alignment of CyaG with *Anabaena* PCC 7120 CyaE, *S. platensis* CyaC, *Rhizobium melliloti* Cya1, rat CAC2, rat atrial natriuretic peptide receptor GC-A, human RetGC-1, rat GC-S α1 (GC-S alpha-1), and rat GC-S β1 (GC-S beta-1). The rat AC2 sequence represents two conserved regions (C1 and C2). Amino acid residues identical in half or more of the sequences are shown in black, and conservative substitutions are shown in gray. Gaps introduced for good alignment are indicated by dashes. Numbers are amino acid positions for each protein sequence. Amino acid residues involved in recognizing the purine ring of ATP or GTP are indicated by asterisks. B, phylogenetic analysis of the catalytic domains of Class III ACs and GCs. Phylogenetic analysis was performed using the MegAlign module of the Lasergene software package (DNASTAR, Inc.). The DDBJ/GenBank/EMBL Data Bank accession numbers of the protein sequences used are as follows: human RetGC-1, M92432; human RetGC-2, L37378; *Oryzias* GC, AB000899; human GC-C, U20230; rat GC-C, M56566; rat KSGC (kinase-like domain-containing soluble GC), AB000899; rat GC-A, J05677; *Drosophila* AC, L35598; sea urchin GC, M22444; rat GC-S α1, U60835; rat GC-S β1, P20595; *Drosophila* AC (ruthaeba), M1857; rat AC2, M80550; rat AC5, M96158; *Dicyostelium* ACA, L35498; *Dicyostelium* ACC, M37278; *Spirulina* CyaG, D49531; *Spirulina* CyaA, D49530; *Synechocystis* Cya1, S75018; *Anabaena* CyaA, D49531; *Spirulina* CyaA, D49530; *Synechocystis* Cya1, S75018; *Anabaena* CyaB1, D89623; *Anabaena* CyaB2, D89624; *Stigmatella* CyaA, AJ223798; *Spirulina* CyaA, D49530; *Synechocystis* Cya1, S75018; *Anabaena* CyaB1, D89623; *Anabaena* CyaB2, D89624; *Stigmatella* CyaA, AJ223798; *Rhizobium* Cya1, M35096; rat soluble AC, AAD04035; *Neurospora* AC, Q01631; and *Saccharomyces* AC, P08678.
The catalytic domain of CyaG is more similar to that of the novel domains of Class III ACs and GCs. One (Met1–Gly31) is near the N-terminal end, and the other (Ile365–Ile376) is located in the central region of the protein. The N-terminal hydrophobic region is closely related to the signal peptides of bacteria required for the targeting of proteins to membranes (20–22). The latter hydrophobic region probably represents a membrane-spanning region.

Comparison of the C-terminal Region of CyaG with the Catalytic Domains of Class III ACs and GCs—The amino acid sequence of CyaG exhibited similarities to the catalytic domains of Class III ACs and GCs. Fig. 2A shows the alignment of the C-terminal region (Val467–Gly663) of CyaG with the catalytic domains of various Class III ACs and GCs. The putative catalytic domain of CyaG has 29–44% identity to those of Class III ACs and 35–48% identity to those of GCs. Asterisks in Fig. 2A indicate essential amino acid residues required for interacting with the adenine or guanine substrate ring (9). The essential amino acid residues present in CyaG were more similar to those of Class III ACs than to those of GCs.

The complete genome sequence of a filamentous cyanobacterium, *Anabaena* sp. PCC 7120, has recently been determined. In addition to those five AC genes that have already been isolated from *Anabaena* 7120 (23), we found a novel AC gene by searching the database at the Kazusa DNA Institute and named this gene *cyaE*. Among the six *Anabaena* ACs, the catalytic domain of CyaG is more similar to that of the novel *Anabaena* AC, CyaE (Fig. 2A).

We performed a phylogenetic analysis of the catalytic domains of the Class III ACs and GCs. The catalytic domain of CyaG was found to be more closely related to those of eukaryotic Class III ACs and GCs than to those of cyanobacterial and bacterial ACs (Fig. 2B).

Existence of the Dimerization Domain in CyaG and *Anabaena* CyaE, Commonly Found in GCs—We detected a unique amino acid sequence homologous to the so-called dimerization domain of GCs in the N-terminal region next to the catalytic domains of CyaG and *Anabaena* CyaE (Fig. 3). The dimerization domain is present in all GCs except two exceptional GCs (6, 24), but not in Class III ACs. Rat GC-A forms a homodimer by interacting with the dimerization domain of each subunit (12). The domain is predicted to form an amphipathic α-helix, a feature that is associated with mediating protein-protein interactions. A helical diagram of the putative dimerization domain of CyaG using DNASTAR software showed that this region could form an amphipathic α-helix (data not shown). Hydrophobic amino acid residues that form a cluster at one side of the helix are shown in Fig. 3 (asterisks). Three mutations are identified in conserved amino acid residues in the dimerization domain of human retinal GC-1, RetGC-1, in patients with cone-rod dystrophy (Fig. 3, arrowheads) (25, 26). By characterizing a mutant RetGC-1 protein with Arg substituted for Cys in the conserved amino acids, Tucker *et al.* (13) showed that mutant RetGC-1 had altered responses to GC-activating proteins and Ca2+.* They concluded that the mutation in the dimerization domain stabilized the active form of RetGC-1. The corresponding amino acid residues were also found to be conserved in the dimerization domain of...
method may be useful for expressing ACs in protein, we were able to construct pGEX-CyaG-CD. This GST-CyaG-CD protein by the addition of 0.1 mM isopropyl-
CD) were grown at 27 °C and induced for the production of the rat AC2, rat soluble GC (GC-S)
a and Mn2
S. platensis
found for Class III ACs and GCs from eu-
Spirulina
CyaC,
cya
(27). By using strain TP2339 E. coli
cationic ACs and GCs (9). We investigated whether the substrate of CyaG could be changed from ATP to GTP by site-directed mutagenesis of the purine ring-binding site. Liu et al. (9) iden-
tified amino acid residues involved in recognizing the purine ring of the substrate by a modeling study based on the three-
dimensional structure of a mammalian AC (8). Lys533, Ile603, and Asp605 of CyaG correspond to the amino acid residues related to purine ring binding. A mutant CyaG protein (GST-
CyaG-CD-K533E) in which Lys533 was replaced by glutamate was produced as described under “Experimental Procedures.” Lys533 is replaced by glutamate in all GCs (1). We found that GST-CyaG-CD-K533E lost AC activity completely, and no GC activity was detected (data not shown). Next, we changed two more amino acid residues of GST-CyaG-CD-K533E, Ile603 and Asp605, to arginine and cysteine, respectively. The resultant mutant protein, GST-CyaG-CD-K533E/I603R/D605C, has triple mutations. GST-CyaG-CD-K533E/I603R/D605C lost AC activity, but obtained significant GC activity (Fig. 6). CyaG as an AC was therefore changed to GC by the site-directed mutations.

**AC and GC activities of wild-type and mutant CyaG proteins.** AC and GC activities were measured as described under “Experimental Procedures.” Partially purified wild-type (GST-CyaG-
and mutant (GST-CyaG-CD-K533E/I603R/D605C) proteins were used for the assays. AC and GC activities are shown as black and gray bars.

CyaG.

Fig. 4 shows domain organizations of CyaG, Spirulina CyaC, rat AC2, rat soluble GC (GC-S) α1, and rat GC-A. It is worth noting that CyaG contains a dimerization domain like those of GCs.

**Expression and Purification of CyaG—**To investigate whether CyaG exhibits AC and/or GC activity, we purified the C-terminal domain of CyaG, which contains both the catalytic and dimerization domains, as a GST fusion protein (GST-
CyaG-CD). We failed to construct the expression vector encoding the GST-CyaG-CD protein (pGEX-CyaG-CD) when using an E. coli strain, JM109 (cya
, crp
), for cloning. This was probably due to an inhibitory effect of the high concentration of cAMP on the growth of E. coli (27). By using strain TP2339 (cya
, crp
), which is defective in AC and the cAMP receptor protein, we were able to construct pGEX-CyaG-CD. This method may be useful for expressing ACs in E. coli cells.

The transformants (TP2339 cells harboring pGEX-CyaG-
were grown at 27 °C and induced for the production of the GST-CyaG-CD protein by the addition of 0.1 mM isopropyl-
-thiogalactopyranoside. The majority of the GST-CyaG-CD protein formed was found to be in the insoluble fraction (data not shown). Nevertheless, the 150,000 g fraction, in which we detected the GST-CyaG-CD protein by immunoblotting against CyaG, was recognized. The size was consistent with the predicted theoretical value for the GST fusion protein.

**AC and GC Activities of the GST-CyaG-CD Protein—**The effects of divalent cations on the AC activity of the GST-
protein were measured. The addition of 0.1 mM isopropyl-
and Mn2
were 0.036 and 11 nmol of cAMP formed per min/mg in the presence of 10 μM ATP, respectively. The observation that Mn2
stimulates CyaG catalytic activity is similar to results found for Class III ACs and GCs from S. platensis and eu-
ykaryotes (15, 28, 29). AC and GC activities were assayed in the presence of Mn2
as described under “Experimental Procedures.” Significant AC activity was detected, but no GC activity was detected (Fig. 6).

**Site-directed Mutations within the Predicted Nucleotide-binding Site of CyaG—**The cyclase catalytic consensus sequence is conserved among the Class III AC and GC families (30). Residues interacting with the ribose and triphosphate of ATP or GTP and the metal ion are conserved between Class III ACs and GCs. However, interactions with the purine ring are different between the ATP- and GTP-binding sites of Class III ACs and GCs (9). We investigated whether the substrate of CyaG could be changed from ATP to GTP by site-directed mutagenesis of the purine ring-binding site. Liu et al. (9) iden-
tified amino acid residues involved in recognizing the purine ring of the substrate by a modeling study based on the three-
dimensional structure of a mammalian AC (8). Lys533, Ile603, and Asp605 of CyaG correspond to the amino acid residues related to purine ring binding. A mutant CyaG protein (GST-
CyaG-CD-K533E) in which Lys533 was replaced by glutamate was produced as described under “Experimental Procedures.” Lys533 is replaced by glutamate in all GCs (1). We found that GST-CyaG-CD-K533E lost AC activity completely, and no GC activity was detected (data not shown). Next, we changed two more amino acid residues of GST-CyaG-CD-K533E, Ile603 and Asp605, to arginine and cysteine, respectively. The resultant mutant protein, GST-CyaG-CD-K533E/I603R/D605C, has triple mutations. GST-CyaG-CD-K533E/I603R/D605C lost AC activity, but obtained significant GC activity (Fig. 6). CyaG as an AC was therefore changed to GC by the site-directed mutations.

**DISCUSSION**

In this study, we isolated a novel AC gene, cyaG, from the cyanobacterium S. platensis. The CyaG protein contains a catalytic domain homologous to the Class III ACs and GCs at the C-terminal region. It contains a dimerization domain conserved among GCs at the N-terminal side of the catalytic domain and two hydrophobic regions, one at the N terminus and the other in the middle of the protein. The N-terminal hydrophobic region has characteristics of a bacterial signal sequence for localizing the protein to the membrane and would be digested after targeting. CyaG is thought to be a single transmembrane protein.

When we performed the BLAST search to find homologies using the amino acid sequence of the cyaG gene product, we found that the protein sequences most closely related to the C-terminal regions of CyaG were those of GCs rather than ACs. Since we previously showed that the cDNA of cyaG produced cAMP (14), we speculated that CyaG may exhibit GC activity as well as AC activity. Furthermore, investigating the CyaG sequence carefully, we found the dimerization domain highly conserved among all GCs.

Wilson and Chinkers (12) predicted that the dimerization domain formed an amphiphilic α-helix and showed that GC activity was lost upon the deletion of the dimerization domain. The dimerization domains of both soluble and membrane-bound GCs are located on the N-terminal side next to the catalytic domain. To our knowledge, the dimerization domain exists in all GCs except two GCs recently found (6, 24), but not in ACs. Tucker et al. (13) have shown that point mutations within the dimerization domain of human RetGC-1 result in reduced catalytic activity of RetGC-1 and reduced stimulation by GC-activating protein-2. These findings indicate that the dimerization domain is essential for the catalytic activity of GCs. CyaG contains the dimerization domain (Fig. 3), but cannot form cGMP; and thus, CyaG represents a unique AC with a characteristic structure of GCs.

To investigate the catalytic activity of CyaG, we produced a recombinant protein (GST-CyaG-CD) containing the catalytic
changing certain amino acid residues. First, we changed Lys533 to Glu since the Lys residue is highly conserved in ACs, but the same position contains Glu in GCs (1). GST-CyaG-CD-K533E showed no AC activity. GC activity was also not detectable. We therefore concluded that additional replacements were required to obtain GC activity. In addition to the K533E substitution, we replaced two other amino acid residues, Ile603 and Glu605, with Arg and Cys, respectively. Ile603 and Glu605 are conserved within the purine ring-binding site. The triple mutant GST-CyaG-CD-K533E/I603R/D605C was found to exhibit significant GC activity (Fig. 6), but no AC activity. Therefore, CyAG, an AC, was converted to a GC by replacing these three amino acid residues.

The primary structure of the catalytic domain of CyAG is more closely related to those of eukaryotic transmembrane ACs and GCs than to those of other cyanobacterial ACs (Fig. 2). The catalytic domains of cyanobacterial ACs are divided into two groups. The first group includes CyAG and Anabaena CyaE (group I), and the second includes the remaining cyanobacterial Cya proteins (group II). These two groups might have evolved independently just like mammalian soluble AC and transmembrane AC (31). Interestingly, mammalian transmembrane ACs and a soluble AC are related to the cyanobacterial group I and II ACs, respectively (Fig. 2B). Thus, CyA-type ACs may possibly share a common ancestor with the transmembrane ACs and GCs.

Class III ACs and GCs are thought to be evolutionarily related to each other because of the similarity in the primary structures of their catalytic domains. Two GCs that have the unusual property of lacking the dimerization domain were recently found in Paramecium (24) and Synechocystis (6). The catalytic domain of Paramecium GC has a topology identical to that of mammalian membrane-bound ACs (Fig. 4, rat AC2) and thus was proposed to be evolved from a eukaryotic transmembrane AC. Synechocystis GC (Fig. 2B, Cya2) is the first example of a prokaryotic GC whose catalytic domain is more similar to those of bacterial ACs than to those of eukaryotic GCs. Thus, it is likely that Synechocystis GC has evolved from a bacterial AC. The other GC members that contain the dimerization domain seem to have an ancestor other than Paramecium GC and Synechocystis GC. CyAG is a unique AC in that it contains the dimerization domain essential for GC activity. Also, the catalytic activity of CyAG is changed to a GC by replacing only three amino acid residues. Based on these findings, it seems likely that most eukaryotic GCs, with the exception of Paramecium GC, have evolved from an AC such as CyAG that contains the dimerization domain.

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CyaG, a Novel Cyanobacterial Adenylyl Cyclase and a Possible Ancestor of Mammalian Guanylyl Cyclases
Masahiro Kasahara, Tsuyoshi Unno, Kumiko Yashiro and Masayuki Ohmori

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