differential synthesis of embryonic U1 RNA's (*7*). It remains to be established how—or whether—these changes in composition of U1 snRNP's might alter the specificity of splice site recognition in different tissues or during development (or both).

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9. Mitotic division of germline cells in the developing testis stops around day 12 or 13 of gestation and is not resumed until shortly after birth. During that period the actual number of germ cells in the testis decreases by about 50 percent. Spermatogenesis is initiated a few days after birth coincident with the beginning of mitotic division of germ cells [A. R. Bellvé *et al.*, *J. Cell Biol.* **74**, 69 (1977); H. Peters, *Philos. Trans. R. Soc. London Ser. B*, **259**, 91 (1970)].
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Molecular Cloning of Complementary DNA for the Alpha Subunit of the G Protein That Stimulates Adenylate Cyclase

Abstract. *A complementary DNA clone encoding the α subunit of the adenylate cyclase stimulatory G protein (G_s) was isolated and identified. A bovine brain complementary DNA library was screened with an oligonucleotide probe derived from amino acid sequence common to known G proteins. The only clone that was obtained with this probe has a complementary DNA insert of approximately 1670 base pairs. An antibody to a peptide synthesized according to deduced amino acid sequence reacts specifically with the α subunit of G_s. In addition, RNA that hybridizes with probes made from the clone is detected in wild-type S49 cells; however, cyc$^-$ S49 cells, which are deficient in $G_{s\alpha}$ activity, are devoid of this messenger RNA.*

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The activity of hormone-sensitive adenylate cyclase is regulated by at least two guanine nucleotide–binding proteins (G proteins) (*1*). The stimulatory G protein, G_s, which has been purified to homogeneity (*2*), is a heterotrimer (*2, 3*). The guanine nucleotide–binding α subunit exists as two predominant species (M_r, 52,000 and 45,000), which are thought to be products of a single gene. The α subunit of G_s is the activator of the catalytic moiety of the adenylate cyclase complex. The molecular sizes of the β and γ subunits are approximately 35,000 and 8,000 daltons, respectively. A homologous oligomer, G_i, mediates hormonal inhibition of adenylate cyclase (*4*); it has a distinct α subunit (41,000 daltons), while its β and γ subunits are functionally indistinguishable from those of G_s. Dissociation of the shared βγ subunit complex of G_i and its subsequent interaction with $G_{s\alpha}$ are thought to be responsible for inhibition of adenylate cyclase (*4, 5*). A third G protein, G_o, is abundant in brain, and its α subunit is 39,000 daltons. The α subunits of G_i and G_o may interact with other specific effectors that are distinct from adenylate cyclase (*1*). G_o may also function as an inhibitor of adenylate cyclase, since it too shares an indistinguishable βγ subunit complex (*6*). The G protein family also includes transducin, which mediates the activation by rhodopsin of a cyclic GMP–specific (guanosine monophosphate) phosphodiesterase in the retinal rod outer segment (*7*). The sequences of complementary DNA's (cDNA's) for the α and γ subunits of transducin have been reported (*8, 9*). Structural and functional relations between G proteins and the products of the *ras* genes in yeast and in higher organisms have also been noted (*10–12*).

Comparison of partial amino acid sequences of polypeptides near the NH_2-termini of the α subunits of bovine transducin and G_o (*12*) reveals a region con-

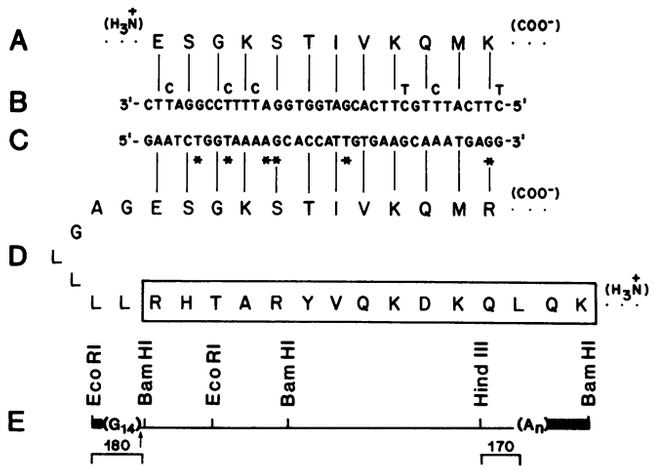


Fig. 1. Derivation of the mixed oligonucleotide probe and partial sequence analysis of $pBBG_{s\alpha}$. (A) Amino acid sequence of a region common to bovine brain $G_{o\alpha}$ and the α subunit of bovine retinal transducin (*12*). (B) A synthetic 36-base oligonucleotide probe was made corresponding to the amino acid sequence shown in (A) with the indicated codon redundancy. The probe was synthesized with an automated oligonucleotide synthesizer (Applied Biosystems), purified from a 20 percent urea-acrylamide gel, and 5′ end-labeled with [γ-^{32}P]ATP; it was then used to screen a bovine brain cDNA library by hybridization. (C) Sequence of the bovine brain cDNA clone in the probe-binding region. Starred nucleotides indicate those residues that do not match the 36-base oligonucleotide probe shown in (B). (D) Inferred amino acid sequence derived from the translation reading frame that yielded the amino acid sequence shown in (A). The enclosed region indicates the sequence used for the synthesis of a peptide for the production of a $G_{s\alpha}$-specific antibody. (E) Restriction map of the $pBBG_{s\alpha}$ cDNA insert. Solid boxed regions indicate vector DNA; arrow indicates the location of the probe-binding region shown in (C). The regions labeled 180 and 170 denote the 5′ and 3′ portions of the clone used for the synthesis of single-stranded probes for hybridization to RNA and DNA blots. "G_{14}" indicates a homopolymer tail of 14 guanine residues used in the cloning of $pBBG_{s\alpha}$. *Cloning.* Poly(A)$^+$ RNA (1.2 μg) from bovine brain was used for cDNA synthesis as described (*16*). The resulting bovine brain library was transformed into *E. coli* DH-1 (*30*) and plated as 12,000 single colonies on each of ten 15-cm nitrocellulose filters for growth on LB agar plates containing ampicillin (50 μg/ml). Duplicate nitrocellulose filters prepared for hybridization by standard procedures (*31*) were hybridized overnight to the mixed, end-labeled [^{32}P]oligonucleotide probe (*17*). Hybridized filters were washed for 15 minutes in 6× SSC (saline sodium citrate) and 0.1 percent SDS at 4°C, followed by washing for 20 minutes in 6× SSC and 0.1 percent SDS at 45°C. Autoradiography for 1 hour produced duplicate signals corresponding to individual bacterial colonies harboring $pBBG_{s\alpha}$. Single-letter abbreviations for the amino acid residues are A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; and Y, tyrosine.

taining an identical stretch of 22 residues. An oligonucleotide probe corresponding to 12 residues of this sequence was synthesized; in most cases the preferred codons for eukaryotes were used (Fig. 1B). The mixed probe is 36 bases in length and contains 64 members; all possible triplet codons were included for amino acids at the 3′ and 5′ ends of the probe, but only a few of the possible codons in the middle were used. The mixed oligonucleotide probe was labeled on the 5′ end with [γ-^{32}P]ATP (adenosine triphosphate) and polynucleotide kinase and was used to screen a bovine brain cDNA library.

Total RNA was prepared from fresh bovine cerebral cortex (*13*). Polyadenylated [poly(A)$^+$] RNA was selected with oligo(dT) (deoxythymidylate) cellulose (*14*); we used this to construct a cDNA library by the plasmid-primed cDNA synthesis method of Okayama and Berg (*15*) with the cDNA expression vector system described by Breathnach and Harris (*16*). The resulting cDNA library (40,000 members) was amplified in *Escherichia coli* DH-1, and approximately 120,000 bacterial colonies were screened on nitrocellulose filters by hybridization to the 5′ end-labeled mixed oligonucleotide probe (*17*). Six colonies that showed reproducible hybridization to the probe were selected for analysis of their plasmids by digestion with restriction endonucleases.

All six cDNA clones displayed an identical pattern of restriction fragments, consistent with the map shown in Fig. 1E. On transfer of restricted cDNA to

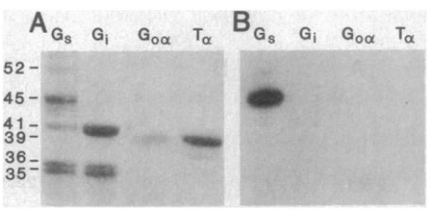


Fig. 2 (left). Immunoblot analysis of the reactivity of purified G proteins with antiserum to the synthetic peptide. (A) Coomassie blue stain of an 11 percent SDS–polyacrylamide gel of G protein preparations (*32*). Lanes contained 1 μg of rabbit liver G_s, 0.5 μg of bovine retinal transducin, and 0.25 μg of bovine brain G_o. (B) G_s and G_i (100 ng each) and $G_{o\alpha}$ and transducin α (50 ng each) were resolved on an 11 percent SDS–polyacrylamide gel and were transferred to nitrocellulose (*24*). Blots were blocked with buffer A consisting of 5 percent nonfat dry milk, 0.2 percent Nonidet P-40, 0.02 percent sodium azide, 50 m*M* tris-HCl, *p*H 8, 2 m*M* $CaCl_2$, and 80 m*M* NaCl. A 1-hour incubation with antiserum to the peptide (1:200 dilution in buffer A) was followed by three 10-minute washings with buffer A. Antibody binding was detected by incubation of the blot with affinity-purified goat antibody to rabbit IgG (Cooper Biomedical) labeled with ^{125}I (10^6 cpm/ml, in buffer A). The blot was washed three times with buffer A for 10 minutes and then with buffer A without milk or Nonidet P-40 for 5 minutes. The autoradiographic image of the blot was obtained with Kodak XAR-5 film after an overnight exposure with an intensifying screen. For the preparation of antisera, peptide (sequence C K Q L Q K D K Q V Y R A T H R) was coupled to keyhole limpit hemocyanin and injected into four New Zealand White rabbits (*23*). Two rabbits each received 600 μg of peptide-hemocyanin conjugate and two rabbits each received 300 μg. Serum from all rabbits reacted positively with peptide and with hemocyanin by an enzyme-linked immunosorbent assay after three injections. Serum from only one rabbit (300-μg injections) reacted with a G protein in either an immunosorbent assay or on immunoblots.

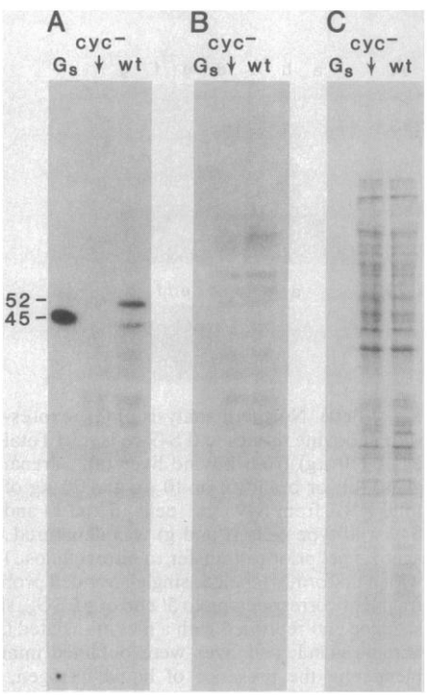


Fig. 3 (right). Immunoblot analysis of the reactivity of the antiserum to the peptide with plasma membrane extracts of cyc$^-$ and wild-type (wt) S49 cells. Methods for gel electrophoresis and immunoblots were the same as in Fig. 2, except that the solution used for incubation with antibody and for washing contained 2 percent Nonidet P-40 and 0.1 percent SDS. (A) Immunoblot with antiserum to peptide (1:200 dilution) of 100 ng of purified G_s and SDS–sample buffer extracts of plasma membranes of S49 cells (*33*) containing 50 μg of total protein. (B) Immunoblot of identical samples with preimmune serum (1:100 dilution). (C) Coomassie blue stain of the protein samples prior to electrophoretic transfer.

nitrocellulose filters and hybridization to the oligonucleotide probe used for screening (*18*), each of the six clones showed an equivalent hybridization signal to a fragment of invariant length that mapped to the 5′ end of the cDNA insert. It is likely that the six clones obtained by screening with this mixed oligonucleotide probe represent copies of one or perhaps two original clones in the amplified library.

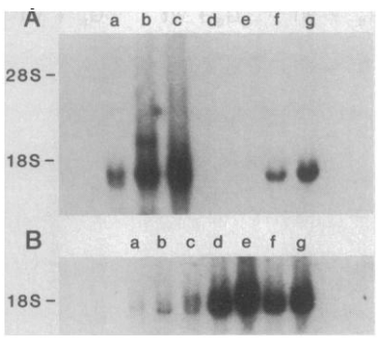
The 5′ end of the cDNA insert containing the probe-binding region was sequenced by the chemical method (*19*) and by the dideoxy chain–termination method (*20*) after subcloning into M13 vectors (*21*). This analysis confirmed the presence of the probe-binding sequence (Fig. 1C). Of 36 bases of probe sequence, there are 30 correct matches and 6 mismatches in the cDNA. Translation of the nucleic acid sequence in the probe-binding region of the cDNA clone yielded amino acid sequence identical to that found in G_o and transducin, with the exception of substitution of an arginyl for a lysyl residue at the carboxyl terminus of this region (Fig. 1D). Translation of the cDNA sequence 5′ to the probe-binding site produced several amino acid residues (Leu-Leu-Leu-Leu-Gly-Ala-Gly) identical to those found NH_2-terminal to the common sequence in transducin and $G_{o\alpha}$. The extent of the amino acid homology between transducin, G_o, and the brain cDNA clone ends NH_2-terminal to the four leucyl residues. At this point the sequences of G_o and transducin also diverge from each other. No ATG sequence that could code for an initiator methionine was found 3′ to the homopolymer G tails; therefore, the bovine brain cDNA clone, which contains approximately 1670 base pairs, is not full length.

As indicated, translation of the bovine brain cDNA sequence in the reading frame that produces amino acid sequence common to $G_{o\alpha}$ and transducin α (*12*) also yields amino acid sequence that occurs only in this clone (Fig. 1D). The peptide containing these 15 amino acids was synthesized commercially (*22*); it has an additional cysteine residue at the NH_2-terminus to allow coupling of the peptide to keyhole limpit hemocyanin with an *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester. Conjugated peptide was injected subcutaneously into rabbits (*23*). Serum was obtained 6 weeks after initial injection of the antigen and was used directly as a probe for purified G protein subunits on immunoblots (*24*). Rabbit polyclonal antibodies to this synthetic peptide specifically recognized the 52,000- and 45,000-dalton α subunits of rabbit liver G_s (Fig. 2B) (and bovine brain G_s). However, the antiserum did not recognize the α subunits of bovine brain G_o, bovine retinal transducin, or rabbit liver G_i. The G protein β subunits also were not visualized with the antiserum.

The murine S49 lymphoma cell line has been used extensively for analysis of the metabolism and action of cyclic AMP because this nucleotide is cytocidal to these cells. The cyc$^-$ S49 cell variant (*25*) is deficient in the activity of $G_{s\alpha}$ (*26*). Extracts of plasma membranes from wild-type and cyc$^-$ S49 cells were resolved on SDS (sodium dodecyl sulfate) polyacrylamide slab gels and transferred to nitrocellulose for immunoblots. Antiserum to the peptide described above recognized 45,000- and 52,000-dalton polypeptides in extracts from wild-type S49 cells, but it did not detect similar proteins in extracts from cyc$^-$ cells (Fig. 3A). The antiserum to the synthetic peptide thus recognizes $G_{s\alpha}$ specifically and to the exclusion of other known G protein α subunits.

Total RNA isolated from bovine tissues and S49 cells (Fig. 4) (*13*) was denatured with glyoxal (*27*) and resolved on a 1.5 percent agarose gel before transfer to a nitrocellulose membrane. The blot was hybridized to a uniformly labeled probe corresponding to the 5′ end of the cDNA clone. The probe hybridized specifically to an RNA species of 1900 bases. Among bovine RNA's, more hybridization was detected with brain and adrenal than with liver. There also appears to be a larger RNA species (2500 bases) in adrenal tissue that hybridizes with the probe.

The 5′ probe from the cloned cDNA also hybridized to an RNA in S49 wild-type cells that migrates at 1900 bases. The detection of this message in a murine cell line indicates a high degree of conservation of sequence displayed by this RNA. Of significance, there is no



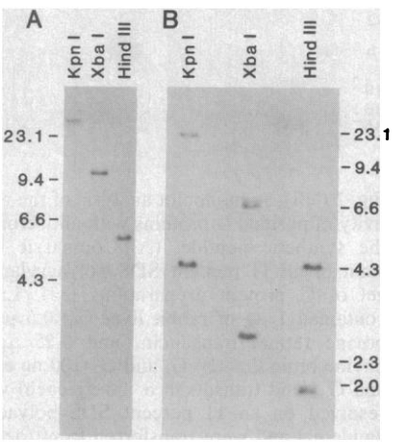


Fig. 4 (left). Northern analysis of $G_{s\alpha}$ expression in bovine tissues and S49 cells. (A) Total RNA (10 μg) from bovine liver (a), adrenal gland (b), or brain (c) or 10 μg and 20 μg of total RNA from S49 cyc$^-$ cells (d and e) and S49 wild-type cells (f and g) was denatured with glyoxal and resolved through a 1.5 percent agarose gel prior to transfer to nitrocellulose (*34*). The blot was hybridized at 42°C for 16 hours with a uniformly labeled, single-stranded probe (5×10^6 cpm/ml) (*35*) from the Eco RI–Bam HI fragment corresponding to 5′ end of $pBBG_{s\alpha}$ shown in Fig. 1E. (B) The Northern blot in (A) was stripped and reprobed with a nick-translated (*36*) chicken actin probe (*37*). Bovine brain cortex, adrenal gland, and liver were obtained immediately after slaughter, powdered in a Waring blender in the presence of liquid nitrogen, and stored at −80°C until they were used for preparation of total RNA (*13*). Procedures for the purification of RNA from S49 lymphoma cells in suspension culture were similar. Hybridizations were performed in 50 percent formamide, 5× SSC, 1× Denhardt's solution, 20 m*M* sodium phosphate, salmon sperm DNA at 100 μg/ml, and 1 percent SDS at 42°C for 16 hours. Blots were washed twice at room temperature in 50 percent formamide, 2× SSC, and 1 percent SDS for 30 minutes; twice at room temperature in 2× SSC and 1 percent SDS; and twice at 62°C in 0.1× SSC and 1 percent SDS for 30 minutes. Autoradiographic signals shown were obtained after an overnight exposure. Fig. 5 (right). Southern analysis of the $G_{s\alpha}$ gene in bovine DNA. Genomic DNA from bovine liver (5 μg) was digested with Kpn I, Xba I, or Hind III and resolved through 0.8 percent agarose gels. After their transfer to nitrocellulose, the blots were probed with a uniformly labeled, single-stranded probe (5×10^6 cpm/ml) corresponding to (A) the Hind III–Bam HI fragment from the 3′ end of $pBBG_{s\alpha}$ or (B) the Eco RI–Bam HI fragment from the 5′ end of the cDNA (Fig. 1E). Blots were hybridized overnight at 42°C in 50 percent formamide, 5× SSC, 1× Denhardt's solution, 20 m*M* sodium phosphate, salmon sperm DNA (100 μg/ml), and 1 percent SDS. Blots were washed twice at room temperature in 2× SSC and 1 percent SDS, followed by a 3-hour washing in 0.5× SSC and 1 percent SDS at 65°C. Autoradiographic signals were obtained by an overnight exposure of the blots with intensifying screens. Size markers are from a Hind III digest of bacteriophage λ DNA. High molecular weight DNA was prepared from bovine liver nuclei essentially by the method of Blin and Stafford (*28*) and transferred to nitrocellulose as described by Southern (*18*).

hybridization signal detectable with RNA from cyc$^-$ S49 cells, even though equivalent amounts of RNA were present on the nitrocellulose; this is shown by the similarity of signal obtained with a chicken actin cDNA probe (Fig. 4B). The presence of an RNA in wild-type S49 cells that hybridizes to the bovine brain cDNA clone and the lack of a hybridization signal in RNA of cyc$^-$ cells confirms the identity of the cDNA clone as one that encodes bovine brain $G_{s\alpha}$. The bovine brain $G_{s\alpha}$ clone, designated pBBG$_{s\alpha}$, was then used as a probe to investigate the gene encoding the message for $G_{s\alpha}$.

Genomic DNA was prepared from bovine liver (*28*) and 5 μg was digested with Kpn I, Xba I, or Hind III; resolved on 0.8 percent agarose gels; and transferred to nitrocellulose (*18*). Blots were hybridized to uniformly labeled, single-stranded probes corresponding to the 3′ or the 5′ end of pBBG$_{s\alpha}$. The 3′ probe probably corresponds to an untranslated region of $G_{s\alpha}$ messenger RNA (mRNA), while the 5′ probe includes the region where amino acid sequence is strongly conserved among G_s, G_o, and transducin. A single band was obtained in each of the three restriction digests analyzed with the 3′ probe, while two bands were obtained with the 5′ probe (Fig. 5). It thus seems likely that one or at most two genes contain sequences that are revealed by such hybridization analysis. This is consistent with the observation that the cDNA sequence for $G_{s\alpha}$ differs significantly from that for transducin in the region where the amino acid sequence is identical (*8*).

The oligonucleotide probe corresponding to a region of shared amino acid sequence among G proteins detected only $G_{s\alpha}$ in the bovine brain cDNA library and, surprisingly, did not detect bovine brain $G_{o\alpha}$ or $G_{i\alpha}$. A bovine adrenal cDNA library was also screened with the mixed 36-base oligonucleotide probe, and again clones corresponding to $G_{s\alpha}$ were the only ones that were identifiable under the conditions utilized. The limited sequence redundancy allowed in the synthesis of the oligonucleotide probe may have caused it to be more "$G_{s\alpha}$-like" than "$G_{o\alpha}$- or $G_{i\alpha}$-like." Alternatively, it may be difficult to synthesize long cDNA's for $G_{o\alpha}$ or $G_{i\alpha}$ with reverse transcriptase, and oligonucleotide probes corresponding to carboxyl terminal regions of these proteins may have advantages for cDNA cloning.

The immunoblot of S49 cell membrane extracts with $G_{s\alpha}$-specific antibody (Fig. 3) and analysis of RNA from these cells (Fig. 4) address the nature of the S49 cyc$^-$ mutant; this phenotypic variation occurs at a high frequency (*29*). The absence of a detectable amount of protein that is immunologically reactive at this NH_2-terminal epitope does not support the idea that an altered $G_{s\alpha}$ molecule contributes to low basal adenylate cyclase activity in the cyc$^-$ mutant. Because no RNA was found in the cyc$^-$ cells that would hybridize to a $G_{s\alpha}$-specific probe, it is likely that the gene encoding $G_{s\alpha}$ is not transcribed in these cells or that the mRNA is unable to accumulate to levels detectable by hybridization to total RNA.

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Genetic Damage, Mutation, and the Evolution of Sex

Abstract. *The two fundamental aspects of sexual reproduction, recombination and outcrossing, appear to be maintained respectively by the advantages of recombinational repair and genetic complementation. Genetic variation is produced as a by-product of recombinational repair, but it may not be the function of sexual reproduction.*

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Why organisms reproduce sexually is a major unsolved problem in evolutionary biology (*1, 2*). Evolutionary explanations have usually appealed to the genetic variation produced by sex (*1–3*). In a recent review of the problem Bell concluded that the consequence of increased genetic diversity of progeny is "a category which includes all hypotheses of interest" (*3*). There is, however, widespread skepticism that any particular variation argument can provide a general explana-

*The order of authors is strictly alphabetical and does not imply seniority.