Vol. 179, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1991 Pages 455-462

SEQUENCE OF A HUMAN BRAIN ADENYLYL CYCLASE PARTIAL cDNA: Evidence for a consensus cyclase specific domain

Jasmine PARMA*, Dominique STENGEL, Marie-Hélène GANNAGE, Madeleine POYARD, Robert BAROUKI and Jacques HANOUNE

*Unité 99 INSERM, Hôpital Henri Mondor, 94010 Créteil, France

Received July 4, 1991

SUMMARY. A cDNA coding for a human brain adenylyl cyclase was isolated and sequenced. The deduced partial 675 amino-acid sequence was compared with those of other known adenylyl and guanylyl cyclases. Comparison of this predicted amino-acid sequence with that of bovine brain (type I) and rat olfactory (type III) adenylyl cyclase indicated a significant homology with the carboxyl-terminal halves of both enzymes. The homology between the human adenylyl cyclase and the other two mammalian adenylyl cyclase also appears at the topographic level. Indeed, the human enzyme includes an extremely hydrophobic region containing six potential membrane-spanning segments followed by a large hydrophilic domain. At the beginning of the hydrophilic domain, there is a 250 amino-acid region which shows not only a striking homology with the bovine and rat adenylyl cyclase (86% of similarity and 57% of identity), but also a significant homology with non-mammalian adenylyl cyclase and guanylyl cyclases. We found that this 250 amino-acid domain contains a sequence of about 165 amino-acids which is highly conserved in most of the known nucleotide cyclases suggesting that it includes residues that are critical for the function of the enzymes.

INTRODUCTION. Adenosine 3',5'-cyclic monophosphate (cAMP) is one of the most important regulatory elements in living cells. Adenylyl cyclase is responsible for the transformation of ATP in cAMP (1-3). It is now clear that the hormone-sensitive adenylyl cyclase system of higher eukaryotes cells is made up of several distinct polypeptides. Stimulatory and inhibitory receptors (Rs,Ri) modulate the activity of the catalytic subunit (AC) indirectly *via* the G proteins (Gs, Gi) (see ref 2,3 for review). In mammals, the AC appears to exist in multiple forms, varying by their apparent molecular weight (around 150,000 in liver, 120,000 in brain or 45,000 in spermatozoa), or by their sensitivity to calmodulin (CaM). Unlike the other components of the cAMP synthesizing machinery, the AC has been difficult to purify. Only recently have two AC cDNA been cloned and sequenced from the bovine brain (1,134 amino-acids, type I AC, ref. 4) and the rat olfactory system (1,144 amino-acids, type III AC, ref. 5). Both proteins have two hydrophobic regions, each comprising six membrane spanning segments, and three cytosolic regions, two of which correspond to large domains that show a high degree of similarity, and are probably responsible for the catalytic activity. The first of these large domains lies between the two hydrophobic regions and the second corresponds to the carboxyl terminal tail of the protein. We report here the first cloning, and sequencing, of a cDNA coding for a human enzyme using a brain cDNA library. This opens up the way for analyzing the expression of human AC in different physiological and pathological conditions.

0006-291X/91 $1.50

MATERIALS AND METHODS

Isolation of a cDNA clone coding for a human adenylyl cyclase. A λgt 10 cDNA library (containing 3 x 10^6 unique recombinants) derived from mRNA purified from the human frontal lobe was kindly provided by Dr. Yves Le Bouc (INSERM U. 142, Paris) with approval of Transgene SA. Approximately 2 x 10^6 plaque forming units from the library were screened with the first 625 base pairs from the p 1-8 bovine adenylyl cyclase clone (type I AC) kindly provided to us by Pr. A. Gilman (University of Texas, Dallas), labeled to 3-5 x 10^8 cpm/μg by nick-translation with [α -^{32}P]dATP (Amersham). After a 2-hr prehybridization in [5 x Denhardt, 5 x SSC, 0.1% SDS, 5 mM EDTA, 50 μg/ml ssDNA] at 65°C, the filters were hybridized in the same medium containing labeled probe (~ 5 x 10^5 cpm/ml) for 15hr at 65°C. After a 5 min wash at room temperature, a 20 min wash was performed at 37°C, followed by two 20 min washes at 42°C. All washes were in 500 ml of 2 x SSC, 0.1% SDS. One positive recombinant (CYC1) was identified by autoradiography (hyperfilm -MP, Amersham), plaque purified and reprobed. This recombinant phage was amplified and the EcoRI insert was subcloned into plasmid pGEM-3Z f + (Promega) using standard procedures (6). The sequence of the cDNA insert was determined by the dideoxy chain termination method (7) modified for double-stranded DNA (8) by using Sequenase (United States Biochemical Corporation) (9). The SP6 and T7 primers (Promega) were used initially. Subsequent sequencing reactions used oligonucleotide primers designed from previously determined sequence and synthesized on an Applied Biosystems 381A DNA Synthesizer.

Northern blot. Total RNA was isolated from frozen human frontal cerebral cortex using the guanidine thiocyanate extraction method described by Chirgwin et al (10). Poly A+ RNA from human brain was purchased from Clontech Laboratories (Palo Alto, Ca). Total RNA (30 μg) and poly A+ RNA (5 μg) were electrophorezed on 2.2 M formaldehyde -1 % agarose gel (11) overnight at 40 V, tranferred to a Hybond N+ membrane (Amersham), and fixed with 0.05 M NaOH. The blot was prehybridized overnight at 60°C in 25 ml of 50 % formamide, 5 % sodium dodecyl sulfate, 5 x SSPE containing 100 μg/ml denatured salmon sperm DNA. The hybridization was carried out in the same medium at 60° for 20 h. Adenylyl cyclase RNA probe complementary to RNA strand (cRNA) was transcribed from T7 promotor of pGEM-3Z f + recombinant plasmid (see below) linearized by SacI and yield cRNA of 2,200 bases which corresponds to coding part and 3' untranslated region. The blot was washed two to four times in 0.5 x SSPE, O.5 % SDS, and finally in 0.2 x SSPE, 0.5 % SDS for 30 min at 70° and exposed to an X-ray film Amersham MP for 1 to 7 days.

Computer Analysis. A computer program based on the method described by Kanehisa (12) and using the matrix MDM 78 of Dayhoff (13) was used to define regions of homology between the amino-acid sequence of the human brain adenylyl cyclase and the other sequences of guanylyl or adenylyl cyclases cloned so far. These regions of homology were then extended, whenever possible by scanning by eye the surrounding amino-acid sequences. Finally, they were aligned using the Clustal program (14,15).

RESULTS

Adenylyl cyclase cDNA from human brain. One clone (CYC1) from a human brain library (frontal lobe) was identified and the sequence of both strands was determined (Fig.1). The size of the cDNA insert was 2,183 base pairs and an open reading frame of 2,025 bases was observed, ending with a TAA codon 93 bases upstream from a poly(A) tail. Two AATAAA polyadenylation signals were found. It is very likely that this cDNA is incomplete and lacks the 5' end of the mRNA including part of the coding sequence. This is deduced from the absence of a conserved translation start sequence and from the comparison with other adenylyl cyclases in different species. In addition, the length of this cDNA does not correspond to any human brain mRNA revealed by Northern blots (two mRNAs of 10.5 and 5.2 kb are labeled by the cRNA probe, see Fig.2). Since this cDNA includes the polyadenylation site, it is at least 3 kb shorter than the complete mRNA on its 5' end. Screening of the frontal lobe and six other human cDNA libraries (including random

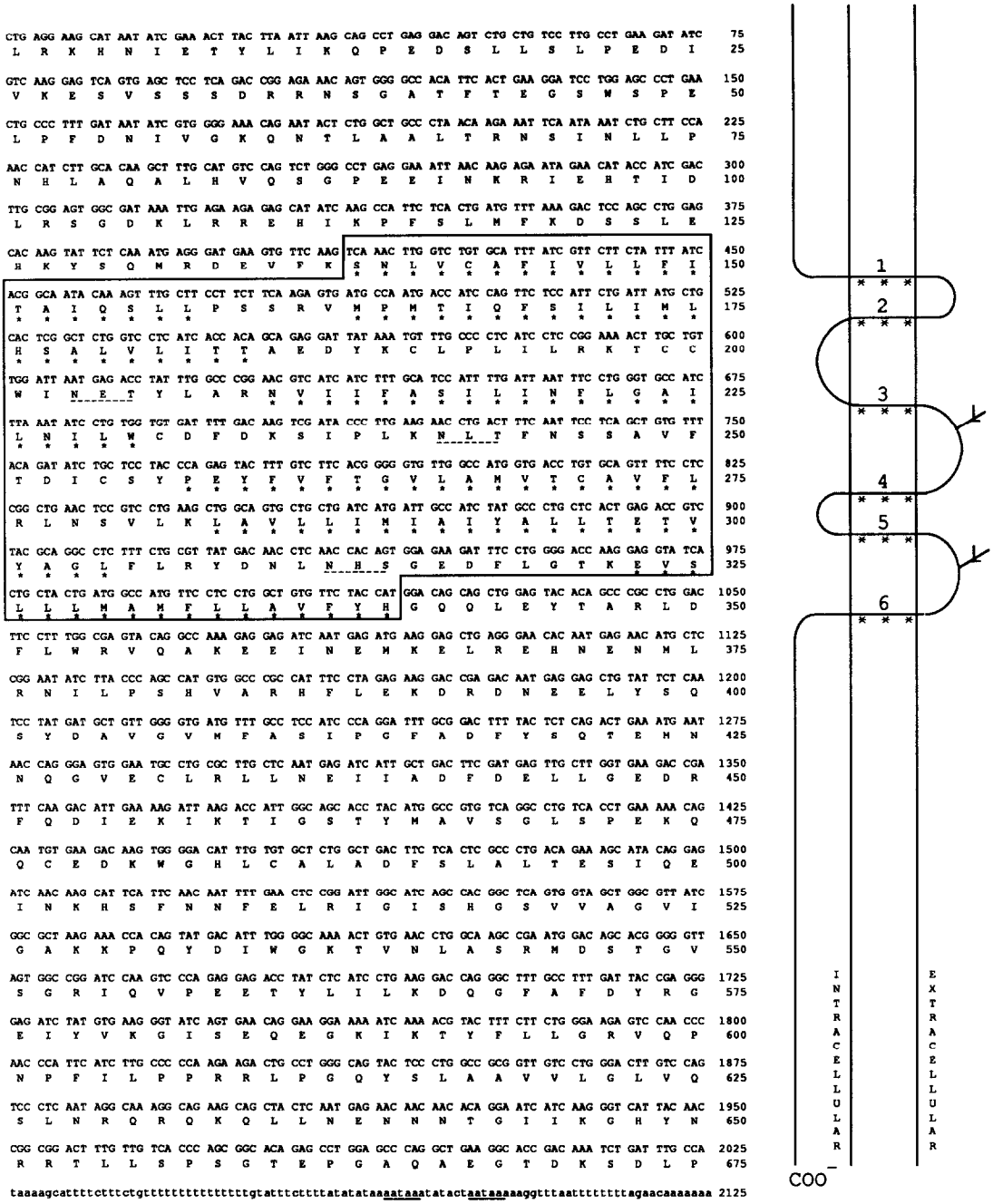
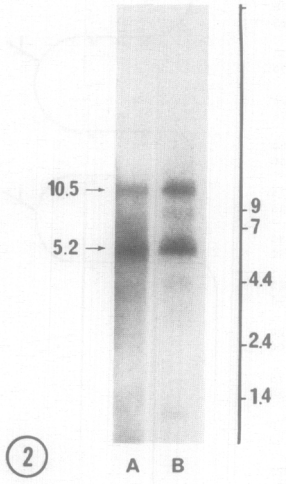


Figure 1. Nucleic acid and deduced amino-acid sequences for the carboxyl-terminal part of the human brain adenylyl cyclase. Nucleotides and amino-acids (in one letter amino-acid notation) are numbered on the right. Boxed residues represent the hydrophobic region. Polyadenylation signals are underlined. Putative transmembrane sequences and potential N-glycosylation sites are indicated by stars and dashed lines under the amino-acid sequences respectively. Two of the 3 potential N-glycosylation sites are situated in the putative extracellular domain. On the right, the putative topology of the carboxyl-terminal part of the human adenylyl cyclase is shown.

priming libraries) using a 5' end fragment of CYC1 did not allow us to isolate full length cDNAs or cDNAs spanning the region of the mRNA upstream of CYC1. When translated into amino-acids, this 675 amino-acid sequence provides the first data on a human adenylyl cyclase (HBAC).



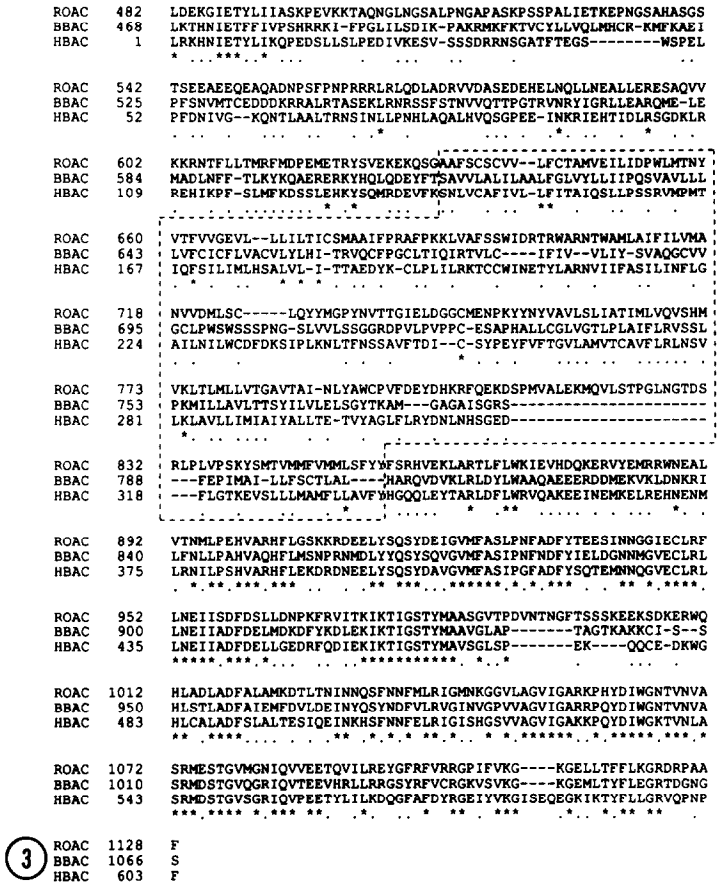


Figure 2. Northern blot analysis of human brain mRNA. Human brain poly A+ RNA (5 µg, lane A) and total RNA (30 µg, lane B) were electrophoresed on 1 % agarose gel, transferred to a nylon membrane and hybridized to the cRNA HBAC probe as described under "Methods". Autoradiography was performed using two intensifying screens at -80°C for one night for total mRNA (lane B) and for a week for poly A+ RNA (lane A). The sizes of the fragments were determined from RNA ladder markers on the same gel.

Figure 3. Alignment of deduced amino-acid sequences of the carboxyl-terminal part of the human brain (HBAC), bovine brain (BBAC, 4) and rat olfactory (ROAC, 5) adenylyl cyclases. Identical amino-acids in the 3 sequences are indicated by asterisks and similar amino-acids by points (conservative substitutions corresponding to a minimal score of 8 in the amino-acid score matrix of Dayhoff (13)). The number of the first residue for each raw is on the left. Dashes indicate gaps for optimal alignment. Hydrophobic region is boxed.

Comparison with other mammalian adenylyl cyclases. Using computational matching programs, the encoded protein sequence (HBAC) was screened for the presence of segments presenting homologies with the bovine brain type I (4) and the rat olfactory type III (5) adenylyl cyclases (BBAC and ROAC). The deduced amino-acid sequence revealed an homology with the carboxyl-terminal halves of both enzymes (see Fig.3). The homology begins slightly upstream of the second hydrophobic region which is present in these enzymes and finishes near the end of the carboxyl-terminal cytoplasmic tail. The region [1-603] of HBAC shows an identity of about 37% with the region [468-1066] of BBAC and the same identity with the region [482-1128] of ROAC. The similarity between the human sequence and each of the other sequences increases to about 75% when amino-acids (aa) are compared on the basis of conservative substitutions (conservative

substitutions corresponding to a minimal score of 8 in the amino-acid score matrix of Dayhoff (13)). There is a 250 aa region in which the homology is especially pronounced. Indeed, the region [348-601] of HBAC shows an identity of about 57% and a similarity of about 86% with the regions [813-1064] and [865-1125] of BBAC and ROAC respectively. These regions are within the long cytoplasmic tail of the bovine and rat enzymes. We have determined the hydropathy profile of the sequenced portion of the human enzyme by the method of Kyte and Doolittle (16). A comparison of the hydrophobicity profile of the human enzyme with those of the bovine and rat enzymes indicated a similar distribution of hydrophobic and hydrophilic residues (data not shown). In particular, the region [138-339] of HBAC, which is homologous to the [613-803] and [632-855] regions of BBAC and ROAC respectively, displays a strong hydrophobicity and contains 6 segments with an average hydrophobicity characteristic of transmembrane domains (Fig.1) (16). This region is followed by a hydrophilic domain which, according to the model of Krupinski et al (4), would be cytoplasmic. Therefore, the carboxyl-terminal 675 aa fragment of the HBAC probably contains 6 transmembrane spans followed by a long cytoplasmic tail. The latter domain contains a 250 aa region which shows not only a high degree of homology with BBAC and ROAC but also homologies with guanylyl cyclases (GC) and non-mammalian AC (see below).

Homologies with guanylyl cyclases and non-mammalian adenylyl cyclases. The most extensive homology between HBAC and other cyclases is found in the carboxyl-terminal cytoplasmic tail. Since the 250 aa sequence in this region, which is highly homologous to the bovine and rat enzymes might be related to the catalytic activity of the enzyme, we compared this sequence with different guanylyl cyclases (GC) and non-mammalian AC. The comparison shows that this 250 aa human sequence presents an identity comprised between 30 to 40% with the carboxyl-terminal part of all known GC (17-29) except one, the *A. punctulata spermatozoan* GC (28). This 250 aa region shows also a lower but significant homology with three non-mammalian AC : the region [345-581] of HBAC shows an identity of about 20 % and a similarity of about 67 % with the regions [1611-1836] and [1262-1527] of the *Saccharomyces cerevisæ* AC (SCAC ; 30) and *Schizosaccharomyces pombe* AC (SPAC, 31) respectively while the region [415-600] of HBAC shows an identity of 25.3% and a similarity of 67.6% with the region [2-183] of the bacterial AC from *Rhizobium meliloti* (RMAC, 32) (data not shown). It appears that one region of about 165 amino-acids (corresponding to the region [415-581] of HBAC) seems to be common to most of the known adenylyl and guanylyl cyclases. Alignment of all the GC and AC sequences possessing this common region indicated that four subdomains present striking homologies. This observation suggests that these subdomains are important for the common function of these enzymes which is the catalysis of a cyclic-3'-5' nucleotide monophosphate formation (see Fig.4).

DISCUSSION. We describe here the isolation of the first human cDNA clone of an adenylyl cyclase (AC). Screening of a human cDNA library (frontal lobe) using a fragment of the bovine calmodulin sensitive type I AC cDNA allowed us to isolate a human cDNA clone encompassing the carboxyl-terminal 675 aa of a human brain AC (see Fig.1). Comparison of the predicted amino-acid sequence from the human brain AC with that of the bovine brain (4) and rat olfactory AC (5) indicated an homology with the carboxyl-terminal halves of both enzymes (see Fig.3). The strong

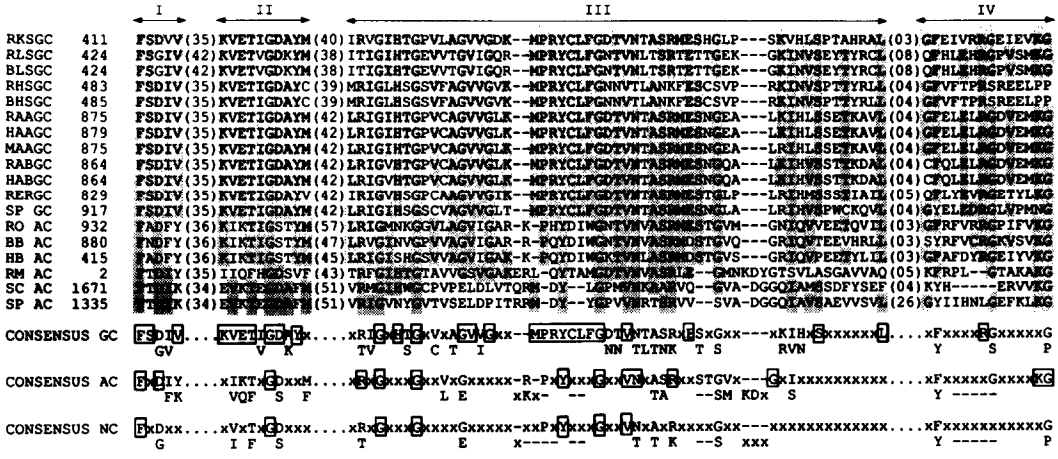


Figure 4. Alignment of the four highly conserved subdomains present within the 165 amino-acid common cyclase domain. Alignment of the four highly conserved subdomains present within the 165 amino-acid common cyclase domain of the GC from rat kidney (RKSGC, 29), the light subunit of the soluble GC from rat (RLSGC, 17) and bovine lung (BLSGC, 18) ; the heavy subunit of the soluble GC from rat (RHSGC, 19) and bovine lung (BHSGC, 20), the ANP-A receptor which possesses guanylyl activity from rat brain (RAAGC, 21), human kidney (HAAGC, 22) and murine Leydig tumor cells (MAAGC, 23), the ANP-B receptor from rat brain (RABGC, 24) and human placenta (HABGC, 25), the enterotoxin receptor which is a plasma membrane form of GC from rat intestine (RERGC, 26), the membrane form of GC from *S. purpuratus* testis (27), the rat olfactory AC (ROAC, 5), the bovine and human brain AC (BBAC, 4 and HBAC), the *R. meliloti* AC (RMAC, 32) the yeast AC from *S. cerevisæ* (SCAC, 30) and *S. pombe* (SPAC, 31). One letter amino-acid notation is used. Amino-acids which are identical in at least half of the sequences are shadowed. Numbers in parentheses denote the number of amino-acids in less conserved regions. Numbers on both sides of the sequences indicate the amino-acid position of the first and the last residues (position 1 corresponding to the initiating Met except for HBAC, see Fig.1). At the bottom, are reported the consensus sequence for the guanylyl cyclase (GC), the adenylyl cyclase (AC) and both cyclases (NC).

homology between HBAC and the other two mammalian AC appears also at the topographic level. Indeed, a comparison of the hydropathy profiles revealed a similar distribution of hydrophobic and hydrophilic residues (data not shown). As in the carboxyl-terminal halves of the bovine and rat AC, we found, in the sequenced fragment of the human enzyme, an extremely hydrophobic region containing six potential membrane-spanning segments (corresponding to the bovine transmembrane regions seven to twelve) followed by a large hydrophilic domain which, according to the model of Krupinsky et al, has a cytoplasmic localization (4). The very high similarity of this putative cytoplasmic domain with the corresponding domain of the bovine brain and rat olfactory AC is a strong indication that our cloned cDNA corresponds indeed to a human adenylyl cyclase. However, sequence comparisons do not allow us to state whether it is the human homolog of the type I or type III cyclase or of an as yet unidentified mammalian AC. The bovine enzyme, which is glycosylated, contains a potential N-glycosylation site between transmembrane regions 9 and 10 on the putative extracellular face of the protein. The human AC contains not only a potential N-glycosylation site at this position but, also an additional site between the last two transmembrane regions such as in the rat olfactory AC.

At the beginning of the hydrophilic domain, there is a 250 aa region which shows not only a striking homology with the bovine and rat AC (57% of identity and 86% of similarity), but also a

significant homology with three other AC : the yeast AC from *Saccharomyces cerevisae* (30) and *Schizosaccharomyces pombe* (31) and the bacterial AC from *Rhizobium meliloti* (32). The region of homology between SCAC and the mammalian enzymes is located in the carboxyl-terminal catalytic part of the yeast enzyme. The *Rhizobium meliloti* AC differs from the other AC by a lower molecular weight (20,000), and is therefore presumably constituted by a simple catalytic domain (32). Regions of homology between this bacterial enzyme and the mammalian AC span over almost the whole bacterial amino-acid sequence. In all cases, it is clear that the regions of homology correspond to the putative catalytic domain of each enzyme.

The amino-acid sequences for several membrane-bound guanylyl cyclases (GC) and the two subunits of cytoplasmic GC have been reported (17-28). Comparison of these amino-acid sequences revealed a highly homologous 250 aa region in the C-terminal part of these enzymes. Interestingly, this common carboxyl-terminal region of GC is homologous to the conserved 250 aa region of mammalian AC suggesting that this region represents a common cyclase domain. This common cyclase domain contains itself a 165 aa region which is conserved not only between GC and mammalian AC but also in the three non-mammalian AC mentioned above. This 165 aa common region contains some invariant residues ; in particular, there are 4 blocks (I to IV in Fig.4) presenting striking homologies between all cyclases suggesting that these subdomains are very important for the catalytic function of these cyclase enzymes. Some residues are conserved in both AC and GC (see consensus NC in Fig.4) whereas others seem specific to AC or GC respectively (see consensus AC and GC in Fig.4). The first could be important for the catalysis of the cyclic -3'-5'-nucleotide monophosphate formation, while the second could be involved in the determination of substrate specificity. Various observations suggest that this domain is, at least in part, the cyclase catalytic domain. First, some studies show that the cyclase common domain is essential for adenylyl (30,32) or guanylyl (33,34) cyclase catalysis. Second, the small AC from *R. meliloti* (194 aa) which possesses an AC activity contains this 165 aa domain ; further it was shown that a deletion of a 50 aa region (distroying the block III) abolished the AC activity (32). Finally, although the adenylyl cyclase from *Bordetella pertussis* (35) and *Bacillus anthracis* (36) do not show striking homologies with other cyclases, a careful examination of these bacterial sequences and of the 165 aa domain reveals conserved residues between a 60 aa region present in both bacterial enzymes (regions [14-73] and [302-361] of *Bordetella pertussis* and *Bacillus anthracis* respectively) and the block III (data not shown). Interestingly this region of the bacterial enzymes contains the ATP-binding site which is essential for catalysis. However, the relevance of such weak homologies awaits a further understanding of the role of each amino-acid in catalysis. These observations strongly suggest that the 165 aa common cyclase domain is important for catalysis but do not exclude that other regions can be also important. This seems true at least for some mammalian cyclases. First, the formation of cyclic GMP by soluble GC, which is a heterodimer, requires both subunits (37). Since each subunit contains the common cyclase domain, we conclude that this domain is not active alone. Second, using a recombinant baculovirus expression system, it was shown that the carboxyl-terminal half of the calmodulin sensitive (type I) AC which contains this domain does not have catalytic activity by itself while coexpression of the amino and carboxyl halves results in enzymatic activity, suggesting that an interaction between the two halves of AC

may be necessary for catalysis (38). Further studies will be necessary to determine the importance of the cyclase common domain as well as importance of the other regions.

ACKNOWLEDGMENTS. We are grateful to Georges Guellaën, Isao Matsuoka and Martine Aggerbeck for critical reading of the manuscript and to Edith Grandvilliers and Lydie Rosario for expert secretarial assistance. This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Fondation de France and the Université Paris-Val-de-Marne.

REFERENCES

1. Levitzki, A. (1986) Physiol. Rev. 66, 819-842.
2. Gilman, A.G. (1987) Annu. Rev. Biochem. 56, 615-649.
3. Levitzki, A. (1988) Trends Biochem. Sci. 13, 298-301.
4. Krupinski, J., Coussen, F., Bakalyar, H.A., Tang, W-J., Feinstein, P.G., Orth, K., Slaughter, C., Reed, R.R. and Gilman, A.G. (1989) Science 244, 1558-1564.
5. Bakalyar, H.A. and Reed, R.R. (1990) Science 250, 1403-1406.
6. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) In Molecular Cloning : A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), 1st Ed.
7. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
8. Chen, E.Y. and Seeburg, P.H. (1985) DNA 4, 165-170.
9. Tabor, S. and Richardson, C.C. (1987) Proc. Natl. Acad. Sci. USA 84, 4767-4771.
10. Chirgwin, J.M., Pnzybyla, A.E., Mc Donald, R.J. and Rutter, W.J. (1979) Biochemistry. 18, 5294-5299.
11. Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA. 77, 5201-5205.
12. Kanehisa, M. (1984) Nucl. Acids. Res. 12, 203-213.
13. Dayhoff, M.O., Barker, W.C., Hunt, L.T. (1983) Methods Enzymol. 91, 524-545.
14. Higgins, D.G. and Sharp, P.M. (1988) Gene 73, 237-244.
15. Higgins, D.G. and Sharp, P.M. (1989) Cabios 5, 151-153.
16. Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105-132.
17. Nakane, M., Saheki, S., Kuno, T., Ishii, K. and Murad, F. (1988) Biochem. Biophys. Res. Commun. 157, 1139-1147.
18. Koesling, D., Herz, J., Gausepohl, H., Niroomand, F., Hinsch, K-D., Mülsch, A., Böhme, E., Schultz, G. and Frank, R. (1988) Febs Lett. 239, 29-34.
19. Nakane, M., Arai, K., Saheki, S., Kuno, T., Buechler, W. and Murad, F. (1990) J. Biol. Chem. 265, 16841-16845.
20. Koesling, D., Harteneck, C., Humbert, P., Bosserhoff, A., Frank, R., Schultz, G. and Böhme, E. (1990) Febs Lett. 266, 128-132.
21. Chinkers, M., Garbers, D.L., Chang, M-S., Lowe, D.G., Chin, H., Goeddel, D.V. and Schulz, S. (1989) Nature, 338, 78-83.
22. Lowe, D.G., Chang, M-S., Hellmiss, R., Chen, E., Singh, S., Garbers, D.L. and Goeddel, D.V. (1989) Embo J. 8, 1377-1384.
23. Pandey, K.N. and Singh, S. (1990) J. Biol. Chem. 265, 12342-12348.
24. Schulz, S., Singh, S., Bellet, R.A., Singh, G., Tubb, D.J., Chin, H. and Garbers, D.L. (1989) Cell 58, 1155-1162.
25. Chang, M-S., Lowe, D.G., Lewis, M., Hellmiss, R., Chen, E. and Goeddel, D.V. (1989) Nature 341, 68-72.
26. Schulz, S., Green, C.K., Yuen, P.S.T. and Garbers, D.L. (1990) Cell 63, 941-948.
27. Thorpe, D.S. and Garbers, D.L. (1989) J. Biol. Chem. 264, 6545-6549.
28. Singh, S., Lowe, D.G., Thorpe, D.S., Rodriguez, H., Kuang, W-J., Dangott, L.J., Chinkers, M., Goeddel, D.V. and Garbers, D.L. (1988) Nature 334, 708-712.
29. Yuen, P.S.T., Potter, L.R. and Garbers, D.L. (1990) Biochem. 29, 10872-10878.
30. Kataoka, T., Broek, D. and Wigler, M. (1985) Cell 43, 493-505.
31. Young, D., Riggs, M., Field, J., Vojtek, A., Broek, D. and Wigler, M. (1989) Proc. Natl. Acad. Sci. USA 86, 7989-7993.
32. Beuve, A., Boesten, B., Crasnier, A., Danchin, A. and O'Gara, F. (1990) J. Bacteriol. 172, 2614-2621.
33. Chinkers, M. and Garbers, D.L. (1989) Science 245, 1392-1394.
34. Thorpe, D.S. and Morkin, E. (1990) J. Biol. Chem. 265, 14717-14720.
35. Glaser, P., Ladant, D., Sezer, O., Pichot, F., Ullmann, A., and Danchin, A. (1988) Mol . Microbiol. 2, 19-30.
36. Escuyer, V., Duflot, E., Sezer, O., Danchin, A., and Mock. M. (1988) Gene, 71, 293-298.
37. Harteneck, C., Koesling, D., Söling, A., Schultz, G. and Böhme, E. (1990) Febs Lett. 272, 221- 22
38. Tang, W-J., Krupinski, J. and Gilman, A.G. (1991) J. Biol. Chem., 266, 8595-8603.