A Defined Subset of Adenylyl Cyclases Is Regulated by Bicarbonate Ion*§

Received for publication, March 25, 2003, and in revised form, June 23, 2003
Published, JBC Papers in Press, June 26, 2003, DOI 10.1074/jbc.M303025200

Martin J. Cann‡‡§, Arne Hammer¶, Jie Zhou†, and Tobias Kanacher¶

From the ‡Department of Biological and Biomedical Sciences, University of Durham, South Road, Durham, DH1 3LE, United Kingdom and ¶Pharmazeutische Biochemie, Pharmazeutisches Institut, Morgenstelle 8, D-72076 Tübingen, Germany

The molecular basis by which organisms detect and respond to fluctuations in inorganic carbon is not known. The cyaB1 gene of the cyanobacterium Anabaena sp. PCC7120 codes for a multidomain protein with a C-terminal class III adenylyl cyclase catalyst that was specifically stimulated by bicarbonate ion (EC50 9.6 mM). Bicarbonate lowered substrate affinity but increased reaction velocity. A point mutation in the active site (Lys-646) reduced activity by 95% and was refractory to bicarbonate activation. We propose that Lys-646 specifically coordinates bicarbonate in the active site in conjunction with an aspartate to threonine polymorphism (Thr-721) conserved in class III adenylyl cyclases from diverse eukaryotes and prokaryotes. Using recombinant proteins we demonstrated that adenylyl cyclases that contain the active site threonine (cyaB of Stigmatella aurantiaca and Rv1319c of Mycobacterium tuberculosis) are bicarbonate-responsive, whereas adenylyl cyclases with a corresponding aspartate (Rv1264 of Mycobacterium) are bicarbonate-insensitive. Large numbers of class III adenylyl cyclases may therefore be activated by bicarbonate. This represents a novel mechanism by which diverse organisms can detect bicarbonate ion.

*cAMP is one of the most prevalent signaling molecules among prokaryotes and eukaryotes, modulating the responses of an organism to diverse environmental stimuli. The enzyme adenylyl cyclase (AC) synthesizes cAMP and belongs to a large gene family consisting of six phylogenetically defined classes (1–4). Class I ACs are found in the Enterobacteria, e.g. Escherichia coli; class II ACs are exclusive to certain toxin-producing bacteria, e.g. Bacillus anthracis; class III (the universal class) ACs are the only class found among higher eukaryotes and also include the mammalian guanylyl cyclases and prokaryotic members; class IV enzymes are found in certain prokaryotic thermophiles, e.g. Aeromonas hydrophila; class V consists of a single member from the obligate anaerobe, Prevotella ruminicola; and the recently described class VI ACs are found in the genomes of the Rhizobiaceae.

cAMP is synthesized in mammals by a seemingly ubiquitous family of class III plasma membrane-spanning ACs (transmembrane adenylyl cyclase, tmAC) that mediates cellular responses to extracellular signals. Additionally, a cytosolic form of AC (soluble adenylyl cyclase, sAC) has been identified in mammals that was demonstrated to be molecularly and biochemically distinct from the tmACs (5). Although most abundantly expressed in testis, sAC is expressed ubiquitously (6, 7) and is directly activated by bicarbonate ion in a pH-independent manner (8).

The HCO3⁻-regulated mammalian sAC is more closely related to other prokaryotic class III ACs than to other mammalian tmACs (5, 9). Consistent with this phylogenetic relationship, it was demonstrated that a single cyanobacterial class III AC, cyaC of Spirulina platensis (Arthrospira), was also stimulated by HCO3⁻ (8). If HCO3⁻ stimulation were a general feature of at least a subset of class III ACs, they would represent the first family of HCO3⁻-responsive signaling molecules. HCO3⁻ is fundamental to prokaryotic biology; accumulated cytoplasmic HCO3⁻ is the primary source of inorganic carbon transported to the cyanobacterial carboxysomes for photosynthesis (10) and is also hypothesized to have been the predominant carbon source utilized by oxygenic photrophs in the generation of Earth’s oxygen atmosphere (11).

To define the extent to which class III ACs may be stimulated by HCO3⁻ we have utilized the cyaB1 AC gene of the nitrogen-fixing freshwater cyanobacterium Anabaena sp. PCC7120 as a model system. Cyanobacteria are dependent upon the accumulation of intracellular HCO3⁻ for growth, but the mechanism by which they detect HCO3⁻ is unknown and a major stumbling block in the study of this environmentally important class of organisms. The genome of Anabaena sp. PCC7120 encodes six AC genes (12, 13), and cyaB1 codes for a protein that has an N-terminal autoregulatory GAF (found in GMP-phosphodiesterases, adenylyl cyclases, and FhlA (formate hydrogen lyase transcriptional activator) domain) that binds cAMP and up-regulates catalytic activity (14). Biochemical analysis of the catalytic center of cyaB1 revealed that HCO3⁻ stimulates the catalytic activity of AC by an increase in reaction velocity. In addition we have defined a residue (Lys-646) essential for HCO3⁻ action within the catalytic center. We have examined the catalytic centers of a number of other prokaryotic class III ACs and demonstrated that an active site lysine coordinates HCO3⁻ in the catalytic cleft of the subset of ACs that contain an aspartate to threonine active site polymorphism. On the basis of this hypothesis, we propose that a large number of prokaryotic class III AC catalytic domains are...
HCO₃⁻/H₁₁₀₀₂-responsive. HCO₃⁻/H₁₁₀₀₂ signaling through cAMP synthesis is established as a mechanism by which a variety of eukaryotic and prokaryotic organisms can respond to environmental carbon. This knowledge is of fundamental importance in understanding the global impact of bicarbonate on organismal biology.

EXPERIMENTAL PROCEDURES

Recombinant DNAs—The cyaB₁ gene of Anabaena sp. PCC7120 with associated single amino acid point mutations and the M. tuberculosis H₃7Rv Rv1264 gene were assembled as previously described (14, 15). Full details of the Mycobacterium Rv1319c gene will be reported elsewhere. Nucleotides 1349–1930 of the S. aurantiaca B₁₁₀₂₀ cyaB gene (GenBank™ accession number AJ223795; gift from Dr. O. Sismeiro, Institut Pasteur) were amplified by PCR, and cloning was performed using standard molecular biology techniques. A discrepancy from the published sequence was noted that gave an amino acid change (P₁₆₃R). A BamHI and a HinDIII site were added at the 5' and 3'-end, respectively. The cyaB fragment was cloned between the BamHI and HinDIII sites of pQE30. The resulting open reading frame codes for amino acids 160–353 of the cyaB adenylyl cyclase with an MRGSH₆GS metal-affinity tag at the N-terminal end. Primer sequences are available on request.

Expression and Purification of Bacterially Expressed Proteins—Anabaena cyaB1 wild type and mutant proteins and Mycobacterium Rv1264₁–₃₉₇ protein were expressed and purified as previously described (14, 15). Full details of the Mycobacterium Rv1319c protein will be reported elsewhere. The Stigmatella pQE30-cyaB construct was transformed into E. coli BL21(DE3)pREP4. A culture was grown in Luria Bertani broth medium containing 100 mg/liter ampicillin and 25 mg/liter kanamycin at 30 °C to an A₆₀₀ of 0.5. 60 μl isopropyl-β-D-thiogalactopyranoside was added and the culture kept at room temperature for 3 h. Cells were harvested by centrifugation at 4,000 g and washed once with 10 mM Tris-HCl, pH 7.5. The cell pellet was resuspended in 20 ml of buffer A (50 mM Tris-HCl, pH 8.0, 2.5 mM 1-thioglycerol, 50 mM NaCl) and disrupted by two treatments in a French Press at 1000 psi. Particulate material was removed at 31,000 g for 30 min. The supernatant was supplemented with 250 mM NaCl, 15 mM imidazole, and 200 μl of Ni²⁺-nitrilotriacetic acid slurry (Qiagen) for 30 min. The resin was washed with 3 ml each of buffer B (Tris-HCl, pH 8.0, 2.5 mM 1-thioglycerol, 2 mM MgCl₂, 400 mM NaCl, 5 mM imidazole), buffer C (buffer B with 15 mM imidazole), and buffer D (buffer C with 10 mM NaCl). The enzyme was eluted with 0.4 ml of buffer E (Tris-HCl, pH 8.0, 2.5 mM 1-thioglycerol, 2 mM MgCl₂, 400 mM NaCl, 5 mM imidazole). The preparation was stabilized with 20% glycerol and stored at 4 °C.

AC Assay—The AC activity of cyaB₁ wild type and mutant proteins and Mycobacterium Rv1264₁–₃₉₇ protein were expressed and purified as previously described (14, 15). Full details of the Mycobacterium Rv1319c protein will be reported elsewhere.

The Stigmatella pQE30-cyaB construct was transformed into E. coli BL21(DE3)pREP4. A culture was grown in Luria Bertani broth medium containing 100 mg/liter ampicillin and 25 mg/liter kanamycin at 30 °C to an A₆₀₀ of 0.5. 60 μl isopropyl-β-D-thiogalactopyranoside was added and the culture kept at room temperature for 3 h. Cells were harvested by centrifugation at 4,000 g and washed once with 10 mM Tris-HCl, pH 7.5. The cell pellet was resuspended in 20 ml of buffer A (50 mM Tris-HCl, pH 8.0, 2.5 mM 1-thioglycerol, 2 mM MgCl₂, 400 mM NaCl, 5 mM imidazole) and disrupted by two treatments in a French Press at 1000 psi. Particulate material was removed at 31,000 g for 30 min. The supernatant was supplemented with 250 mM NaCl, 15 mM imidazole, and 200 μl of Ni²⁺-nitrilotriacetic acid slurry (Qiagen) for 30 min. The resin was washed with 3 ml each of buffer B (Tris-HCl, pH 8.0, 2.5 mM 1-thioglycerol, 2 mM MgCl₂, 400 mM NaCl, 5 mM imidazole), buffer C (buffer B with 15 mM imidazole), and buffer D (buffer C with 10 mM NaCl). The enzyme was eluted with 0.4 ml of buffer E (buffer B with 10 mM NaCl and 150 mM imidazole). The preparation was stabilized with 20% glycerol and stored at 4 °C.

AC Assay—The AC activity of cyaB₁ wild type protein, cyaB₁ mutant proteins, and other prokaryotic AC recombinant proteins was assessed in a final volume of 100 μl (16). Reactions typically contained 22% glycerol, 50 mM MOPS-Na as buffer, 2 mM MnCl₂ as divalent metal ion...
cofactor, and 75 μM [α-32P]ATP (25 kBq) and 2 mM [2,8-3H]cAMP (150 Bq) to determine yield during production isolation (cAMP was not added to assays for cyaB1 holoenzyme). Details of pH, temperature, and enzyme concentration are provided in the figure legends. Differences in buffer or cofactor usage are also indicated in the text. Protein concentration was adjusted to keep substrate conversion at <10%. Kinetic constants were determined over a concentration range of substrate of 1–100 μM. The data represent the means of several independent experiments, and error bars represent the S.E.

RESULTS

The cyaB1 (alr2266; www.kazusa.or.jp/cyano/Anabaena/) gene of Anabaena sp. PCC7120 codes for a protein consisting of two tandem GAF (GAF-A and GAF-B) domains, a PAS domain, and error bars represent the S.E.

Fig. 2. A, dose response of cyaB1595–859 AC-specific activity in the presence of NaHCO3 (squares) or NaCl (triangles) (assayed at pH 8.5 and 45 °C with 53 nM enzyme). B, time dependence of cyaB1595–859 AC-specific activity in the presence (squares) or absence (triangles) of 10 mM KHCO3 (assayed at pH 7.5 (Tris-HCl-buffered) and 37 °C with 7.8 nM enzyme and 75 μM Mg-ATP as substrate). Note that the time-dependent increase in cAMP formation is accelerated in the presence of KHCO3.

cyaB1 (cyAB1595–859) to include a region of the C terminus (amino acids 795–828) that had some similarity to a tetrapeptide repeat and is essential for production of functional soluble protein in E. coli (14). The activity of cyaB1595–859 was measured in the presence or absence of various salts (Fig. 1B). Specific activity was unchanged in the presence of NaCl and KCl, whereas NaHCO3 and KHCO3 both gave a ~2-fold increase of cyaB1595–859-specific activity, demonstrating that HCO3− activation of cyaB1595–859 was independent of the associated cation. We measured the specific activity of cyaB1595–859 over a range of HCO3− concentrations with Cl− as a control for nonspecific ionic effects (Fig. 2A). A maximal 2-fold stimulation was seen in the presence of HCO3− with an EC50 of 9.6 mM. The GAF-B domain of cyaB1 binds cAMP and activates the AC catalytic domain (14). cyaB1 therefore acts as a self-activating switch. We asked whether the behavior of this switch is affected by HCO3− and expressed recombinant protein corresponding to the cyaB1 holoenzyme (cyAB1595–859) that contains the GAF domains and examined its specific activity in the presence or absence of HCO3−. cyaB1595–859-specific activity showed a non-linear time dependence as previously reported (14); the rate of cAMP formation was significantly accelerated in the presence of 10 mM KHCO3, indicating that HCO3− activated the GAF-B-mediated positive feedback mechanism of cyaB1 (Fig. 2B). The rate of cAMP formation was also stimulated in the presence of 10 mM NaHCO3 but inhibited in the presence of higher concentrations of NaHCO3, indicating that Na+ may block GAF-B binding of cAMP or intramolecular signaling.2

cyaB1595–859-specific activity showed a non-linear protein dependence (Fig. 3), indicating that homodimerization was necessary for formation of the active site. This has been independently confirmed by titration of complementary mutant cyaB1595–859 proteins that are inactive as homodimers but restored catalytic activity as heterodimers (14). To determine whether HCO3− up-regulated cyaB1595–859-specific activity by increasing homodimer formation, we examined the ratio of the HCO3− and Cl−-specific activities as a function of protein concentration. Interestingly, this ratio remained constant over the range of protein concentrations tested, indicating that HCO3− did not affect homodimer formation. The protein concentration independence of HCO3− up-regulation of specific activity al-

* M. J. Cann and T. Kanacher, unpublished observations.
Bicarbonate Ion-regulated Adenylyl Cyclases

The kinetic data implied that HCO₃⁻ interacts with the catalytic center to alter substrate-binding kinetics. The catalytic center is in close agreement with a canonical class III catalytic cleft (17, 18) except for the replacement of an aspartate (Asp-1018 in AC IIC₂; Ref. 17) with a threonine (Thr-721 in cyaB1). Asp-1018 is involved in substrate definition in AC by forming a hydrogen bond with N⁶ of the adenine ring of ATP (17). Thr-721 functionally replaced this aspartate and may act as a hydrogen acceptor from the purine ring (14). When assayed at pH 7.5 to eliminate problems with divalent metal ion depletion, cyaB1₅₉₅–₈₅₉-specific activity was stimulated ~3-fold relative to the Cl⁻ activity over the tested range (0–60 mM HCO₃⁻) (Fig. 4A). We investigated the involvement of the canonical active site residues of a class III AC in HCO₃⁻ stimulation using point mutations. Although the basal-specific activities of cyaB1₅₉₅–₈₅₉ were significantly reduced compared with wild type enzyme, their fold stimulation by HCO₃⁻ was equivalent (Supplemental Data Fig. 1). A key difference between Thr-721 of cyaB1 and Asp-1018 of AC IIC₂ is the loss of the aspartate carboxyl group. We reasoned that HCO₃⁻ possibly mimics the carboxyl group within the active site but, interestingly, HCO₃⁻-mediated up-regulation of cyaB1₅₉₅–₈₅₉T721A-specific activity was equivalent to wild type despite a >99% reduction in basal activity (Fig. 4B). We noted that Lys-938 of AC IIC₂ (substrate definition and equivalent to Lys-646 of cyaB1; Ref. 17) was proposed to act not only as a hydrogen acceptor for the N⁷ of the ATP purine ring but also as a hydrogen donor to the carboxyl group of the adjacent Asp-1018 residue (19). Thus, Lys-646 may form a stabilizing hydrogen bond with HCO₃⁻ at a position equivalent to the carboxyl group of AC IIC₂. Although basal activity was reduced by ~95%, HCO₃⁻ activation was completely abolished in cyaB1₅₉₅–₈₅₉K646A in support of this hypothesis (Fig. 4C). If HCO₃⁻ mimics a carboxyl group within the active site, reintroduction of this carboxyl group should ablate HCO₃⁻ responsiveness. A cyaB1₅₉₅–₈₅₉T721D mutant protein

![Figure 4](image)

A. dose response of cyaB1₅₉₅–₈₅₉-specific activity in the presence of KHCO₃ (squares) or KCl (triangles) (assayed at pH 7.5 and 45 °C with 53 nM enzyme). B. dose response of cyaB1₅₉₅–₈₅₉T721A-specific activity (662 nM enzyme). C. dose response of cyaB1₅₉₅–₈₅₉K646A-specific activity (662 nM enzyme). D. dose response of cyaB1₅₉₅–₈₅₉T721D-specific activity (662 nM enzyme). Symbols and assay conditions for panels B, C, and D are as for panel A above. Specific activities dropped at HCO₃⁻ concentrations above the tested range due to depletion of divalent metal ion cofactor (M. J. Cann, unpublished data).
Bicarbonate Ion-regulated Adenylyl Cyclases

35037

Although the amino acid equivalent to Lys-646 of cyaB1 and Lys-938 of AC IIC2 is conserved in all the ACs examined (Fig. 1A), we reasoned that an adjacent threonine or aspartate within the catalytic cleft of a class III enzyme (i.e. at the position corresponding to Thr-721) could be a marker for HCO₃⁻ AC responsiveness or non-responsiveness, respectively. To test this hypothesis we generated recombinant proteins corresponding to diverse prokaryotic class III ACs with either a threonine or aspartate at the position equivalent to cyaB1 Thr-721 (Fig. 1A) and examined them for their response to HCO₃⁻.

S. aurantiaca B17R20 is a myxobacterium from which two ACs have been identified (20). We expressed amino acids 160–353 of cyaB as a recombinant protein (cyAB160–353) that contained a threonine residue (Thr-293) at the position corresponding to cyaB1 Thr-721 (Fig. 1A). cyAB160–353-specific activity was up-regulated by HCO₃⁻ ~2-fold relative to the Cl⁻-dependent activity (EC₅₀ 8.6 mM) (Fig. 5A), consistent with the hypothesis that the threonine at amino acid 293 is a marker for HCO₃⁻ responsiveness. This stimulation was maintained in the presence of alternative anions to Cl⁻, indicating that cyAB160–353 was most likely stimulated by HCO₃⁻ rather than inhibited by Cl⁻.

M. tuberculosis H37Rv is a Gram-negative bacterium and important human pathogen for which the genome has revealed a number of putative class III ACs (15, 21, 22). We expressed two ACs that contain either a threonine (amino acids 356–535 of Rv1319c) or an aspartate (Rv1264 holoenzyme) at the position corresponding to Thr-721 of cyaB1 (Fig. 1A). Consistent with our hypothesis that the threonine residue is a marker for AC HCO₃⁻ responsiveness, Rv1319c356–535-specific activity was up-regulated ~3-fold in the presence of HCO₃⁻ over the concentration range tested (Fig. 5B), whereas Rv1264c397-specific activity did not respond to HCO₃⁻ over an identical concentration range (Fig. 5C). The data of Fig. 5 support the hypothesis posited in Fig. 4 and indicate that HCO₃⁻-responsive class III AC domains are widespread in biology and represent the sole candidate mechanism for HCO₃⁻ detection in an organism.

**DISCUSSION**

cyaB1 of Anabaena sp. PCC7120 is a class III AC whose catalytic center is functionally equivalent to that identified for the mammalian tmACs (17, 18) except for a threonine residue (Thr-721) that replaces an aspartate highly conserved among the tmACs. Thr-721 functionally replaces aspartate and is suggested to act as a hydrogen acceptor from the purine ring (14).

cyaB1 catalytic activity was demonstrated to be responsive to HCO₃⁻, extending the number of identified class III ACs that are stimulated by HCO₃⁻: stimulation was cation-independent and anion-dependent. The measured EC₅₀ of 9.6 mM is well within the range of calculated intracellular HCO₃⁻ concentrations for cyanobacteria (23). Although the inorganic carbon pool for Anabaena sp. PCC7120 has not been measured, the related heterocyst-forming species Anabaena variabilis M5 can accumulate up to 50 mM internal inorganic carbon depending upon the growth conditions (24). cAMP production through cyaB1 is therefore likely to be responsive to variations in intracellular HCO₃⁻. Intracellular cAMP has previously been correlated with the rate of HCO₃⁻ uptake in the cyanobacterium Anabaena flos-aquae (25), indicating that the protein chemistry we describe is functional in vivo. HCO₃⁻ was able to functionally activate not only the catalytic domains but also the entire holoenzyme with its associated GAF and PAS domains. The GAF-B-mediated positive feedback loop created by cyaB1 may therefore be accelerated by the availability of a fixable carbon source in Anabaena sp. PCC7120.

HCO₃⁻ did not affect cyaB1 homodimer formation or lower the activation energy for transition state formation but did...
significantly alter substrate binding kinetics by increasing the $K_m$ for ATP and $V_{max}$. The cyanobacterium *Synechococcus PCC6301* (*Anacystis nidulans*) has an intracellular ATP concentration of $1 \text{ mM}$ (value calculated from data in Ref. 26). Because the $K_m$ (ATP) for both cyaB1$_{Th721D}$ and holoenzyme is of the order of $<50 \mu\text{M}$, it is likely that the effect of HCO$_3^-$ on $K_m$ is biologically irrelevant and that cyaB1 is activated by HCO$_3^-$ in the intracellular environment by an increase in reaction velocity. Point mutations revealed that loss of Thr-721 did not affect cyaB1$_{Th721D}$ HCO$_3^-$ responsiveness. We demonstrated, however, that loss of Lys-646 (equivalent to Lys-938 of AC IIC2) ablated HCO$_3^-$ stimulation of specific activity. In class III ACs that contain an aspartate residue corresponding to the position of Thr-721, the adjacent lysine in the catalytic center has been demonstrated to be HCO$_3^-$-regulated class III AC domains among diverse prokaryotes and eukaryotes represents the sole mechanism by which organisms may respond to environmental carbon.

Acknowledgments—We thank Joachim Schultz and Jurgen Linder for the kind gift of recombinant proteins and for helpful discussions and comments on the manuscript. We also thank David Garbers, Anthony O'Sullivan, Roy Quinlan, and Lonny Levin for comments on the manuscript.

REFERENCES

1. Barzu, O., and Danchin, A. (1994) Prog. Nucleic Acids Res. Mol. Biol. 49, 241–283
2. Cotta, M. A., Whitehead, T. R., and Wheeler, M. B. (1998) *FEMS Microbiol. Lett.* 164, 257–260
3. Sismeiro, O., Trostet, P., Riville, F., Viveares, C., and Danchin, A. (1998) *J. Bacteriol.* 180, 3339–3344
4. Tellez-Susa, J., Soberon, N., Vega-Segura, A., Torres-Marquez, M. E., and Cevallos, M. A. (2002) *J. Bacteriol.* 184, 3560–3568
5. Buck, J., Sinclair, M. L., Schapal, L., Cann, M. J., and Levin, L. R. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 79–84
6. Sinclair, M. L., Wang, X.-Y., Mattin, M., Conti, M., Buck, J., Wolgemuth, D. J., and Levin, L. R. (2000) *Mol. Reprod. Dev.* 56, 6–11
7. Zippin, J. H., Chen, Y., Nahirney, P., Kamenetsky, M., Wuttke, M. S., Fischman, D. A., Levin, L. R., and Buck, J. (2003) *FASEB J.* 17, 82–94
8. Chen, Y., Cann, M. J., Litvin, T. N., Iourgenko, V., Sinclair, M. L., Levin, L. R., and Buck, J. (2000) *Science* 289, 625–628
9. Roelofs, J., Meima, M., Schaap, P., and Van Haastert, P. J. (2001) *EMBO J.* 20, 4341–4348
10. Bhaya, D., Schwarz, R., and Grossman, A. (2000) in *The Ecology of Cyanobacteria* (Whitton, B. A., and Potts, M., eds), pp. 398–442, Kluwer Academic Publishers, Dordrecht, The Netherlands
11. Dammakke, G. C., Klimov, V. V., Baranov, S. V., Kozlov, Y. N., DasGupta, J., and Tsyryshkin, A. (2001) *Proc. Natl. Acad. Sci. U. S. A.* 98, 2170–2175
12. Katayama, M., and Ohmori, M. (1997) *J. Bacteriol.* 179, 3588–3593
13. Ohmori, M., Ikuuchi, M., Sato, N., Wolk, P., Kaneko, T., Ogawa, T., Kanehisa, M., Goto, S., Kawashima, S., Okamoto, S., Yoshimura, H., Katoh, H., Fujisawa, T., Ehira, S., Kamei, A., Yoshihara, S., Narikawa, R., and Tabat, S. (2001) *DNA Res.* 8, 271–284
14. Kanachev, T., Danchin, A., Linder, U. J., and Schultz, J. (2002) *EMBO J.* 21, 3672–3680
15. Linder, J. U., Schultz, A., and Schultz, J. E. (2002) *J. Biol. Chem.* 277, 19271–19276
16. Salomon, Y., Londos, C., and Rodbell, M. (1974) *Annu. Rev. Biochem.* 53, 541–548
17. Tesmer, J. J., Sunahara, R. K., Gilman, A. G., and Sprang, S. R. (1997) *Science* 278, 1907–1916
18. Zhang, G., Liu, Y., Rusha, A. E., and Hurley, J. H. (1997) *Nature* 386, 247–253
19. Tucker, C. L., Hurley, J. H., Miller, T. R., and Hurley, J. B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 5993–5997
20. Coward-Cavalli, M. P., Sismeiro, O., and Danchin, A. (1997) *Biochimie* 79, 757–767
21. Guo, Y. L., Seebacher, T., Kurz, U., Linder, J. U., and Schultz, J. E. (2001) *EMBO J.* 20, 3667–3677
22. Reddy, S. K., Kamireddi, M., Dhanireddy, K., Young, L., Davis, A., and Reddy, P. T. (2001) *J. Biol. Chem.* 276, 35141–35149
23. Price, G., Sultemeyer, D., Klughammer, B., Ludwig, M., and Badger, M. (1998) *Can. J. Bot.* 76, 973–1002
24. Kaplan, A., Badger, M., and Berry, J. (1980) *Arch. Microbiol.* 127, 13–21
25. Litvin, T. N., Kamenetsky, M., Zarfayan, A., Buck, J., and Levin, L. R. (2003) *J. Biol. Chem.* 278, 15922–15926

---

3 M. J. Cann, unpublished data.
4 M. J. Cann and D. L. Garbers, unpublished observations.