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Human cDNA clones for four species of G_{α_s} signal transduction protein

(alternative RNA splicing/receptors/adenylate cyclase)

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ABSTRACT **λgt11 cDNA libraries derived from human brain were screened with oligonucleotide probes for recombinants that code for α subunits of G signal transduction proteins. Eleven α_s clones were detected with both probes and characterized. Four types of α_s cDNA were cloned that differ in nucleotide sequence in the region that corresponds to amino acid residues 71–88. The clones differ in the codon for α_s amino acid residue 71 (glutamic acid or aspartic acid), the presence or absence of codons for the next 15 amino acid residues, and the presence or absence of an adjacent serine residue. S1 nuclease protection experiments revealed at least two forms of α_s mRNA. A mechanism for generating four species of α_s mRNA by alternative splicing of precursor RNA is proposed.**

Membrane-associated guanine nucleotide binding proteins (G proteins) act as signal transducers, coupling receptors for light, hormones, or neurotransmitters to effectors such as adenylate cyclase or cGMP phosphodiesterase, and possibly ion channels (1, 2). Known G proteins include G_s and G_i required for receptor-mediated activation or inhibition, respectively, of adenylate cyclase, two species of transducin (TD)—one in rod photoreceptor outer segments (3), the other in cones (4, 5)—and G_o, a G protein of unknown function, abundant in brain (1). There is immunochemical (6) and functional (7) evidence suggesting the existence of additional G proteins. Known G proteins are composed of three protein subunits, α, β, and γ; the α subunits bind guanine nucleotides, catalyze GTP hydrolysis, and couple, directly or indirectly, receptors with effector molecules (1). Comparison of the amino acid sequences of different species of G α-subunits shows that sequences are highly conserved in some, but not all, regions of the protein (8) and that α-subunits are related to the *ras* family of proteins (8) and to other GTP binding proteins such as elongation factor Tu (9).

Bovine (10–12) and rat (13) α_s cDNAs have been cloned and sequenced. Robishaw *et al.* (14) recently described two types of cloned bovine α_s cDNA, which correspond to two forms of α_s protein with apparent M_rs of 45,000 and 52,000, and suggested that two species of α_s mRNA are formed by alternative splicing.

In this report, we present the sequence of human α_s cDNA, describe four species of α_s cDNA, and propose a mechanism for their synthesis.

METHODS AND MATERIALS

A λgt11 cDNA library was constructed by a modification of the method of Huynh *et al.* (15) using poly(A)$^+$ RNA prepared from basal ganglia dissected from a 1-day-old human female brain. Duplex DNA >800 nucleotide pairs in length was ligated to dephosphorylated λgt11 arms and packaged. The resulting library contained 1×10^6 cDNA recombinants; >90% of the phage contained DNA inserts. Another λgt11 cDNA library (adult human brain temporal cortex) was obtained from Clontech (Palo Alto, CA).

Petri dishes (150 mm), each containing 25,000 phage and 1×10^9 *Escherichia coli* Y1090, were incubated at 42°C for 2 hr and then at 38°C for 4 hr. Phage DNA was transferred to replicate nitrocellulose filters for hybridization. Filters were prehybridized in a solution containing 1.5 M NaCl/150 mM sodium citrate, pH 7.0, 1 mg of bovine serum albumin per ml, 1 mg of polyvinylpyrrolidone per ml, 1 mg of Ficoll per ml, 50 mM sodium phosphate (pH 6.8), 1 mM sodium pyrophosphate, 50 μg of yeast tRNA per ml, and 20% formamide for 16 hr at 42°C. One ^{32}P-labeled probe consisted of 32 species of oligodeoxynucleotides, 43 nucleotide residues in length, containing six to eight dI residues (5′ TCAT$^{T}_{C}$TGCTT$^{C}_{I}$AC-IATIGT$^{A}_{G}$CT$^{T}_{C}$TT$^{C}_{I}$CCIGATTCICCIGCICC 3′). The other ^{32}P-labeled probe was 50 nucleotides in length (5′ ACCTTG-AAGATGATGGCGGTCACGTCCTCGAAGCCGT-GGATCCACTTCTT 3′). Each probe (≈1.5×10^6 cpm/ml, 175 fmol/ml) was added to a set of replicate filters and incubated for 16 hr at 42°C. Each filter was washed three times in a solution containing 60 mM NaCl/6 mM sodium citrate and 0.1% $NaDodSO_4$ at 23°C for 20 min per wash, washed once at 42°C in 60 mM NaCl/6 mM sodium citrate and 0.1% $NaDodSO_4$ for 3 min, and then subjected to autoradiography.

Insert DNA was excised with *Eco*RI endonuclease and subcloned into M13mp18. Additional DNA fragments were obtained by incubating insert DNA with *Bam*HI or *Hae* III endonuclease and subcloning into M13mp18 or M13mp19. Nucleotide sequences were obtained by the dideoxynucleotide chain-termination method (16). Maxam–Gilbert sequencing (17) was used to clarify ambiguous sequences.

Total RNA for transfer blots was prepared (18) from mouse S49 lymphoma wild-type and mutant cyc$^-$ cells and primary cultures of human skin fibroblasts. Ten micrograms of total RNA from each sample was fractionated by formaldehyde/agarose gel electrophoresis (19) and transferred to nitrocellulose. ^{32}P-labeled probes were prepared by nick-translation of gel-purified 413-base-pair (bp) (5′) and 869-bp (3′) *Eco*RI fragments of BG-3 with specific activities of 4–5 $\times 10^7$ Cerenkov cpm/pmol. The RNA was hybridized for 20 hr at 42°C in a solution containing 3×10^5 cpm of [^{32}P]DNA per ml, 1.2 M NaCl/120 mM sodium citrate, 40 mM sodium phosphate

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Abbreviations: α_s and α_i, α-subunits of G proteins that activate (G_s) or inhibit (G_i) adenylate cyclase; α_{TD}, α-subunit of transducin, a G protein of rod photoreceptor cells that activates cGMP phosphodiesterase; α_o, α-subunit of G_o, a G protein of unknown function; TD, transducin; bp, base pair(s).

(pH 7.0), 1× Denhardt's solution, 250 μg of denatured salmon sperm DNA per ml, and 50% formamide. Blots were washed four times with 0.6 M NaCl/60 mM sodium citrate and 0.1% $NaDodSO_4$ at room temperature for 5 min per wash and two times with 30 mM NaCl/3 mM sodium citrate and 0.1% $NaDodSO_4$ at 50°C for 30 min per wash.

For S1 nuclease digestion (20), 7.5 μg of total RNA and single-stranded [^{32}P]DNA probe (3000–5000 Cerenkov cpm) were hybridized in 30 μl of a solution containing 40 mM Pipes (pH 6.4), 400 mM NaCl, 10 mM EDTA, and 80% formamide at 52°C for 16 hr. The RNA/DNA mixture was diluted to 300 μl in a solution containing 7 units of S1 nuclease (Pharmacia P-L Biochemicals), 25 μg of denatured salmon sperm DNA per ml, 250 mM NaCl, 30 mM sodium acetate (pH 4.6), 1 mM zinc sulfate, and 5% glycerol and incubated at 37°C for 30 min. Products were denatured at 95°C for 1 min and fractionated by electrophoresis through an 8% acrylamide/8.3 M urea gel.

RESULTS

Nucleotide Sequences of Human α_s cDNAs. A λgt11 cDNA library, prepared from total cellular poly(A)$^+$ RNA from 1-day-old human basal ganglia, and another λgt11 cDNA library from adult human brain were screened with two ^{32}P-labeled oligodeoxynucleotide probes for recombinants that correspond to α subunits of G proteins. The probes, 43 and 50 deoxynucleotide residues in length, were designed to hybridize to conserved regions of α_o, α_s, α_i, and α_{TD} cDNA (8). Fourteen of the 575,000 recombinant clones screened from the basal ganglia library (BG clones) and 12 of the 400,000 clones screened from the brain library (HB clones)

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CAG CGC AAC GAG GAG AAG GCG CAG CGT GAG GCC AAC AAA AAG ATC GAG AAG CAG CTG CAG   60
Gln Arg Asn Glu Glu Lys Ala Gln Arg Glu Ala Asn Lys Lys Ile Glu Lys Gln Leu Gln   20

AAG GAC AAG CAG GTC TAC CGG GCC ACG CAC CGC CTG CTG CTG CTG GGT GCT GGA GAA TCT  120
Lys Asp Lys Gln Val Tyr Arg Ala Thr His Arg Leu Leu Leu Leu Gly Ala Gly Glu Ser   40

GGT AAA AGC ACC ATT GTG AAG CAG ATG AGG ATC CTG CAT GTT AAT GGG TTT AAT GGA GAG  180
Gly Lys Ser Thr Ile Val Lys Gln Met Arg Ile Leu His Val Asn Gly Phe Asn Gly Glu   60

GGC GGC GAA GAG GAC CCG CAG GCT GCA AGG AGC AAC AGC GAT GGC AGT GAG AAG GCA ACC  240
Gly Gly Glu Glu Asp Pro Gln Ala Ala Arg Ser Asn Ser Asp Gly Ser Glu Lys Ala Thr   80

AAA GTG CAG GAC ATC AAA AAC AAC CTG AAA GAG GCG ATT GAA ACC ATT GTG GCC GCC ATG  300
Lys Val Gln Asp Ile Lys Asn Asn Leu Lys Glu Ala Ile Glu Thr Ile Val Ala Ala Met  100

AGC AAC CTG GTG CCC CCC GTG GAG CTG GCC AAC CCC GAG AAC CAG TTC AGA GTG GAC TAC  360
Ser Asn Leu Val Pro Pro Val Glu Leu Ala Asn Pro Glu Asn Gln Phe Arg Val Asp Tyr  120

ATT CTG AGT GTG ATG AAC GTG CCT GAC TTT GAC TTC CCT CCC GAA TTC TAT GAG CAT GCC  420
Ile Leu Ser Val Met Asn Val Pro Asp Phe Asp Phe Pro Pro Glu Phe Tyr Glu His Ala  140

AAG GCT CTG TGG GAG GAT GAA GGA GTG CGT GCC TGC TAC GAA CGC TCC AAC GAG TAC CAG  480
Lys Ala Leu Trp Glu Asp Glu Gly Val Arg Ala Cys Tyr Glu Arg Ser Asn Glu Tyr Gln  160

CTG ATT GAC TGT GCC CAG TAC TTC CTG GAC AAG ATC GAC GTG ATC AAG CAG GCT GAC TAT  540
Leu Ile Asp Cys Ala Gln Tyr Phe Leu Asp Lys Ile Asp Val Ile Lys Gln Ala Asp Tyr  180

GTG CCG AGC GAT CAG GAC CTG CTT CGC TGC CGT GTC CTG ACT TCT GGA ATC TTT GAG ACC  600
Val Pro Ser Asp Gln Asp Leu Leu Arg Cys Arg Val Leu Thr Ser Gly Ile Phe Glu Thr  200

AAG TTC CAG GTG GAC AAA GTC AAC TTC CAC ATG TTT GAC GTG GGT GGC CAG CGC GAT GAA  660
Lys Phe Gln Val Asp Lys Val Asn Phe His Met Phe Asp Val Gly Gly Gln Arg Asp Glu  220

CGC CGC AAG TGG ATC CAG TGC TTC AAC GAT GTG ACT GCC ATC ATC TTC GTG GTG GCC AGC  720
Arg Arg Lys Trp Ile Gln Cys Phe Asn Asp Val Thr Ala Ile Ile Phe Val Val Ala Ser  240

AGC AGC TAC AAC ATG GTC ATC CGG GAG GAC AAC CAG ACC AAC CGC CTG CAG GAG GCT CTG  780
Ser Ser Tyr Asn Met Val Ile Arg Glu Asp Asn Gln Thr Asn Arg Leu Gln Glu Ala Leu  260

AAC CTC TTC AAG AGC ATC TGG AAC AAC AGA TGG CTG CGC ACC ATC TCT GTG ATC CTG TTC  840
Asn Leu Phe Lys Ser Ile Trp Asn Asn Arg Trp Leu Arg Thr Ile Ser Val Ile Leu Phe  280

CTC AAC AAG CAA GAT CTG CTC GCT GAG AAA GTC CTT GCT GGG AAA TCG AAG ATT GAG GAC  900
Leu Asn Lys Gln Asp Leu Leu Ala Glu Lys Val Leu Ala Gly Lys Ser Lys Ile Glu Asp  300

TAC TTT CCA GAA TTT GCT CGC TAC ACT ACT CCT GAG GAT GCT ACT CCC GAG CCC GGA GAG  960
Tyr Phe Pro Glu Phe Ala Arg Tyr Thr Thr Pro Glu Asp Ala Thr Pro Glu Pro Gly Glu  320

GAC CCA CGC GTG ACC CGG GCC AAG TAC TTC ATT CGA GAT GAG TTT CTG AGG ATC AGC ACT 1020
Asp Pro Arg Val Thr Arg Ala Lys Tyr Phe Ile Arg Asp Glu Phe Leu Arg Ile Ser Thr  340

GCC AGT GGA GAT GGG CGT CAC TAC TGC TAC CCT CAT TTC ACC TGC GCT GTG GAC ACT GAG 1080
Ala Ser Gly Asp Gly Arg His Tyr Cys Tyr Pro His Phe Thr Cys Ala Val Asp Thr Glu  360

AAC ATC CGC CGT GTG TTC AAC GAC TGC CGT GAC ATC ATT CAG CGC ATG CAC CTT CGT CAG 1140
Asn Ile Arg Arg Val Phe Asn Asp Cys Arg Asp Ile Ile Gln Arg Met His Leu Arg Gln  380

TAC GAG CTG CTC TAA  GAAGGGAACCCCCAAATTTAATTAAAGCCTTAAGCACAATTAATTAAAAGTGAAACGT 1213
Tyr Glu Leu Leu Term                                                            384

AATTGTACAAGCAGTTAATCACCCACCATAGGGCATGATTAACAAAGCAACCTTTCCCTTCCC                 1276
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FIG. 1. Nucleotide sequence of human BG-3 α_s cDNA. The first nucleotide residue shown corresponds to residue 34 in the coding portion of bovine α_s. The underlined nucleotides represent the sites of hybridization of the 43-mer or 50-mer oligodeoxynucleotide probes. BG-1 α_s cDNA contains eight nucleotide residues, CCGAGGAC, preceding the first nucleotide residue of BG-3 α_s cDNA shown here, which are identical to nucleotide residues 26–33 in the coding portions of bovine (11, 12) and rat (13) α_s cDNA. The first six nucleotide residues found in BG-3 α_s cDNA are CCGAGG (not shown here); nucleotide residues 7 and 8, AC, are missing. We do not know whether the absence of AC is an artifact of cloning and therefore do not show the first six nucleotide residues, CCGAGG, here.

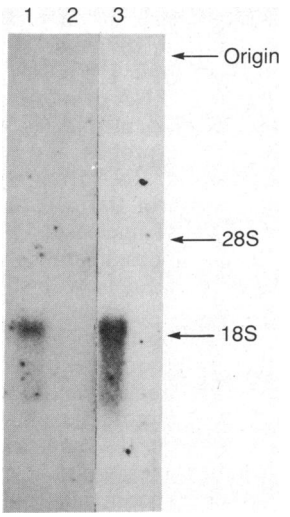

FIG. 2. Transfer analysis of total RNA (10 μg per lane). Lane 1, mouse S49 lymphoma cells; lane 2, mutant S49 cyc⁻ cells; and lane 3, primary cultures of normal human skin fibroblasts with a [^{32}P]DNA probe corresponding to the 413-bp 5′ *Eco*RI fragment of BG-3. Identical results were obtained with the 869-bp 3′ *Eco*RI fragment of BG-3 as a ^{32}P-labeled probe (not shown). Hybridization of a β-actin probe to the RNA in lane 2 resulted in a band (not shown) similar in density to those in lanes 1 and 3.

were detected with both ^{32}P-labeled probes. DNA inserts from 14 positive BG clones and 1 HB clone were sequenced partially; this information was sufficient to identify 11 clones as α_s cDNA, and 2 clones as α_i (the latter will be described elsewhere).

Both strands of DNA from one of the α_s clones, BG-3, were sequenced (Fig. 1). The first nucleotide residue of BG-3 corresponds to the 34th residue of the coding portion of bovine α_s cDNA (11, 12). An open reading frame of 1152 nucleotide residues was found that codes for 384 amino acid residues, followed by a termination codon and 121 additional nucleotide residues in the 3′-untranslated region. The nucleotide sequence of BG-3 human α_s cDNA is 95% homologous to bovine (11, 12) or rat (13) α_s cDNA sequences (1213 and 1207 of 1276 human α_s nucleotide residues match bovine or rat α_s cDNA, respectively). However, the amino acid sequence predicted for human BG-3 α_s differs from the bovine α_s sequence (11, 12) by the presence of an extra amino acid residue, Ser-76, which is not present in bovine α_s, and the substitution of Ala-177 in human α_s for Asp-188. In addition, BG-3 human α_s cDNA codes for Ala-7 and Phe-353 instead of Gly-18 and Ser-364, respectively, reported for bovine α_s cDNA by Robishaw *et al.* (12). Rat α_s cDNA (13) also lacks the codon for Ser-87, and the codon for Asn-139 is replaced in human BG-3 α_s cDNA by a codon for Asp-129. The high homology between human and bovine or rat α_s cDNA nucleotide sequences that code for protein (95% homology) also was found in the 3′-untranslated regions [91% and 90% homology between human BG-3 cDNA and bovine (11, 12) or rat (13) α_s cDNA (113 and 112 of 124 BG-3 nucleotide residues match, respectively)].

*Eco*RI fragments from the 5′ and 3′ regions of BG-3 α_s DNA were labeled and used as probes for transfer blots with RNA from wild-type and mutant cyc⁻ S49 mouse lymphoma cells and RNA from normal human skin fibroblasts (Fig. 2). Bands of α_s RNA were detected with wild-type S49 RNA and RNA from human fibroblasts but not with RNA from cyc⁻ S49 cells. These results show that human α_s RNA is ≈1900 nucleotide residues in length, similar to the chain length reported for mouse α_s mRNA, and confirm the demonstration that cyc⁻ S49 cells lack α_s RNA (10).

Comparison of partial nucleotide sequences of 10 other human α_s cDNA clones revealed four species of α_s cDNA, shown in Table 1, that differ in nucleotide sequence in the region that corresponds to amino acid residues 71–87 of bovine α_s. Four of the 11 human α_s cDNA clones are α_s-1 cDNAs (BG-1, HB-2, BG-8, and BG21-5), which code for the same sequence of amino acids, with respect to residues 71–87, as bovine α_s (11, 12). Only one α_s-2 cDNA clone was found (BG-3, which was sequenced completely), which differs from α_s-1 in the codon for Gly-86 (GGC instead of GGT) and the presence of three additional nucleotide residues (AGT) that code for Ser-87. Two α_s-3 clones were found (BG-6 and BG-20), which have the codon GAT for Asp-71 instead of GAG for Glu-71 and lack codons for amino acid residues 72–86 of α_s-1. Three of the 10 α_s cDNA clones were identified as the α_s-4 type, which have a GAC rather than a GAT codon for Asp-71, lack codons for amino acid residues 72–86, and contain an AGT codon for Ser-72. These results reveal unexpected diversity in α_s cDNA clones[§] and suggest

[§]Several additional differences were found between the sequences of human α_s cDNA clones: BG-3, BG-8, BG-12, and BG-21-5 contain thymidylate residues at positions 135 and 363 (Fig. 1); BG-6 and BG-20 contain cytidylate rather than thymidylate at both positions; and BG 13 and BG 21-1 contain thymidylate at position 135 and cytidylate at position 363.

Table 1. Nucleotide and amino acid sequences of cloned species of human α_s cDNA

α_s cDNA species	cDNA clones		Nucleotide and amino acid sequences	
α_s-1	BG-1, HB-2	211	GAG GGC GGC GAA GAG GAC CCG CAG GCT GCA AGG AGC AAC AGC GAT GGT --- GAG	261
	BG-8, BG21-5		GLU GLY GLY GLU GLU ASP PRO GLN ALA ALA ARG SER ASN SER ASP GLY --- GLU	
			71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87	
α_s-2	BG-3	211	GAG GGC GGC GAA GAG GAC CCG CAG GCT GCA AGG AGC AAC AGC GAT GGC AGT GAG	264
			GLU GLY GLY GLU GLU ASP PRO GLN ALA ALA ARG SER ASN SER ASP GLY SER GLU	
			71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88	
α_s-3	BG-6	211	GAT --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- GAG	216
	BG-20		ASP --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- GLU	
			71 72	
α_s-4	BG-12	211	GAC --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- AGT GAG	219
	BG-13		ASP --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- SER GLU	
	BG21-1		71 72 73	

The numbers before and after the nucleotide sequences correspond to nucleotide residues of the coding sequences of bovine (11, 12) and rat (13) α_s. The numbers under the amino acid sequence of α_s-1 correspond to the amino acid residues of bovine (11, 12) and rat (13) α_s starting from the initial Met residue.

that the four species of cloned α_s cDNA found correspond to four species of α_s mRNA.

S1 Nuclease Protection Experiments. To determine whether human cells contain multiple species of α_s mRNA, S1 nuclease protection experiments were performed, using the 5′ *Eco*RI fragments of BG-21-5 or BG-3 DNA as ^{32}P-labeled α_s-1 or ^{32}P-labeled α_s-2 probe, respectively. Diagrams of the probes and the expected fragments resulting from S1 nuclease digestion of α_s-1 or α_s-2 DNA·α_s RNA duplexes are shown in Fig. 3 *A* and *B*, respectively, and in Fig. 3 *C* and *D* are shown the results of S1 nuclease protection experiments. Hybridization of the α_s-1 [^{32}P]DNA probe with α_s-1 mRNA should protect a fragment ≈412 bp in length. Hybridization of the α_s-1 probe to α_s-2 mRNA should yield DNA·RNA duplexes with single-stranded loops of three unpaired nucleotide residues, which, if cleaved by S1 nuclease, should result in [^{32}P]DNA·RNA fragments approximately 232 and 180 bp in length, whereas hybridization of the α_s-1 probe to α_s-3 or α_s-4 mRNA should yield fragments approximately 187 and 180 bp in length. Similarly, treatment of α_s-2 [^{32}P]DNA·RNA hybrids with S1 nuclease should yield DNA fragments approximately 413, 230, 185, 183, and 180 bp in length.

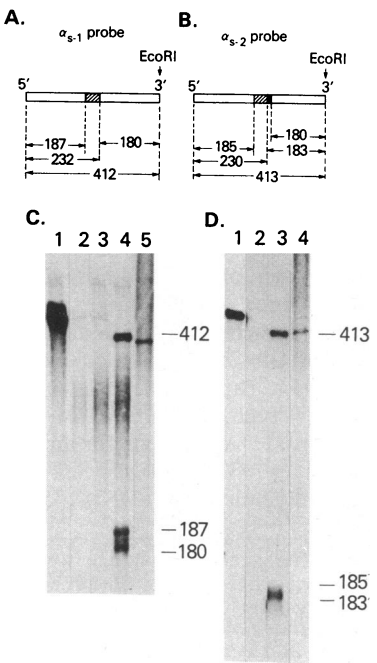

FIG. 3. S1 nuclease analysis of human and mouse α_s RNA. (*A* and *B*) Diagrams of α_s-1 and α_s-2 DNA probes, respectively, and the approximate chain lengths of the fragments expected after hybridization of the [^{32}P]DNA probes with α_s RNA and digestion with S1 nuclease. The single-stranded [^{32}P]DNA probes contain 47 bases of flanking vector sequence. The hatched boxes represent the 45 nucleotide residues present in α_s-1 and -2 (nucleotide residues 213–259 in Table 1) but not in α_s-3 or -4. The black box to the right of the hatched region in *B* represents the three nucleotide residues (CAG) that are present in α_s-2 and -4 but not in α_s-1 or -3. (*C* and *D*) Autoradiograms of the α_s-1 and α_s-2 [^{32}P]DNA·α_sRNA hybrids, respectively, that had been treated with S1 nuclease and subjected to electrophoresis. (*C*) The α_s-1 [^{32}P]DNA probe was used. Lane 1, [^{32}P]DNA probe without RNA and S1 nuclease; lane 2, [^{32}P]DNA without RNA treated with S1 nuclease; lane 3, total RNA from S49 cyc$^-$ mouse lymphoma cells hybridized with [^{32}P]DNA and treated with S1 nuclease; lane 4, total RNA from human skin fibroblasts incubated with [^{32}P]DNA and digested with S1 nuclease; lane 5, the double-stranded *Eco*RI fragment of BG-3 DNA labeled by nick-translation, without RNA or S1 nuclease. (*D*) The α_s-2 [^{32}P]DNA probe was used. Other conditions for lanes 1 and 2 are as described for *C*, lanes 1 and 2; lanes 3 and 4, as described for *C*, lanes 4 and 5, respectively. DNA chain length was estimated by comparing the migration of nucleic acid fragments to those of *Hpa* II fragments of pBR322 DNA.

Incubation of α_s-1 or α_s-2 [^{32}P]DNA probes with S1 nuclease in the absence of RNA or in the presence of RNA from cyc$^-$ cells, which lack α_s mRNA (9), resulted in almost complete degradation of the probes, and no protected bands of [^{32}P]DNA were detected. RNA from normal human skin fibroblasts protected some of the α_s-1 and α_s-2 [^{32}P]DNA probes from cleavage by S1 nuclease. Three bands of α_s-1 [^{32}P]DNA were detected, approximately 400, 187, and 180 bp in length. Two bands were found with the ^{32}P-labeled α_s-2 probe: a 400-bp band and a broad band ≈180–185 bp in length. The 232- and 230-bp fragments were not detected, which suggests that heteroduplexes with three unpaired bases were not cleaved appreciably by S1 nuclease under the conditions used. DNA heteroduplexes with short single-stranded regions are more resistant to cleavage by S1 nuclease than heteroduplexes with longer single-stranded regions (21). In other experiments (not shown), human fibroblast RNA protected the entire 5′ *Eco*RI [^{32}P]DNA fragment of BG-20, an α_s-3 probe. These results show that human fibroblasts contain at least two species of α_s RNA that differ in chain length and/or sequence in the region corresponding to α_s amino acid residues 71–88.

DISCUSSION

Four species of human α_s cDNA were cloned that differ in nucleotide sequence in the region that codes for amino acid residues 71–88. One species of α_s cDNA, α_s-1, closely resembles the reported sequences of bovine (11, 12) and rat (13) α_s. Another species of α_s cDNA, α_s-4, is similar to a short form of bovine α_s reported by Robishaw *et al.* (14). Our results support the hypothesis (14) that different forms of α_s mRNA are derived from a single precursor by alternative splicing. In addition, we describe two other forms of α_s and, based on the nucleotide sequences of the four species of cloned human α_s cDNA and known constraints on splice sequences (22), propose an alternative RNA splicing mechanism shown in Fig. 4 to account for the formation of four species of α_s mRNA. α_s-1 mRNA coding for amino acid residues 71–87 could be formed by splicing exons 1, 2, and 3 together; α_s-2, by splicing exons 1, 2, and 3′ using the alternative right 2′ (acceptor) splice site shown in Fig. 4; α_s-3, by splicing exon 1 to exon 3 by means of the left 1 (donor) and

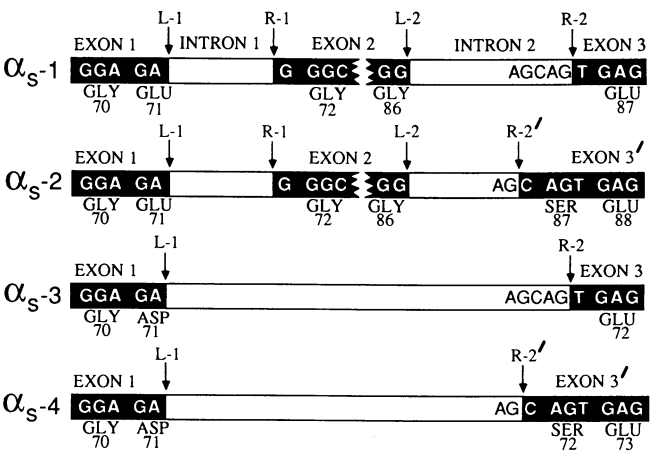

FIG. 4. Alternative splicing of α_s RNA: Proposed mechanism of generating four species of human α_s mRNA from a common precursor RNA. The filled boxes represent exons, arbitrarily numbered 1–3; open boxes represent introns. Left splice donor sites are represented by L-1 and L-2; right splice acceptor sites are represented by R-1, R-2, and R-2′. Sequences found in the + strands of cloned α_s DNA are shown, rather than α_s RNA.

right 2 (acceptor) splice sites; and α_s-4, by splicing exon 1 to exon 3′ by means of the left 1 (donor) and alternative right 2′ (acceptor) splice sites. Thus, two types of splicing events are predicted: the optional removal of exon 2 (45 nucleotide residues) and the use of alternative acceptor splice sites R-2 and R-2′.

The alternative acceptor sites are separated by three nucleotide residues, AG↓CAGTG or AGCAG↓TG (AG↓TAG↓TG in bovine α_s); thus, CAG would be present in some molecules of mature α_s mRNA but not in others. Interestingly, alternative splice acceptor sites separated by CAG have been identified at an intron–exon boundary of cloned genomic prolactin precursor DNA (23). The three additional nucleotide residues, CAG, that were found in α_s-2 and α_s-4 cDNA are distributed between two codons: cytidylate is the third nucleotide of the codon for Gly-86 in α_s-2 and Asp-71 in α_s-4; AG serves as the first and second residues of a newly inserted codon, AGT, for Ser-87 (α_s-2) or Ser-72 (α_s-4). The proposed mechanism of generating four species of α_s mRNA completely accounts for the nucleotide sequences of the four species of α_s cDNA found, with respect to the region corresponding to amino acid residues 71–88. Nucleotide sequences of exon–intron junctions of genomic α_s DNA clones are required to establish unequivocally the origin of the multiple forms of α_s cDNA found.

The amino acid sequence derived from the nucleotide sequence of human BG-3 α_s cDNA is highly homologous to bovine (11, 12) and rat (13) α_s sequences. The conservation in amino acid sequence presumably reflects the constraints against evolutionary divergence in protein structure dictated by the multiple functions of the G_s α-subunit. In addition, the 3′-untranslated regions of human bovine (11, 12) and rat (13) α_s cDNAs also are highly conserved, which suggests that the 3′-untranslated sequence has a function that has not been defined.

The number of cDNA clones for each species of α_s found in the basal ganglia λgt11 library may reflect the relative abundance of each species of α_s mRNA. Although only nine basal ganglia α_s cDNA clones were characterized, the relative abundance of the different types of α_s mRNA in 1-day-old human basal ganglia is estimated to be approximately 33%, 11%, 23%, and 33% for α_s-1, α_s-2, α_s-3, and α_s-4, respectively.

Relatively simple patterns of α_s probe hybridization to bovine (10) and human (C. Van Dop and M. Levine, personal communication) genomic DNA restriction fragments have been reported that suggest that the bovine and human genomes contain single α_s genes. However, we do not rule out the possibility that multiple α_s genes give rise to different species of α_s mRNA.

α_s-1 and α_s-4 forms of α_s cDNA correspond to M_r 52,000 and 45,000 forms of α_s protein (14). Our results suggest that the M_r 52,000 form of α_s protein is composed of α_s-1 and α_s-2 protein, and the M_r 45,000 form of α_s is composed of α_s-3 and α_s-4 protein. The M_r 52,000 and 45,000 forms of α_s are able to activate adenylate cyclase; however, the available evidence suggests that the M_r 52,000 form is more efficient in reconstituting adenylate cyclase activity in α_s-deficient S49 cyc$^-$ membranes than the M_r 45,000 form (24). Some of the 15 or 16 amino acid residues that are present in α_s-1 or α_s-2 protein, but not in α_s-3 or α_s-4, are unique to α_s and do not align with amino acid residues in α_o, α_i, or α_{TD} (13, 25). The 15 or 16 amino acid residues constitute a relatively hydrophilic, negatively charged region of α_s-1 or α_s-2 protein, and residues 83–86 (Asn-Ser-Asp-Gly) are predicted to have the conformation of a β-turn, based on the conformational parameters of Chou and Fasman (26). The four species of human α_s also differ in the number of serine residues in this region that are potential sites for phosphorylation. Two serine residues are present in α_s-1, three in α_s-2, none in α_s-3, and one in α_s-4. Ser-82, present in α_s-1 and α_s-2, but not in α_s-3 or α_s-4, is a potential site for phosphorylation catalyzed by cAMP-dependent protein kinase A but not by protein kinase C (27). In contrast, Ser-87 in α_s-2 and Ser-72 in α_s-4 are potential sites for phosphorylation catalyzed by protein kinase C but not by cAMP-dependent protein kinase A. Further work is required to define the functional consequences of the different nucleotide sequences found in the four types of cloned α_s cDNA.

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