Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1251–1255, March 1986
Biochemistry

Deduced primary structure of the α subunit of the GTP-binding stimulatory protein of adenylate cyclase

(guanine nucleotide-binding regulatory proteins/transducin/ras proteins)

JANET D. ROBISHAW*, DAVID W. RUSSELL†, BRUCE A. HARRIS*, MURRAY D. SMIGEL*, AND ALFRED G. GILMAN*

Departments of *Pharmacology and †Molecular Genetics, Southwestern Graduate School, University of Texas Health Science Center, Dallas, TX 75235

Contributed by Alfred G. Gilman, October 21, 1985

ABSTRACT **A bovine adrenal cDNA clone encoding the entire α subunit of the GTP-binding regulatory protein that stimulates adenylate cyclase (G_s) was isolated and sequenced. This cDNA directed the synthesis of the larger, 52-kDa form of the polypeptide in COS cells, even though the clone appeared to encode a 46-kDa protein. Comparison of the deduced amino acid sequence of $G_{s\alpha}$ with the α subunit of another G protein, transducin, revealed striking homologies.**

The activity of adenylate cyclase is modulated by members of a family of guanine nucleotide-binding regulatory proteins (G proteins). G_s is the protein responsible for transduction of stimulatory signals from receptors to adenylate cyclase, whereas G_i and possibly G_o mediate inhibition of the enzyme (1, 2). In the retinal rod outer segment, regulation of cyclic GMP phosphodiesterase activity in response to photolyzed rhodopsin involves another G protein, termed transducin (3). These four G proteins have been purified (3–7), and each has been found to be a heterotrimer with subunits designated α, β, and γ. The α subunit is unique to each oligomer; the apparent sizes of these polypeptides are 52 or 45 kDa for G_s, 41 kDa for G_i, and 39 kDa for G_o and transducin. The β and γ subunits may be common to all four G proteins; they have apparent sizes of 36 kDa and 8 kDa, respectively.

In response to interaction with specific receptors, the α subunit of each G protein binds GTP and dissociates from the βγ subunit complex (2, 3). The free α subunit is then able to interact with a specific effector in the cell to alter its activity. After hydrolysis of bound GTP, the α subunit reassociates with the βγ complex and is inactive. The α subunit of G_s interacts directly with adenylate cyclase to stimulate its activity. In contrast, the α subunits of G_i and G_o interact weakly, if at all, with the enzyme (8). Instead, the inhibitory effect of these proteins on adenylate cyclase activity appears to be due to the dissociation of the βγ subunit complex and its subsequent association with the α subunit of G_s, thereby reducing the amount of $G_{s\alpha}$ available to stimulate adenylate cyclase (9). The α subunits of G_i and G_o are presumed to interact with other specific effectors that are important in transmembrane signaling reactions (10–12). In the visual transduction system, the α subunit of transducin stimulates the phosphodiesterase directly (3).

Since the specificity of the interaction of each G protein with its effector appears to reside with the α subunit, an intense effort is underway to determine the primary structure of the α subunit of each of these proteins (13–16). We have reported the isolation of a bovine brain cDNA clone (pBBr) that encodes most of the α subunit of G_s (17). The identity of this clone was determined by immunoblotting, using an antibody to a peptide synthesized according to a portion of the deduced amino acid sequence, and by blot hybridization analysis of RNA from wild-type and cyc$^-$ ($G_{s\alpha}$-deficient) S49 lymphoma cells. In the present paper, we report the use of a probe corresponding to the 5′ end of pBBr to isolate a longer clone (pBAd) from a bovine adrenal cDNA library; pBAd appears to encode the entire $G_{s\alpha}$ protein. The amino acid sequence of $G_{s\alpha}$ deduced from the cDNA sequence has been compared to that of the α subunit of transducin (T_α).

METHODS

Materials. A bovine adrenal cDNA library, prepared by the method of Okayama and Berg (18), was generously provided by T. Yamamoto (University of Texas Health Science Center, Dallas). COS-m6 cells were obtained from T. Osborne (University of Texas Health Science Center, Dallas). Other materials were purchased from commercial sources.

Isolation of a cDNA Clone for $G_{s\alpha}$ from Bovine Adrenal. After transformation into *Escherichia coli* HB101 cells, a bovine adrenal cDNA library was plated at high density onto nitrocellulose filters and grown on agar plates containing ampicillin for 6–12 hr, followed by amplification on agar plates containing chloramphenicol for 12 hr (19). Replicate filters were prepared and ≈10^6 colonies were screened with a uniformly labeled probe, about 100 bases long, corresponding to the 5′ end of pBBr. Hybridization of the probe to filters was performed overnight at 42°C in a solution containing 50% (vol/vol) formamide, 5× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/15 mM sodium citrate), 5× Denhardt's solution (1× = 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone), 0.1% $NaDodSO_4$, and heat-denatured *E. coli* DNA at 150 μg/ml (19). Filters were washed twice at room temperature in 2× NaCl/Cit/0.1% $NaDodSO_4$ and once at 45°C in 0.1× NaCl/Cit/0.1% $NaDodSO_4$ for 15 min per wash, dried at room temperature, and subjected to autoradiography. Plasmid DNA was isolated from positive clones (five) by standard procedures (19) and digested with *Bam*HI to determine the size of the cDNA insert. The plasmid with the longest insert (≈1500 base pairs) was designated pBAd.

DNA Sequence Analysis of $G_{s\alpha}$ cDNA Clones. The nucleotide sequences of the cDNA inserts of both the pBBr and pBAd clones were determined. In both cases, appropriate restriction fragments were subcloned in bacteriophage M13 vectors (20) and sequenced by the dideoxynucleotide chain-termination method (21) using M13- and $G_{s\alpha}$-specific oligonucleotides as primers. The amino acid sequence of $G_{s\alpha}$ was deduced from the nucleotide sequence. Protein sequence homology was analyzed using the SEARCH and ALIGN

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: G proteins, GTP-binding regulatory proteins; G_s and G_i, G proteins that mediate stimulation and inhibition, respectively, of adenylate cyclase; G_o, a G protein of unknown function purified from bovine brain; $G_{s\alpha}$ and T_α, α subunit of G_s and of transducin, respectively; EF-Tu and IF-2, bacterial elongation and initiation factors involved in protein synthesis.

programs of the National Biomedical Research Foundation (Washington, DC) (22).

Transfection of a $G_{s\alpha}$ cDNA into COS-m6 Cells. pBAd, which contains a simian virus 40 origin of replication, was transfected into COS-m6 cells (23) by the calcium phosphate precipitation method (24). Proteins from transfected cells and control cells that had been transfected with salmon sperm DNA were solubilized 48 hr later in 75 μl of 1% (vol/vol) Triton X-100/10 mM Hepes, pH 7.4/200 mM NaCl/2.5 mM $MgCl_2$/2 mM $CaCl_2$/0.5 mM dithiothreitol/1% (wt/vol) phenylmethylsulfonyl fluoride/0.1 mM leupeptin. After centrifugation at 12,000 × *g* for 10 min, 25 μg of protein in extracts from control and transfected cells was resolved on a $NaDodSO_4$/11% polyacrylamide gel and transferred to nitrocellulose paper for immunoblotting. Following incubation with buffer containing 10% goat serum, the blot was incubated with a rabbit polyclonal antibody directed against $G_{s\alpha}$, as described (17). Antibody binding was detected by incubation of the blot with buffer containing goat anti-rabbit IgG labeled with ^{125}I. After washing, the blot was subjected to autoradiography, and the autoradiographic image of the blot was scanned with a densitometer.

RESULTS

Sequence of cDNA Encoding the α Subunit of G_s. The strategy that was used to obtain the nucleotide sequence of the pBBr and pBAd cDNA inserts is shown in Fig. 1. In sequencing the cDNA insert of pBBr, an open reading frame extending to the 5′ end of the cDNA was found, with no evidence of an ATG codon 5′ of known protein sequence (17) that could code for the initiator methionine. Therefore, pBBr does not encode the entire α subunit of G_s. To isolate longer cDNAs, a probe corresponding to sequence at the 5′ end of the pBBr was synthesized and used to screen a large cDNA library from bovine adrenal. From this library, a cDNA clone (pBAd) was isolated that appears to be identical to the pBBr clone by restriction mapping (Fig. 1), except that it is 40 base pairs longer at the 5′ end and includes a potential initiator methionine codon.

The nucleotide sequence of the cDNA encoding $G_{s\alpha}$, determined using both of these clones, is shown in Fig. 2. In the sequence common to both clones, we detected a single

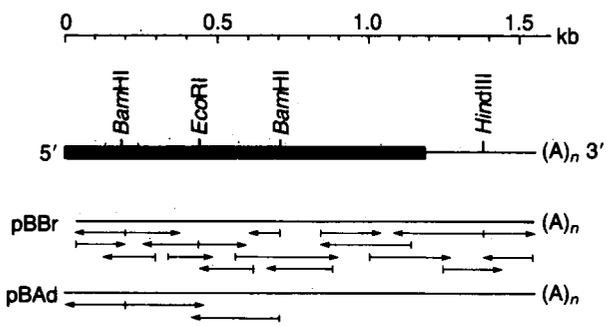

FIG. 1. Restriction endonuclease map and sequencing strategy for the bovine $G_{s\alpha}$ cDNAs. The scale above the restriction map indicates the nucleotide positions in kilobases (kb) relative to the first ATG codon in the cDNA. The thick black line depicts the region of the mRNA that codes for $G_{s\alpha}$ protein, while the thin black line depicts the 3′ untranslated region of the mRNA. The extents to which the cDNA plasmids pBBr and pBAd represent the mRNA are shown below the restriction map. The arrows indicate the direction and extent of DNA sequence that was determined by the dideoxynucleotide method (21), using bacteriophage M13 subclones as templates (20) and M13- and $G_{s\alpha}$-specific oligonucleotides as primers. For pBBr, the coding sequence and 3′ untranslated sequence were determined for both strands. For pBAd, ≈60% of the coding sequence was determined for either one or both strands.

nucleotide difference that occurs at positions 286–290; the pBBr clone has only four adenosine residues, while pBAd has five. The deletion of an adenosine residue in the pBBr clone leads to loss of the reading frame 6 bases downstream. Since the sequences of several M13 subclones of pBBr have only four adenosine residues, it seems likely that the deletion occurred during the construction of the bovine brain cDNA library. Several other $G_{s\alpha}$ clones from the bovine adrenal library have since been sequenced in this region and found to be identical to pBAd. The complete nucleotide sequence of pBAd, excluding the poly(A) track, is 1491 bases.

Translation of the nucleotide sequence reveals a single open reading frame of a length sufficient to encode a protein of at least 45 kDa. The deduced amino acid sequence of the protein product, starting at the first available ATG codon (nucleotide positions 1–3) in the open reading frame and ending at the TAA termination codon (nucleotide positions 1183–1185), is shown below the DNA sequence. The predicted size (46 kDa) and amino acid composition of the 394-residue protein agree closely with values reported for $G_{s\alpha}$ (4, 25). In addition, the deduced amino acid sequence was confirmed by generation of an antibody to a synthetic peptide corresponding to the portion of the sequence underlined in Fig. 2. The antibody to the peptide recognizes both the 45-kDa and the 52-kDa α subunits of G_s (17).

Transient Expression of the $G_{s\alpha}$ cDNA in COS-m6 Cells. COS-m6 cells were transfected with pBAd and analyzed 48 hr later for expression of $G_{s\alpha}$ by immunoblotting, using the peptide-directed antibody described above. To quantitate the relative amounts of $G_{s\alpha}$ in control and transfected cells, the blot was scanned with a densitometer. As expected, control COS cells contain both the 45- and the 52-kDa α subunits of G_s (Fig. 3). In addition, these cells contain a somewhat larger protein (≈55 kDa) that is recognized by the peptide antibody; the identity of this protein is not known. The 55-kDa protein is not seen in membrane extracts of wild-type S49 cells (data not shown) or several bovine tissues (26), and it is presumed to be irrelevant to the present discussion. Although control and transfected cells contain roughly the same amount of the 45-kDa and 55-kDa proteins, the amount of the 52-kDa species of $G_{s\alpha}$ was increased at least 2-fold in COS cells transfected with pBAd in two separate experiments. The increased amount of the 52-kDa protein is presumed to represent synthesis of protein directed by the pBAd plasmid. Since there was no detectable difference in size between the $G_{s\alpha}$ synthesized in response to transfection with pBAd and the larger form of $G_{s\alpha}$ synthesized by control COS cells, we suggest that pBAd encodes the entire sequence of $G_{s\alpha}$. Further, since pBAd encodes a protein with a predicted size of 46 kDa but directs the synthesis of the 52-kDa form of the protein, the possibility of posttranslational modification of $G_{s\alpha}$ is raised. Although estimates of molecular size from gel electrophoresis may certainly be in error, this was not the case for T_α (13–16).

DISCUSSION

Homologies with T_α. The complete amino acid sequence of $G_{s\alpha}$ allows comparison with T_α as a step toward elucidation of the functional domains of these proteins. Alignment of the two sequences reveals striking homology (Fig. 4). More than half of the $G_{s\alpha}$ sequence shows at least 30% identity to the corresponding sequence of T_α. This homology presumably reflects properties that are shared by the α subunits of these two proteins, such as binding and hydrolysis of guanine nucleotides, interaction with βγ, and ADP-ribosylation by bacterial toxins.

To identify regions that might be involved in the binding of guanine nucleotides, Halliday (27) and others (13–16) have searched for regions of sequence that are common to a

```
CCATGGGCTGTCTCGGAAACAGCAAGACCGAGGACCAGCGCAACGAGGAGAAGGGCCAGCGCGAGGCCAACAAGAAGATCGAGAAGCAGCTGCAGAAGGACAAGCAGGTCTACCGGGCC  117
  MetGlyCysLeuGlyAsnSerLysThrGluAspGlnArgAsnGluGluLysGlyGlnArgGluAlaAsnLysLysIleGluLysGlnLeuGlnLysAspLysGlnValTyrArgAla
                10                  20                  30

ACGCACCGTCTGCTGCTGCTGGGTGCTGGAGAATCTGGTAAAAGCACCATTGTGAAGCAAATGAGGATCCTGCATGTTAATGGGTTTAATGGAGAGGGCGGCGAAGAGGACCCGCAGGCT  237
ThrHisArgLeuLeuLeuLeuGlyAlaGlyGluSerGlyLysSerThrIleValLysGlnMetArgIleLeuHisValAsnGlyPheAsnGlyGluGlyGlyGluGluAspProGlnAla
40                  50                  60                  70

GCAAGGAGCAACAGCAGTGGTGAGAAGGCCACCAAAGTGCAGGACATCAAAAACAACCTGAAAGAGGCCATTGAAACCATCGTGGCCGCCATGAGCAACCTGGTGCCCCCTGTGGAGCTG  357
AlaArgSerAsnSerSerGlyGluLysAlaThrLysValGlnAspIleLysAsnAsnLeuLysGluAlaIleGluThrIleValAlaAlaMetSerAsnLeuValProProValGluLeu
80                  90                  100                 110

GCCAACCCAGAGAACCAGTTCAGAGTGGATTACATTCTGAGCGTGATGAACGTGCCGGACTTTGATTTCCCTCCCGAATTCTACGAGCATGCCAAGGCTCTCTGGGAGGATGAAGGGGTG  477
AlaAsnProGluAsnGlnPheArgValAspTyrIleLeuSerValMetAsnValProAspPheAspPheProProGluPheTyrGluHisAlaLysAlaLeuTrpGluAspGluGlyVal
120                 130                 140                 150

CGTGCCTGCTATGAGCGCTCCAACGAGTACCAGCTGATTGACTGCGCCCAGTACTTCCTGGACAAGATTGATGTCATCAAGCAGGATGACTACGTGCCCAGCGACCAGGATCTGCTCCGC  597
ArgAlaCysTyrGluArgSerAsnGluTyrGlnLeuIleAspCysAlaGlnTyrPheLeuAspLysIleAspValIleLysGlnAspAspTyrValProSerAspGlnAspLeuLeuArg
160                 170                 180                 190

TGCCGTGTCCTGACTTCTGGAATCTTTGAGACCAAGTTCCAGGTGGACAAAGTCAACTTCCACATGTTTGACGTGGGCGGCCAGCGCGATGAACGCCGCAAATGGATCCAATGCTTCAAT  717
CysArgValLeuThrSerGlyIlePheGluThrLysPheGlnValAspLysValAsnPheHisMetPheAspValGlyGlyGlnArgAspGluArgArgLysTrpIleGlnCysPheAsn
200                 210                 220                 230

GATGTGACTGCCATCATCTTCGTGGTTGCCAGCAGCAGCTACAACATGGTCATTCGGGAGGACAACCAGACCAACCGCCTGCAGGAGGCTCTGAACCTCTTCAAGAGCATCTGGAATAAC  837
AspValThrAlaIleIlePheValValAlaSerSerSerTyrAsnMetValIleArgGluAspAsnGlnThrAsnArgLeuGlnGluAlaLeuAsnLeuPheLysSerIleTrpAsnAsn
240                 250                 260                 270

AGATGGCTGCGCACCATCTCTGTGATTCTGTTCCTCAACAAGCAAGATCTGCTGGCTGAGAAAGTCCTTGCTGGAAAATCGAAGATTGAGGACTACTTTCCAGAATTTGCTCGCTACACT  957
ArgTrpLeuArgThrIleSerValIleLeuPheLeuAsnLysGlnAspLeuLeuAlaGluLysValLeuAlaGlyLysSerLysIleGluAspTyrPheProGluPheAlaArgTyrThr
280                 290                 300                 310

ACTCCTGAGGATGCGACTCCCGAGCCCGGAGAGGACCCACGCGTGACCCGGGCCAAGTACTTCATTCGAGATGAATTTCTGAGAATCAGCACTGCTAGTGGAGACGGGCGCCACTACTGC  1077
ThrProGluAspAlaThrProGluProGlyGluAspProArgValThrArgAlaLysTyrPheIleArgAspGluPheLeuArgIleSerThrAlaSerGlyAspGlyArgHisTyrCys
320                 330                 340                 350

TACCCTCACTCCACCTGCGCTGTGGACACCGAGAACATCCGCCGTGTGTTCAACGACTGCCGTGACATCATCCAGCGCATGCACCTCCGTCAGTATGAGCTGCTCTAAGAAGGGAACCTC  1197
TyrProHisSerThrCysAlaValAspThrGluAsnIleArgArgValPheAsnAspCysArgAspIleIleGlnArgMetHisLeuArgGlnTyrGluLeuLeu
360                 370                 380                 390

CAGATTTAATTAAGGCCTTAAGCGCAATTAATTAAAAGTAAGATATAATTGTACACGCAGTTGATCACCCACCATAGGGCATGATTAACAAAGCAACCTTTCCTTTCCCTCAAGTGATTT  1317

TGCGAAACCCCCTTCACCTTACAGCTTGCTTAAATATTCCAAATTTAGAAAGCTTAAGGCAGCCTATAGATTAAGATTAAGAAAAAAAGGCCACAAATGTTCCCTTCTCTCTTTAAGCAA  1437

CTACAATAATAGCAGCAAACAGAAATAAATAAATGAAATAAATGAAGCAAATAA  1491
```

FIG. 2. Nucleotide sequence of the cDNA corresponding to bovine $G_{s\alpha}$ mRNA and the predicted amino acid sequence of the protein. Numbering starts at the first ATG codon in the open reading frame. The nucleotides are numbered to the right of the sequence in the 5′ to 3′ direction, while amino acids are numbered underneath the sequence. The peptide sequence (15 residues) used to generate a $G_{s\alpha}$-specific polyclonal antibody is indicated by a single solid underline (17). Potential polyadenylylation signals in the 3′ untranslated region are indicated by overlines and underlines. Note: nucleotides 253–255 are GAT, not AGT; amino acid 85 is Asp, not Ser.

number of different GTP-binding proteins, including the mammalian and yeast *ras*-encoded proteins, bacterial elongation factor Tu (EF-Tu), initiation factor 2 (IF-2), and, most recently, T_α. Of the four regions that have been identified, the two that exhibit the strongest homology (regions a and b) are shown in Fig. 5, along with the corresponding regions of $G_{s\alpha}$. The amino acid sequences of $G_{s\alpha}$ and T_α are identical in region a. The α subunit of G_o also contains this sequence (35), suggesting that all three of these G proteins are derived from a common ancestor. Less extensive, but still striking, homologies are observed in region a between $G_{s\alpha}$ and the other GTP-binding proteins; these range from 58% homology between $G_{s\alpha}$ and EF-Tu to 83% homology between $G_{s\alpha}$ and the mammalian and yeast ras proteins. In region a, three residues (Gly-Xaa-Xaa-Xaa-Gly-Lys) are conserved in all these proteins. Lys-24 of EF-Tu has been shown, by x-ray crystallographic analysis, to neutralize partially the charge of one phosphate group on GDP (36). There is also evidence to suggest that this region is involved in hydrolysis of GTP by ras. Mutations that affect Gly-12 of ras result in a decrease in GTPase activity, which, in turn, correlates with an increase in the oncogenic activity of ras (31, 37, 38). $G_{s\alpha}$, $G_{o\alpha}$, and T_α all contain a glycine residue at the corresponding position in region a. However, the glycine residue has been replaced by a valine in EF-Tu and IF-2.

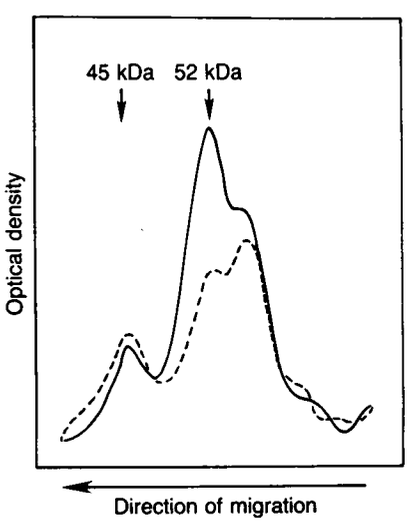

FIG. 3. Transient expression of a cDNA clone for $G_{s\alpha}$ in COS-m6 cells, as detected by immunoblotting. Extracts of control COS cells (——) and COS cells transfected with pBAd (—) were resolved by $NaDodSO_4$/PAGE and transferred to nitrocellulose paper for blotting. The blot was incubated with a $G_{s\alpha}$-specific rabbit polyclonal antibody, followed by goat anti-rabbit IgG labeled with ^{125}I, and autoradiographed. The autoradiograph of the blot was scanned with a densitometer. Arrows mark the positions of the 45-kDa and 52-kDa forms of $G_{s\alpha}$ from wild-type S49 cells.

In region b, the sequence homology ranges from 88% between $G_{s\alpha}$ and T_α or EF-Tu to 62% between $G_{s\alpha}$ and the ras proteins. In region b, three residues (Asn-Lys-Xaa-Asp) are conserved. X-ray crystallographic analysis of EF-Tu indicates that Asn-135 is situated directly over the guanine ring of the bound GDP, whereas Asp-138 interacts with the amino substituent of the guanine ring (36). Since the same amino acid residues that participate in guanine nucleotide binding in EF-Tu have been retained in regions a and b of $G_{s\alpha}$, it seems likely that these two regions form a part of the guanine nucleotide-binding domain in $G_{s\alpha}$. In contrast, the other two regions that have been identified by Halliday (27) as being

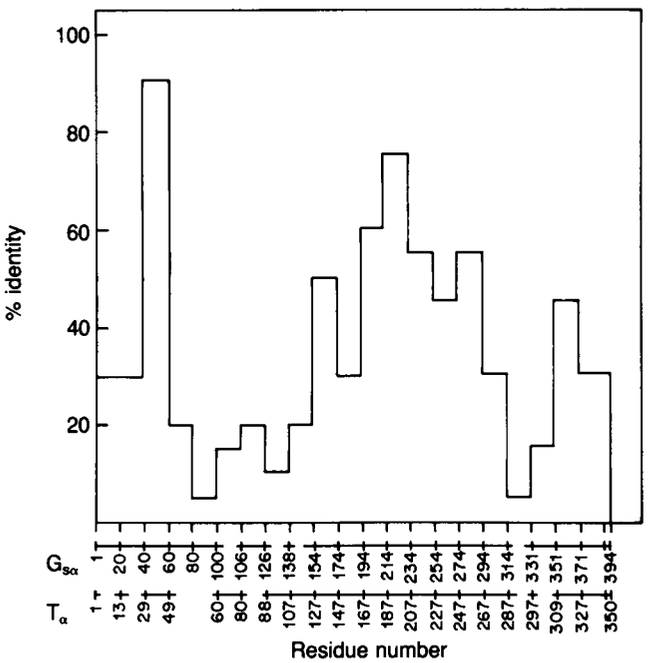

FIG. 4. Identity between the amino acid sequences of $G_{s\alpha}$ and T_α. The predicted amino acid sequences of $G_{s\alpha}$ and T_α (14–16) were aligned by a computer program as described in *Methods*. The statistical significance of the alignment was evaluated by compiling a distribution of alignment scores from 25 random permutations of these sequences (22). A score of 38 SD was obtained for the alignment of $G_{s\alpha}$ (residues 1–394) and T_α (residues 1–350). The number of identical amino acid residues between $G_{s\alpha}$ and T_α in a given segment was expressed as a percentage and was plotted as a function of the amino acid residue number of the respective proteins. Since $G_{s\alpha}$ has 44 amino acids more than T_α, it was necessary to introduce a few gaps (spaces on *x*-axis) into the sequences to obtain maximal homology. Most of the gaps were concentrated in the two regions showing least homology.

Protein	(res. no.)	Sequence	% homology
		Region a	
$G_{s\alpha}$	(43-54)	L L L L G A G E S G K S	
T_α	(32-43)	L L L L G A G E S G K S	100(100)
c-Ha-ras1	(6-17)	L V V V G A G G V G K S	58(83)
YeastRas1	(13-24)	I V V V G G G G V G K S	42(83)
EF-Tu	(15-26)	V G T I G H V D H G K T	25(58)
IF-2	(394-405)	V T I M G H V D H G K T	25(67)
		Region b	
$G_{s\alpha}$	(288-295)	I L F L N K Q D	
T_α	(260-268)	V L F L N K K D	75(88)
c-Ha-ras1	(112-119)	V L V G N K C D	50(62)
YeastRas1	(119-126)	V V V G N K L D	38(62)
EF-Tu	(131-138)	I V F L N K C D	75(88)
IF-2	(494-501)	V V A V N K I D	38(75)
		Region c	
$G_{s\alpha}$	(194-205)	D Q D L L R C R V L T S	
T_α	(167-178)	E Q D V L R S R V K T T	58(83)
c-Ha-ras1	(98-106)	E Q — — I K — R V K D S	33(58)
c-Ki-ras2	(98-106)	E Q — — I K — R V K D S	33(58)
YeastRas1	(105-113)	Q Q — — I Q — R V K D S	33(50)
YeastRas2	(105-113)	Q Q — — I L — R V K D T	25(50)

FIG. 5. Homologies in amino acid sequences between $G_{s\alpha}$ and T_α (14–16), EF-Tu (28, 29), IF-2 (30), and mammalian (31, 32) and yeast (33, 34) ras proteins. The standard one-letter amino acid abbreviations are used. Amino acid residues that have been retained in all the proteins are boxed. The regional percent homology between $G_{s\alpha}$ and each of the other proteins was calculated based on the number of amino acid identities or, as shown in parentheses, the number of amino acid identities plus conservative amino acid substitutions. Conservative amino acid substitutions are grouped as follows: C; S,T,P,A,G; N,D,E,Q; H,R,K; M,I,L,V; F,Y,W.

involved in guanine nucleotide binding appear to be conserved weakly, if at all, in $G_{s\alpha}$ and T_α.

Another region of homology is the site for ADP-ribosylation of $G_{s\alpha}$ and T_α by cholera toxin. The cholera toxin site of T_α has been localized to Arg-174 (13–16, 39). The sequence surrounding this residue and the corresponding sequence of $G_{s\alpha}$ are shown in Fig. 5 (region c). Although the sequence of $G_{s\alpha}$ varies somewhat in this region from that of T_α (83% homology), Arg-201 is retained in $G_{s\alpha}$, consistent with the assertion that it is the site of ADP-ribosylation. Similar sequences containing an arginine residue are present in the mammalian and yeast ras proteins, although these proteins are not good substrates for ADP-ribosylation by cholera toxin (40).

There are regions of sequence that are highly conserved between $G_{s\alpha}$ and T_α that are not found in the other GTP-binding proteins. For example, amino acid residues 217–235 of $G_{s\alpha}$ have 95% homology (15 of 19 identities plus three conservative substitutions) with residues 190–208 of T_α. It is possible that the homology in this region reflects a property shared by $G_{s\alpha}$ and T_α but not by the other GTP-binding proteins, such as interaction with $G_{\beta\gamma}$.

Although the overall homology between these two proteins is striking, at least two regions are notable for their lack of similarity (Fig. 4). The first region is the most extensive and includes residues 70–131 of $G_{s\alpha}$ and 58–80 of T_α, while the second region includes residues 317–340 and 288–298 of $G_{s\alpha}$ and T_α, respectively. These regions appear to represent unique stretches of amino acids present in either $G_{s\alpha}$ or T_α. It is possible that these regions are involved in functions that are specific for $G_{s\alpha}$ or T_α, such as interaction with effectors (adenylate cyclase or phosphodiesterase) and/or receptors.

Homologies with ras Proteins. The mammalian *ras*-encoded proteins are a family of guanine nucleotide-binding proteins of 21 kDa that are encoded by three highly conserved genes first discovered as the oncogenes of the Harvey and Kirsten rat sarcoma viruses (41). More recently, two homologous proteins of 42 kDa were found in yeast (34). Although the precise function of these proteins has not been established, Toda *et al.* (42) have shown that the yeast ras proteins stimulate the activity of adenylate cyclase, leading to speculation that the yeast ras proteins may function in a manner analogous to $G_{s\alpha}$ in the adenylate cyclase system of higher organisms. However, a comparison of the amino acid sequences of these proteins with $G_{s\alpha}$ shows very little overall homology. The alignment scores obtained for $G_{s\alpha}$ with yeast *RAS1* and yeast *RAS2* gene products were 1.9 SD and 0.9 SD, respectively. By way of comparison, the alignment scores for $G_{s\alpha}$ and bovine mitochondrial cytochrome oxidase (subunit 1) or T_α were 3.8 SD or 38 SD, respectively. The homology that is exhibited between $G_{s\alpha}$ and the yeast ras proteins is limited to very short regions of these proteins that appear to be involved in GTP binding and hydrolysis.

A common feature of the ras proteins is the presence of a cysteine as the fourth amino acid residue from the carboxyl terminus (34). This cysteine residue is thought to be involved in the posttranslational events leading to the membrane-associated form of the protein (43). While T_α contains a cysteine residue at the same position (13–16), the cysteine has been replaced by tyrosine in $G_{s\alpha}$.

Relationship Between the 45-kDa and 52-kDa α Subunits of G_s. When transfected into COS-m6 cells, the bovine adrenal cDNA clone directs the synthesis of a protein that is indistinguishable in size from the 52-kDa form of $G_{s\alpha}$. The relationship of the 52-kDa $G_{s\alpha}$ to the 45-kDa form of the

protein is not known. Most cells contain varying amounts of both polypeptides. Functional analysis (4) and peptide mapping (25) show the two proteins to be very similar. In addition, both the 45- and the 52-kDa proteins are recognized by antibodies directed against peptides (17, 26) corresponding to residues 28–42 and 47–61 of the protein sequence shown in Fig. 2. Both forms of $G_{s\alpha}$ may arise from a single RNA transcript by posttranslational modification. Alternatively, the 45- and 52-kDa proteins may be derived from the translation of two different RNA transcripts, whose translation products may or may not undergo posttranslational modification. The two RNA transcripts could arise from one gene by alternative splicing, or from two separate genes. We cannot as yet distinguish between these possibilities.

We thank Tokuo Yamamoto for providing the bovine adrenal cDNA library, Geoff Davis for advice regarding the expression of $G_{s\alpha}$, Susanne Mumby for providing $G_{s\alpha}$ antibody, Jim Cali for synthesizing oligonucleotides, Michael Graziano and Thomas Rall for help with screening, and Wendy Deaner for preparing the manuscript. This work was supported by National Institutes of Health Grants GM34497 and HL31346, American Cancer Society Grant CD225G, American Heart Association Grant G-181, and National Institutes of Health Postdoctoral Fellowship F32 GM09731 (J.D.R.). D.W.R. is the recipient of National Institutes of Health Research Career Development Award HL01278.

1. Gilman, A. G. (1984) *Cell* **36**, 577–579.
2. Smigel, M., Katada, T., Northup, J. K., Bokoch, G. M., Ui, M. & Gilman, A. G. (1984) *Adv. Cyclic Nucleotide Res.* **17**, 1–18.
3. Stryer, L., Hurley, J. B. & Fung, B. K.-K. (1981) *Curr. Top. Membr. Transp.* **15**, 93–108.
4. Sternweis, P. C., Northup, J. K., Smigel, M. D. & Gilman, A. G. (1981) *J. Biol. Chem.* **256**, 11517–11526.
5. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3560–3567.
6. Sternweis, P. C. & Robishaw, J. (1984) *J. Biol. Chem.* **259**, 13806–13813.
7. Neer, E., Lok, J. & Wolf, L. (1984) *J. Biol. Chem.* **259**, 14222–14229.
8. Smigel, M. D. (1986) *J. Biol. Chem.*, in press.
9. Northup, J. K., Sternweis, P. C. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 11361–11368.
10. Gomperts, B. (1983) *Nature (London)* **306**, 64–66.
11. Okajima, F. & Ui, M. (1984) *J. Biol. Chem.* **259**, 13863–13871.
12. Bokoch, G. M. & Gilman, A. G. (1984) *Cell* **39**, 301–308.
13. Lochrie, M. A., Hurley, J. B. & Simon, M. I. (1985) *Science* **228**, 96–99.
14. Tanabe, T., Nukada, T., Nishikawa, Y., Sugimoto, K., Suzuki, H., Takahashi, H., Noda, M., Haga, T., Ichiyama, A., Kangawa, K., Minamino, N., Matsuo, H. & Numa, S. (1985) *Nature (London)* **315**, 242–245.
15. Medynski, D. C., Sullivan, K., Smith, D., Van Dop, C., Chang, F.-H., Fung, B. K.-K., Seeburg, P. H. & Bourne, H. R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4311–4315.
16. Yatsunami, K. & Khorana, H. G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4316–4320.
17. Harris, B. A., Robishaw, J. D., Mumby, S. M. & Gilman, A. G. (1985) *Science* **229**, 1274–1277.
18. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 280–289.
19. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Messing, J. (1983) *Methods Enzymol.* **101**, 20–78.
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
22. Dayhoff, M. O., Barker, W. C. & Hunt, L. T. (1983) *Methods Enzymol.* **91**, 524–545.
23. Horowitz, M., Cepko, C. L. & Sharp, P. A. (1983) *J. Mol. Appl. Genet.* **2**, 147–159.
24. Wigler, M., Sweet, R., Sim, G. K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. & Axel, R. (1979) *Cell* **16**, 777–785.
25. Manning, D. R. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 7059–7063.
26. Mumby, S. M., Kahn, R. A., Manning, D. R. & Gilman, A. G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 265–269.
27. Halliday, K. R. (1984) *J. Cyclic Nucleotide Res.* **9**, 435–448.
28. Laursen, R. A., L'Italien, J. J., Nugarkatti, S. & Miller, D. L. (1981) *J. Biol. Chem.* **256**, 8102–8109.
29. Yokota, T., Sugisaki, H., Takanami, M. & Kaziro, Y. (1980) *Gene* **12**, 25–31.
30. Sacerdot, C., Dessen, P., Hershey, J. W. B., Plumbridge, J. A. & Grunberg-Manago, M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7787–7791.
31. McGrath, J. P., Capon, D. J., Smith, D. H., Chen, E. Y., Seeburg, P. H., Goeddel, D. V. & Levinson, A. D. (1983) *Nature (London)* **304**, 501–506.
32. Capon, D. J., Chen, E. Y., Levinson, A. D., Seeburg, P. H. & Goeddel, D. V. (1983) *Nature (London)* **302**, 33–37.
33. DeFeo-Jones, D., Scolnick, E. M., Koller, R. & Dhar, R. (1983) *Nature (London)* **306**, 707–709.
34. Powers, S., Kataoka, T., Fasano, O., Goldfarb, M., Strathern, J., Broach, J. & Wigler, M. (1984) *Cell* **36**, 607–612.
35. Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D. & Gilman, A. G. (1984) *Science* **226**, 860–862.
36. Jurnak, F. (1985) *Science* **230**, 32–36.
37. Seeburg, P. H., Colby, W. W., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984) *Nature (London)* **312**, 71–75.
38. Gibbs, J., Sigal, I., Poe, M. & Scolnick, E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5704–5708.
39. Van Dop, C., Tsubokawa, M., Bourne, H. & Ramachandran, J. (1984) *J. Biol. Chem.* **259**, 696–698.
40. Beckner, S. K., Hattori, S. & Shih, T. Y. (1985) *Nature (London)* **317**, 71–72.
41. Ellis, R. W., DeFeo, D., Shih, T. Y., Gonda, M. A., Young, H. A., Tsuchida, N., Lowry, D. R. & Scolnick, E. M. (1981) *Nature (London)* **292**, 506–511.
42. Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Brock, D., Cameron, S., Broach, J., Matsumoto, K. & Wigler, M. (1985) *Cell* **40**, 27–36.
43. Willumsen, B. M., Norris, K., Papageorge, A. G., Hubbert, N. L. & Lowry, D. R. (1984) *EMBO J.* **3**, 2581–2585.