MOLECULAR AND CELLULAR BIOLOGY, Feb. 1991, p. 1146–1155
0270-7306/91/021146-10$02.00/0

Vol. 11, No. 2

Different Forms of $G_{o\alpha}$ mRNA Arise by Alternative Splicing of Transcripts from a Single Gene on Human Chromosome 16

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Received 20 April 1990/Accepted 29 November 1990

$G_{o\alpha}$, (gene symbol GNA01), a member of the signal-transducing guanine nucleotide-binding (G) protein family, has been implicated in ion channel regulation. Some tissues contain multiple $G_{o\alpha}$ mRNAs of different sizes that differ in the 3′ untranslated regions (UTRs). Using sequence-specific 48-base oligonucleotides, two complementary to the different 3′ UTRs and one complementary to the coding region, we investigated the origin of the multiple $G_{o\alpha}$ transcripts, the organization of the $G_{o\alpha}$ gene, the interspecies conservation of 3′ UTRs, and the chromosomal localization of $G_{o\alpha}$. Oligonucleotides labeled to high specific activity by using terminal deoxynucleotidyltransferase each hybridized with a single band of restriction enzyme-digested mouse and human DNAs. In three of four digests of human DNA, the two probes specific for the different 3′ UTRs hybridized with the same restriction fragment. Thus, these nucleotide sequences are in close proximity in the human genome. The order of the UTRs in the bovine, human, and mouse genomes was confirmed directly by polymerase chain reaction (PCR) amplification and sequencing. Hybridization of bovine oligonucleotide sequences with mouse and human genomic DNA indicated a high degree of interspecies sequence conservation; conservation was confirmed by PCR amplification and sequencing. Bands detected by both UTR probes, as well as the predominant bands detected by a bovine $G_{o\alpha}$ cDNA, segregated with human chromosome 16 on Southern blot analysis of human-mouse somatic cell hybrids. We conclude that $G_{o\alpha}$ mRNAs with different 3′ UTRs arise by alternative splicing of transcripts from a single gene. The UTRs, which exhibit a high degree of interspecies conservation, may play a role in regulation of $G_{o\alpha}$ expression during differentiation or in specific tissues. The use of oligonucleotide probes of the type described here represents a new strategy, potentially widely applicable for mapping and elucidating structural features of genes.

Guanine nucleotide-binding (G) proteins are members of a family of signal-transducing proteins that link cell surface receptors with intracellular effectors. They include G_s and G_i, the stimulatory and inhibitory proteins of the adenylyl cyclase system, the transducins (G_t) that function in visual excitation, and $G_{o\alpha}$, a protein of unknown function that has been implicated in ion channel regulation (11, 17, 29, 39, 64). These G proteins are heterotrimers with α, β, and γ subunits. The α subunits, which bind and hydrolyze GTP, are specific for and thus identify different G proteins. For each G protein, there is apparently a high degree of interspecies conservation of cDNA sequence in both coding and untranslated regions (UTRs), from which may be inferred selection pressure operating to maintain the structure of both proteins and mRNAs across evolutionary time (9, 34, 37, 69).

Diversity has been found in each of the G-protein subunits, contributing to a large number of possible G-protein heterotrimers and interactions. In the case of the α subunits, four species of $G_{s\alpha}$ arise by alternative splicing of transcripts from a single gene (10, 29, 31, 53), whereas the several members of the G_i family, designated $G_{i\alpha}1$, $G_{i\alpha}2$, and $G_{i\alpha}3$, are products of separate genes (9, 13, 16, 26, 27, 29). The question of whether there is one or multiple forms of $G_{o\alpha}$ (gene symbol GNA01) has been raised in several contexts (7, 20, 25, 38, 48, 49, 63). At least three individual species of $G_{o\alpha}$ mRNA transcripts can be identified on Northern (RNA) analysis using $G_{o\alpha}$-specific probes (8, 40, 41). These mRNA species, which are apparently expressed primarily in neural tissues, are larger and more heterogeneous than is usually seen for mRNA coding for G_α subunits. Evidence of multiple forms of $G_{o\alpha}$ protein has been reported (7, 20, 30, 38). Using Mono Q chromatography and two-dimensional electrophoresis, Goldsmith et al. (20) separated two proteins of 39 and 39.5 kDa from bovine brain that reacted similarly with four different $G_{o\alpha}$-specific antisera. They postulated that these two forms of $G_{o\alpha}$ might result from differences in posttranslational modification, although differences in the primary structures of the molecules as a result of alternative splicing or multiple $G_{o\alpha}$ genes could not be excluded. Milligan et al. (38) described a 39-kDa protein in rat myometrium that reacted with two $G_{o\alpha}$-specific antisera but not with two others that react with rat brain $G_{o\alpha}$ and suggested that there may be a form of $G_{o\alpha}$ in myometrium that differs from that in brain. One or more of the protein variants seen by Goldsmith et al. (20) or Milligan et al. (38) may correspond to clones for a $G_{o\alpha}$ cDNA variant described by both Hsu et al. (25) and Strathmann et al. (63). This cDNA variant, termed $G_{o\alpha}2$, is found to be identical with previously identified $G_{o\alpha}$ clones in regions corresponding to the initial 248 deduced amino acids; the sequence diverges thereafter. The molecular mechanism that generates the two $G_{o\alpha}$ species has not been defined; alternative splicing is clearly a possibility. Expression of the $G_{o\alpha}$ family is tissue specific (3, 6, 8, 12, 18, 19, 32, 48–50, 55, 65, 68), is under hormonal (54) and pharmacologic (35, 44–46, 51) influence, and is developmentally regulated (2, 7, 21, 36, 40, 67). The ratios of different $G_{o\alpha}$ isoforms are also apparently expressed in a tissue-specific manner (7, 21, 63). It has thus been proposed that multiple $G_{o\alpha}$s may have a regulatory role in cellular development.

We have previously demonstrated two different 3′ UTRs (termed UTR-A and UTR-B) in $G_{o\alpha}$ cDNA clones isolated

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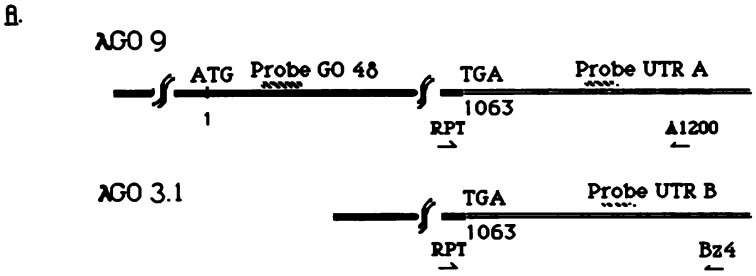

B. 48-base oligonucleotides used as probes:

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UTR-A  AGG CTG TGT GTC TTA ACA AAG GCC AAA AGG TCA TGC TAC CAG GAG ATC
       (UTR-A base 60-107)
UTR-B  TTT GTT ATG CCT TTT TGG AAT TTG TTT ACC AAC TTG CAT TTG  TTA GTG
       (UTR-B base 92-139)
Go-48  GAA GGG CTC CGT GTC TTC CAT CCG ACT CAC CAC GTC ACA CAC CAT CTT
       (Goα ORF base 313-360)
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C. Primers Used for PCR Amplification of Goα

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RPT    CTC CGG GGC TGC GGC TTG TAC (Goα ORF base 1042-1062)
A1200  TAC ACA GCC GTC AGT CAC TCT (UTR-A base 142-162)
Bz4    cccagcctctaga GTA CAG ACT AAA AAA CTT T (UTR-B base 167-185)
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FIG. 1. Two $G_{o\alpha}$ cDNA clones from bovine retina (48, 66), showing loci of sequences used to construct oligonucleotide probes and primers. (A) Sequences of the two clones that diverge 31 bp downstream of the T of the TGA stop codon. λG09 contains UTR-A, and λG03.1 contains UTR-B. (B) The 48-base oligonucleotides used as probes. Positions in λG03.1, which is incomplete, were assigned by alignment with λG09. (C) Oligonucleotides used to amplify and sequence $G_{o\alpha}$ UTR-A and UTR-B from bovine, human, and mouse cDNAs and genomic DNA. Lowercase letters indicate nucleotides included at the 5′ end of primer BZ4 to facilitate subcloning (i.e., to introduce an *Xba*I restriction site). A second version of primer RPT with the sequence ccagcggaattc at the 5′ end (to provide an *Eco*RI restriction site) was used when subcloning was necessary. Positions in the 3′ UTRs are numbered beginning with the T of the termination codon (see Fig. 4).

from a bovine retinal cDNA library in λgt 10 (1, 48, 66). (Each of these clones corresponds to what Strathmann et al. and Hsu et al. refer to as $G_{o\alpha}1$ [25, 63]. For simplicity, we shall continue to refer to cDNA clones containing the initially described coding region as $G_{o\alpha}$.) Hybridization with mRNAs of different lengths that were present in different ratios in different tissues suggested that the UTRs might be important in the tissue-specific transcription or translational regulation of expression of this signal-transducing protein. Since cDNAs for different G_α proteins can have extremely similar coding regions that make positive identification difficult and can sometimes be more readily recognized by differences in UTRs (34, 69), the presence of at least two 3′ UTRs for $G_{o\alpha}$ was consistent with the possibility of a second bovine gene for $G_{o\alpha}$, although it could as well result from alternative splicing. To clarify the origin of the different $G_{o\alpha}$ mRNAs, we turned to genome analysis.

MATERIALS AND METHODS

Oligonucleotide probes. Oligonucleotides used for hybridization were purchased from Pharmacia and purified by full-performance liquid chromatography. Oligonucleotides used as amplification primers were synthesized on an Applied Biosystems 380B synthesizer and used after desalting on Sephadex G-50 (Pharmacia) without further purification. Sequences were complementary to segments of $G_{o\alpha}$ cDNA representing two different 3′ UTRs or to a portion of the coding region (Fig. 1).

DNA hybridization analysis. Mouse, human, and bovine genomic DNAs (purchased from Clontech Laboratories) were digested with restriction endonucleases in buffers suggested by the manufacturer (Boehringer Mannheim Biochemicals). DNA prepared from human-mouse somatic cell lines (57–60) was digested with *Pst*I. Fragments were separated by electrophoresis and transferred to Zetaprobe nylon membranes (62). The $G_{o\alpha}$ cDNA probe was a 1.1-kb *Sty*I-*Eco*RI restriction fragment (nucleotides 262 to 1310, numbered from A of the initiation codon) of the bovine retinal cDNA clone λG09 (66), purified and labeled with [α-^{32}P]dATP (New England Nuclear) by using an oligonucleotide multiprime kit (Boehringer Mannheim). Three different 48-base oligonucleotides were labeled to high specific activity with [α-^{32}P]dATP by tailing with terminal deoxynucleotide transferase (Bethesda Research Laboratories), using buffer and reaction conditions suggested by the manufacturer. Hybridization with the $G_{o\alpha}$ cDNA was done at 42°C in 40% formamide–4× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate)–5× Denhardt solution (1× Denhardt solution is 0.62% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin)–0.5% sodium dodecyl sulfate (SDS)–10 mM Tris hydrochloride (pH 7.4)–10% dextran sulfate containing denatured salmon sperm DNA (100 μg/ml). Hybridizations with the 48-base oligonucleotide probes were done under the same conditions except that the concentration of formamide was 20%. Hybridization was followed by stringent washing in 0.5× SSC–0.5% SDS at 60°C for oligonucleotides and at 65°C for the cDNA probe.

Screening of a human brain λgt11 cDNA library. A total of 1.2×10^6 plaques from a human brain cDNA library, kindly supplied by E. Ginns (National Institute of Mental Health), were screened by using the [α-^{32}P]deoxyadenosine-tailed UTR-B oligonucleotide probe for $G_{o\alpha}$ and the hybridization and washing conditions as described above for the Southern

TABLE 1. Length of PCR product resulting from amplification from mammalian mRNA or genomic DNA between the $G_{o\alpha}$ coding region and oligonucleotides derived from UTR-A or UTR-B[a]

Source of oligonucleotide	Length (bp)					
	mRNA			Genomic DNA		
	Bovine	Human	Mouse	Bovine	Human	Mouse
UTR-A	183	185	184	1,121	1,182	1,109
UTR-B	206	229	237	206	229	237

[a] Amplifications of UTR-A proceeded from primers RPT to A1200 (Fig. 1 and 4). These primers yielded PCR products of smaller size when mRNA was used as a template than when mammalian chromosomal DNA was used; the genomic PCR product consisted of the DNA sequences displayed in Fig. 5, which included the intron-exon splice sites through which it is proposed that UTR-A is brought closer to the coding region in mRNA. Primers RPT and BZ4 yielded DNA fragments of similar size when either poly(A)$^+$ RNA or genomic DNA was used as the amplification template, indicating that UTR-B is adjacent to the $G_{o\alpha}$ coding region in chromosomal DNA.

analysis. Five clones hybridized, but of these only one hybridized with the $G_{o\alpha}$ probe RPT. The insert from this clone (λG0H1) was amplified and directly sequenced by using oligonucleotides flanking the λgt11 cDNA insertion site (oligonucleotides 1222 and 1218; New England BioLabs), as described previously (22, 56).

RT-PCR and genomic PCR amplification of $G_{o\alpha}$ DNA. Reverse transcription polymerase chain reaction (RT-PCR) was performed with human, mouse, and bovine brain poly(A)$^+$ RNAs as described elsewhere (22). Poly(A)$^+$ RNA, purchased from Clontech Laboratories, was reverse transcribed by using avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim Biochemicals). Following alkaline hydrolysis of RNA, the single-stranded cDNA was subjected to amplification with *Thermus aquaticus* DNA polymerase (2 U; Perkin-Elmer Cetus). Reaction mixtures (100 μl), including 50 pmol of each primer (Table 1), were subjected to 40 repeated cycles of 45 s at 94°C, 30 s at 55°C, and 1 min at 72°C, using a Perkin-Elmer Cetus thermal cycler. For sequencing, a modification of these conditions was used. After electrophoretic separation in 3% NuSieve agarose, the gel containing DNA to be sequenced was excised, melted at 55°C, and diluted with 1 ml of distilled water. The DNA was again amplified by using a limiting amount of one primer (0.5 pmol). This procedure preferentially amplified one DNA strand (22). DNA was separated from excess primers and nucleotides with a Centricon 30 filter (Amicon) and sequenced by the dideoxynucleotide termination method using Sequenase.

Genomic PCR was performed in a similar manner, substituting as starting template 300 ng of genomic DNA. In general, direct sequencing of genomic PCR products was less satisfactory than was sequencing of RT-PCR products. The PCR products of genomic DNA were reamplified by using a second set of primers with *Eco*RI (upstream primer) and *Xba*I (downstream primer) restriction sites at the 5′ ends. Products were gel purified and subcloned into M13 phage. At least five M13 phage subclones were sequenced for each sequence reported. Sequences were verified by sequencing both cDNA strands in their entirety. When M13 phage clones were sequenced, the opposite strand was amplified and sequenced by asymmetrical PCR (22). Sequence analyses were performed by using the PC Gene software package and ALIGN program available through Bionet (IntelliGenetics, Mountain View, Calif.) that uses the Smith-Waterman modification of the Needleman-Wunsch algorithm for alignment (42, 61).

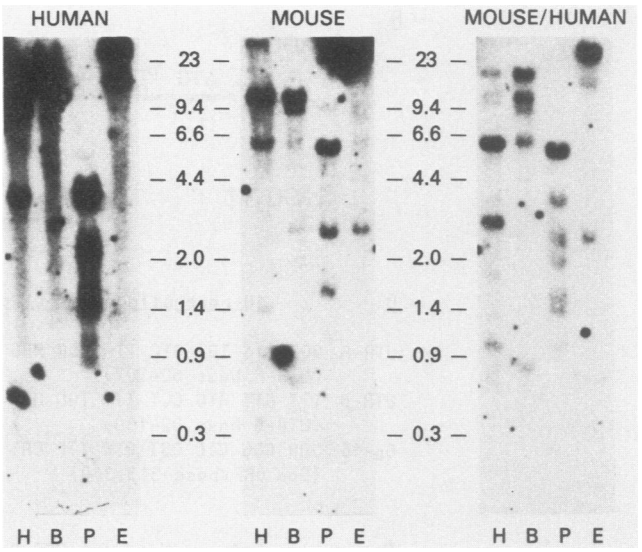

FIG. 2. Restriction fragments of genomic DNA hybridized with bovine λG09 cDNA. Human or mouse genomic DNA (20 μg) or a mixture of mouse DNA (20 μg) and human DNA (4.0 μg) was incubated (3 h, 37°C) with *Hin*dIII (H), *Bam*HI (B), *Pst*I (P), or *Eco*RI (E) (total volume, 100 μl). The reaction mixture was extracted with phenol-chloroform, and the DNA was precipitated with ethanol. After electrophoresis, DNA was transferred to nylon membranes and hybridized with the λG09 cDNA.

Nucleotide sequence accession numbers. The nucleotide sequences of the human $G_{o\alpha}$ 3′ UTR, the bovine $G_{o\alpha}$ 3′ UTR, and the mouse $G_{o\alpha}$ 3′ UTR have been assigned the GenBank accession numbers M59927, M59928, and M59929, respectively.

RESULTS

Hybridization with human and mouse genomic DNAs. To demonstrate that fragments of DNA corresponding to specific regions of the $G_{o\alpha}$ open reading frame and UTRs could be identified, human and mouse DNAs were digested with *Hin*dIII, *Bam*HI, *Pst*I, or *Eco*RI, size fractionated, and transferred to nylon membranes that were hybridized with the *Sty*I-*Eco*RI $G_{o\alpha}$ cDNA fragment (66). Following autoradiography, membranes were stripped of probe and hybridized sequentially with three different 48-base oligonucleotides complementary to UTR-A, to UTR-B, or to a sequence in the $G_{o\alpha}$ coding region that differs in at least 31 bases from corresponding regions reported for other G_α subunits.

The $G_{o\alpha}$ cDNA hybridized with a number of human and mouse DNA fragments (Fig. 2). Mouse and human patterns were most clearly differentiated with *Pst*I digestion, which produced predominant hybridizing fragments of 5.3, 2.4, and 1.6 kb from mouse DNA and 3.9, 2.5, 1.6, and 1.4 kb from human DNA. Other minor bands of variable intensity were visible in DNA from both species, indicating either a large, complicated $G_{o\alpha}$ gene or cross-hybridization of the probe with related genes. Each 48-base oligonucleotide probe hybridized with a single band produced by each enzyme (Fig. 3), consistent with the hypothesis that the probes were complementary to sequence present only once in the haploid genome.

All three probes hybridized with fragments of 18 kb in

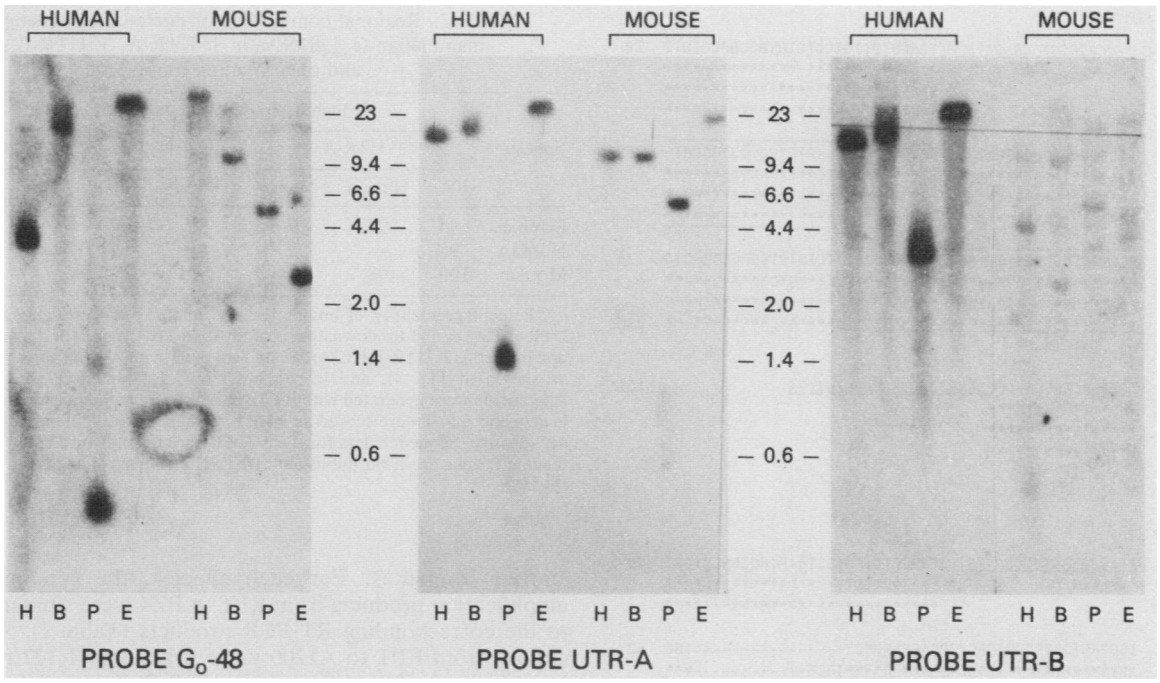

FIG. 3. Restriction fragments of genomic DNA hybridized with Go-48, UTR-A, and UTR-B. Blots of human and mouse genomic DNAs shown in Fig. 2 were sequentially hybridized with probe Go-48 (complementary to the bovine $G_{o\alpha}$-coding region), probe UTR-A, and probe UTR-B after removal of the previously used probe by two washes in 0.1× SSC–0.5% SDS at 95°C for 20 min. Lanes are as indicated for Fig. 2.

*Bam*HI and 23 kb in *Eco*RI digests of human DNA. In *Hin*dIII digests, both UTR probes hybridized with a 16-kb band and the coding region Go-48 probe hybridized with a 3.9-kb band. Each probe hybridized with a different band of human DNA digested with *Pst*I (Fig. 3). These findings are consistent with the conclusion that the two UTRs reside relatively close together in the human genome separated by a *Pst*I site. The 3.9-kb *Pst*I fragment that hybridized with probe UTR-B was a predominant band after hybridization with the cDNA probe. Since probe UTR-B is not related to any reported G-protein cDNA sequence except $G_{o\alpha}$, this finding provides further evidence that the 3.9-kb fragment is, in fact, a part of the human $G_{o\alpha}$ gene and suggests that the coding domain (as detected by the cDNA probe) resides closer to UTR-B than to UTR-A.

The bovine oligonucleotide probes, especially probe UTR-B, hybridized less well with mouse than with human DNA (Fig. 3). Apparently, the bovine nucleotide sequences are more similar to the human than to the mouse sequences, particularly in UTR-B regions.

RT-PCR amplification of $G_{o\alpha}$ 3′ UTRs and isolation of a human cDNA clone, λG0H1, containing 3′ UTR-B. Since UTR-A hybridized with both human and mouse DNA restriction fragments, it appeared that this region might be conserved across species. Sequence data confirming the high degree of interspecies conservation of 3′ UTR-A in human, bovine, and mouse cDNA were obtained by RT-PCR amplification of poly(A)$^+$ RNA from brains of these species. Oligonucleotides complementary to the 3′ end of the $G_{o\alpha}$-coding region and to a 3′-terminal portion of UTR-A (amplification primers RPT and A1200 in Fig. 1) were used to prime the reactions, yielding cDNA fragments of 183 to 185 bases (Fig. 4 and Table 1). These RT-PCR products were sequenced directly. Nucleotide sequences of the human, bovine, and mouse amplification products were >94% identical to each other and to the previously reported 3′ UTR of rat $G_{o\alpha}$ cDNA (26, 28). The sequence of the amplified bovine product was identical to that of the bovine $G_{o\alpha}$ cDNA λG09 (66).

Conservation of UTR-B was evaluated in two ways. First, a partial human cDNA clone, λG0H1, was obtained by screening a human brain λgt11 library with probe UTR-B. This clone has a 350-bp insert corresponding to a partial $G_{o\alpha}$-coding region and 148 bases of 3′ UTR with 89% identity to $G_{o\alpha}$ UTR-B from bovine clone λG03.1 and terminates with a poly(A)$^+$ tail 15 bases downstream of the polyadenylation signal AATAAA (Fig. 4B, arrow). The polyadenylation signal is not present in the corresponding position of bovine UTR-B, which has a C-for-T substitution (i.e., AA CAAA). To extend our observations, amplification primers RPT and BZ4 were selected to synthesize by RT-PCR a cDNA fragment from UTR-B, using human, mouse, and bovine mRNAs as templates (Fig. 4 and Table 1). Sequences of the human PCR product and λG0H1 were identical (with one exception noted below), as were those of the bovine PCR product and clone λG03.1 (Fig. 4). The mouse PCR fragment was slightly longer than the human and bovine fragments; there was 79 to 86% identity among the corresponding regions from the three species (Table 2). As expected from the hybridization data, human and bovine UTR-B were more similar in the area complementary to probe UTR-B (42 of 48 matches) than were the mouse and bovine (39 of 48 matches) UTR-B.

There was some variability in the number of AC repeats in UTR-B from human sources beginning at position 76 (with T of TGA as position 1); the RT-PCR product had 14 repeats; clone λG0H1 has 15 repeats. Whether this variability in tandem repeats was generated during cloning procedures or

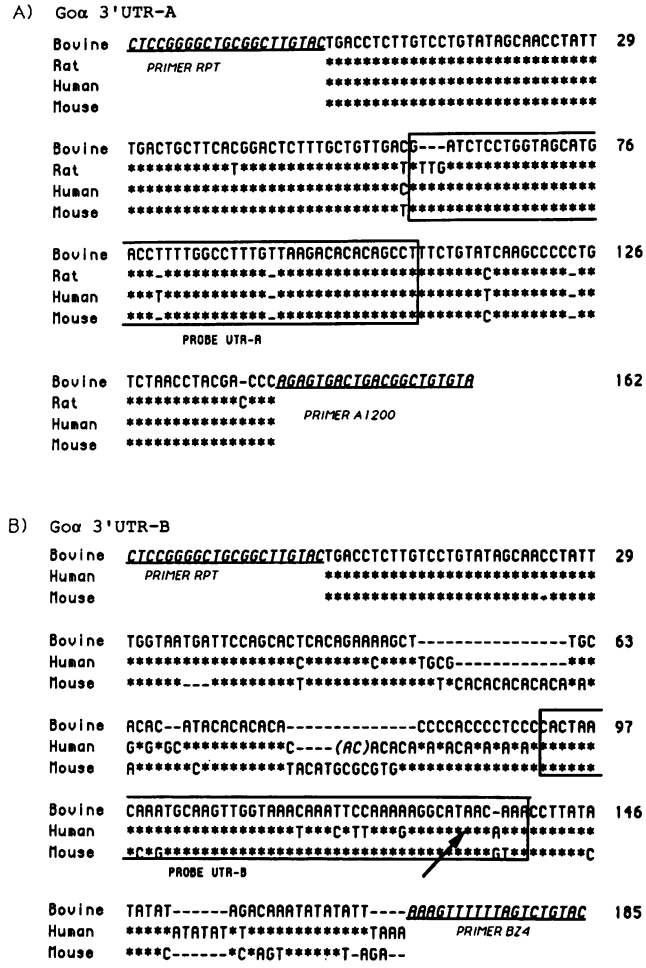

FIG. 4. Sequences of bovine, rat, human, and mouse $G_{o\alpha}$ 3' UTRs derived from cDNA cloning and RT-PCR experiments. (A) Sequences of $G_{o\alpha}$ 3' UTR-A from bovine clone λG09 (66), from a rat brain cDNA (26, 52), and from the RT-PCR products of human and mouse brain poly(A)$^+$ RNAs. Numbering begins with the first base of the termination codon. Gaps (-) were introduced to optimize alignment. Nucleotide identity to the base immediately above a position is indicated by an asterisk. Sequence of the product of RT-PCR of bovine retinal poly(A)$^+$ RNA was identical to that of the bovine retinal clone λG09. Sequences complementary to probe UTR-A are boxed; amplification primers are italicized and underlined. (B) Sequences of $G_{o\alpha}$ 3' UTR-B from bovine clone λG03.1 (66), human clone λG0H1, and the PCR or RT-PCR products of bovine, human, and mouse genomic DNAs and total brain RNA, respectively. Sequences of λG03.1 and of the bovine PCR and RT-PCR products were identical. Sequences of λG0H1 and of the human RT-PCR product were identical except that λG0H1 terminates in a poly(A)$^+$ tail at the point marked with an arrow. A variation was detected in the number of AC repeats found, with an extra AC repeat found in λG0H1 (in parentheses), for a total of 15 AC repeats in λG0H1 starting at position 76 from the top of the TGA, as opposed to 14 AC repeats found in the UTR-B RT-PCR product from human brain poly(A)$^+$ in mRNA.

represents an individual allelic difference requires further study (24, 41).

Structure of the $G_{o\alpha}$ 3' gene. To obtain the 3' structure and sequence of the $G_{o\alpha}$ gene, PCR amplifications were attempted with the primers described above for RT-PCR, using human, bovine, and mouse chromosomal DNAs as starting templates. For each species, the genomic PCR amplification products from RPT to BZ4 were similar in size to the corresponding RT-PCR products (Table 1). Amplifications from RPT to A1200 yielded DNA of 1,122 to 1,175 bp, which was found to contain UTR-B directly adjacent to the stop codon, with UTR-A at the 3' terminus (Table 1 and Fig. 5). Interposed in the bovine sequence between the previously identified UTR-A and UTR-B sequences were 805 bases. A putative intron-exon splice donor site was found at nucleotide 31 (containing the vertebrate consensus sequence TG:GTAA) (47), the point of divergence between $G_{o\alpha}$ cDNAs containing UTR-A or UTR-B. An intron-exon splice acceptor site (PyPyPyPyPyPyPyNCAG:N) was present at the resumption of sequence corresponding to UTR-A (Fig. 5, base 990 in the bovine sequence, marked with a triangle). No other potential splice sites were identified. The AATAAA polyadenylation signal was not present in the bovine sequence and was found only once in the human and mouse sequences (Fig. 5, positions 114 to 119, boxed). As predicted from Southern hybridization analysis, there were *PstI* restriction sites within the human genome in the region separating UTR-B from UTR-A (Fig. 5, positions 195 and 732, boxed). Examination of the genomic DNA thus supports the view that within the $G_{o\alpha}$ gene, the order (5' to 3') is coding region, UTR-B, UTR-A. Splicing out nucleotides 32 to 990 brings UTR-A closer to the TGA stop codon, resulting in the mRNA expected from analysis of clone λG09 and the UTR-A RT-PCR products.

TABLE 2. Regional comparison of nucleotide sequences of mammalian $G_{o\alpha}$ cDNAs in 3' UTR-A, 3' UTR-B, and open reading frame

Species	% Identity in region of cDNA examined[a]						
	3' UTR-A			3' UTR-B		ORF	
	Rat	Human	Mouse	Human	Mouse	Rat	Human
Bovine	94	96	94	86	79	94	95
Human	98	—		—	94	—	
Mouse	100	98	—	86	—		

[a] Percentage identity of bovine, human, mouse, and rat sequences in 3' UTR-A (141 to 143 bp downstream of the T in TGA, not including amplification primers), 3' UTR-B (163 to 186 bp past the T with A, with gaps introduced as shown in Fig. 4), and in the open reading frame (ORF; 1,062 bp). Alignments were generated with the IBI Microgenie computer program. Gaps in sequences (—) were scored as a base mismatch for calculation of percentage identity. Sequence data for the 3' UTRs are taken from Fig. 4. The open reading frames are from published cDNA clones from the species indicated (33, 66).

Nucleotide sequence comparisons revealed that areas of identity among mammalian species are interspersed along the DNA segment, with areas of greatest similarity concentrated in three regions. The first domain extends from bases 1 to 256 of the bovine sequence. This domain contains the TGA stop codon, the initial 31 nucleotides common to UTR-A and UTR-B, as well as the unique nucleotides of UTR-B. This area continues for additional 71 nucleotides and contains several short runs of well-conserved dinucleotide repeats. In total, 206 of the initial 256 nucleotides are identical in the three mammalian species. The second area of nucleotide conservation extends from bovine bases 555 to 722, in which 128 of 167 bases are identical in all three species. Again, recurring motifs of dinucleotide and homopolymeric repeats are found. Finally, a third domain with a high degree of identity extends from bovine base 917 to the end of the sequence. This area contains the intron-exon

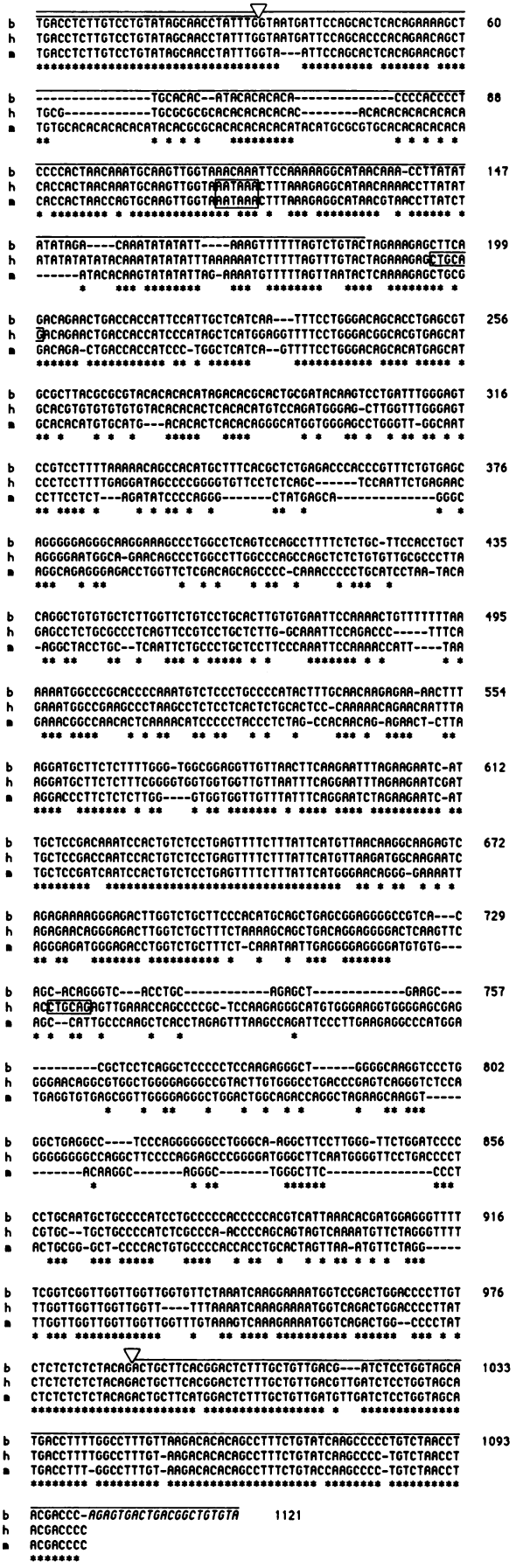

FIG. 5. Sequence of bovine (b), human (h), and mouse (m) $G_{o\alpha}$ 3′ UTRs derived by PCR of mammalian chromosomal DNA. Numbering is from the first base of the termination codon in the bovine sequence. Sequences corresponding to unique domains of UTR-B (bases 32 to 162) and UTR-A (991 to 1121) from Fig. 4 are overlined. The first 31 bases of sequences common to both UTR-A and UTR-B are denoted with a double overline. The proposed splice donor site (following base 31) and splice acceptor site (following position 990) are indicated with triangles. During generation of UTR-A mRNA, it is proposed that bases 32 to 990 are spliced out. Alignments were made by using the CLUSTAL multiple sequence alignment program (23), with a K-tuple size of 2 and a gap penalty of 4. Nucleotides identical in all three species are indicated by asterisks below the sequences. Primer A1200 used for PCR amplification is in italics at the end of the sequence. Boxes indicate the AATAAA polyadenylation signals in human and mouse sequences (nucleotides 114 to 119); two *Pst*I restriction sites, predicted from Southern hybridization data, around positions 195 and 732 of the human sequence are boxed.

acceptor splice site at position 990 (Fig. 5, triangle) and the unique sequences of UTR-A.

Interposed between regions of highest sequence identity are less conserved sequences. In particular, bases 256 to 554 and 723 to 915 have relatively few positions that are identical in the three species, presumably indicating that these sequences are of less functional importance than the highly conserved domains. However, even in these areas, there are regions in which two of three species are identical. Certain DNA motifs, such as dinucleotide and homopolymeric repeats, are also found in the areas of lesser conservation, suggesting that the DNA base composition of these sequences may play some functional role.

Chromosomal localization of human $G_{o\alpha}$ gene. To identify the human $G_{o\alpha}$ gene in human-mouse somatic cell hybrids, DNA was digested with *Pst*I and Southern blots were hybridized with the cDNA and probes UTR-A and UTR-B. The 3.9-kb fragment was used as an indicator of human $G_{o\alpha}$ in hybridizations with the cDNA and UTR-B probes, and the 1.4-kb fragment was used in hybridizations with the UTR-A probe (Fig. 6). The other major fragments of 2.5 and 1.6 kb that hybridized with the cDNA probe segregated with the 3.9-kb restriction fragment, although the presence or absence of these bands was more difficult to score due to variability in intensity. In DNA from 32 independent human-mouse hybrids, the scored bands segregated with chromosome 16 with no discordance (Fig. 7). There were also several minor bands that did not consistently segregate with the major fragments and could not be unambiguously assigned a locus.

DISCUSSION

The data reported here, obtained with sequence-specific oligonucleotide probes, are consistent with the conclusion that multiple forms of $G_{o\alpha}$ mRNA with different 3′ UTRs arise from a single gene on human chromosome 16 and that this pattern of alternative 3′ UTRs is well conserved across species. Based on cDNA hybridization, there was no evidence of a second $G_{o\alpha}$-related gene with a similar coding region, as an explanation for the apparent existence of more than one immunoreactive $G_{o\alpha}$ protein. At least eight different types of G-protein α-subunit cDNAs have been described, and the majority (excluding $G_{o\alpha}$) have been mapped to human chromosomes (4, 5, 43). In some mapping studies, cross-hybridization with related genes was a serious imped-

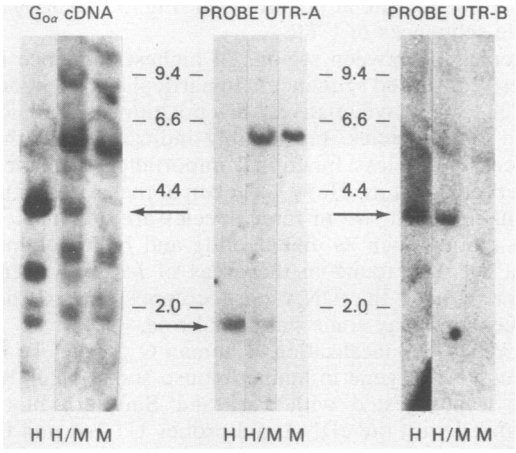

FIG. 6. Detection of human $G_{o\alpha}$ gene in human-mouse somatic cell hybrids. *Pst*I fragments of DNA from human cells (H), mouse cells (M), or a human-mouse somatic cell line (H/M) were hybridized sequentially with λG09 cDNA and the indicated oligonucleotide probe after removal of the previously used probe as described for Fig. 3. For screening of 31 hybrid cell lines (summarized in Fig. 7), the 3.9- or 1.4-kb *Pst*I fragment (arrows) was used as an indicator of human $G_{o\alpha}$ DNA after hybridization with λG09 cDNA and probe UTR-B or probe UTR-A, respectively.

iment to accurate localization, particularly with similarities as extensive as they are among the different types of G_α subunits. By using *Eco*RI restriction digests of mouse-human somatic cell hybrids, a human G_α subunit now referred to as α_{i2} was initially mapped to two different loci (on human chromosomes 3 and 12) by different investigators (5, 43). Because the position on human chromosome 3 was confirmed on an independent screening of mouse-human somatic cell hybrids digested with the restriction endonuclease *Hin*dIII, it appears that the assignment to chromosome 3 is correct (5). The identity of the additional locus on chromosome 12 that hybridized with α_{i2} has not been reported; it may represent a pseudogene or another gene closely related to α_{i2}. This result underscores the necessity for specific probes when one is mapping the members of this highly homologous multigene family.

$G_{o\alpha}$ presented considerable difficulty in mapping. Digestion with four restriction enzymes yielded multiple fragments that hybridized with the cDNA with varying intensity. The patterns appeared to be somewhat more complex than those found with cDNAs for other G_α subunits (5). $G_{o\alpha}$ mouse and human DNA restriction patterns were differentiated most clearly by using *Pst*I digests of human DNA which contained four major bands and at least three minor bands that hybridized with the cDNA probe. To minimize ambiguity, we used, in addition to the cDNA probe, two 3′ UTR oligonucleotide probes that were not significantly similar in sequence to any reported genes. Oligonucleotide probes have the advantage of being highly specific but have not been used for chromosome localization studies because they are difficult to label to the high specific activity necessary for detection of single-copy genes in somatic cell hybrids. This difficulty was overcome by tailing the probes with [α-^{32}P] dATP and terminal deoxynucleotide transferase to a specific activity of 2.5×10^9 dpm/μg, about 10 times the level achieved by 5′ labeling with polynucleotide kinase. Tail lengths were limited to 5 to 15 residues by appropriate labeling conditions. These deoxyadenylate tails do not alter the specificity of oligonucleotide hybridization (14, 15).

As reported here, the oligonucleotide probes hybridized unambiguously with single bands in the *Hin*dIII, *Pst*I, *Bam*HI, and *Eco*RI digests of genomic cDNA. Only bands that hybridized with both the cDNA and a specific oligonucleotide probe were used to identify the $G_{o\alpha}$ gene. Probe UTR-B hybridized with the same restriction fragment that hybridized most strongly with a fragment representing the coding region of $G_{o\alpha}$ cDNA, consistent with the results of the oligonucleotide mapping method and supporting the reliability of using this restriction fragment as an indicator of $G_{o\alpha}$ in screening the human-mouse somatic cell hybrids. Using the oligonucleotide probes and the $G_{o\alpha}$ cDNA, both UTRs and the coding region were localized to chromosome 16. Analogous oligonucleotide probes may have wide applicability in mapping chromosomal locations of other genes, particularly when they represent proteins with considerable similarity, as is the case with the $G_{o\alpha}$ subunits.

The possibility that the family of bovine cDNA clones with two different 3′ UTRs (λG03.1, λG09, and λG01) were derived from two separate genes is not compatible with the finding that an oligonucleotide complementary to a unique sequence in the coding region hybridized with single fragments in four different restriction enzyme digests of human and mouse DNAs. The data are consistent with the conclusion that this coding sequence occurs only once per haploid genome. The fact that probes containing sequences found in the two alternative 3′ UTRs hybridized with the same fragment in *Eco*RI, *Bam*HI, or *Hin*dIII digests of human DNA suggests that the sequences occur relatively close to each other. The coding-region cDNA probe hybridized with a restriction fragment in *Pst*I digests that also hybridized with the UTR-B but not the UTR-A probe. Thus, based on restriction fragment and hybridization studies, the order of $G_{o\alpha}$ sequences in the genome proceeding 5′ to 3′ is protein-coding exons, 3′ UTR-B, 3′ UTR-A.

The structure of the 3′ end of the $G_{o\alpha}$ gene was confirmed by sequencing of RT-PCR and genomic PCR products. As predicted by the oligonucleotide and RT-PCR mapping experiments, the nucleotide sequence unique to UTR-B followed a 31-base common region adjacent to the TGA stop codon, with the start of UTR-A following downstream (at position 991). In each species, intron-exon splice donor sites were conserved and were found at the point of divergence between UTR-A and UTR-B cDNAs. A splice acceptor site was located at the 5′ start of UTR-A. When UTR-B is spliced out, UTR-A is brought adjacent to the 31 bases of the 3′-UTR common region. As AATAAA is not found in the bovine cDNA segment, it is unclear how much of this segment is incorporated into the 2.0-kb $G_{o\alpha}$ mRNA species previously shown to contain $G_{o\alpha}$ UTR-B but not UTR-A. Presumably, this question and others regarding structures of multiple $G_{o\alpha}$ mRNAs resulting from expression of this single gene can be answered only through examination of additional $G_{o\alpha}$ cDNAs.

A variant of $G_{o\alpha}$ cDNA termed $G_{o\alpha}2$ has recently been described (25, 63). Clones for $G_{o\alpha}2$ have 248 deduced amino acids in common with the original $G_{o\alpha}$ and then diverge for the remainder of 3′ coding and untranslated regions. Although the present study focused on alternative splicing of $G_{o\alpha}$ 3′ UTRs, it has relevance to the mechanism of the generation of the carboxy-terminal variant in $G_{o\alpha}2$. Presumably, since all oligonucleotide probes for $G_{o\alpha}$, including probe Go-48 (complementary to sequence found in both $G_{o\alpha}$ and $G_{o\alpha}2$) detected single bands on multiple Southern blots

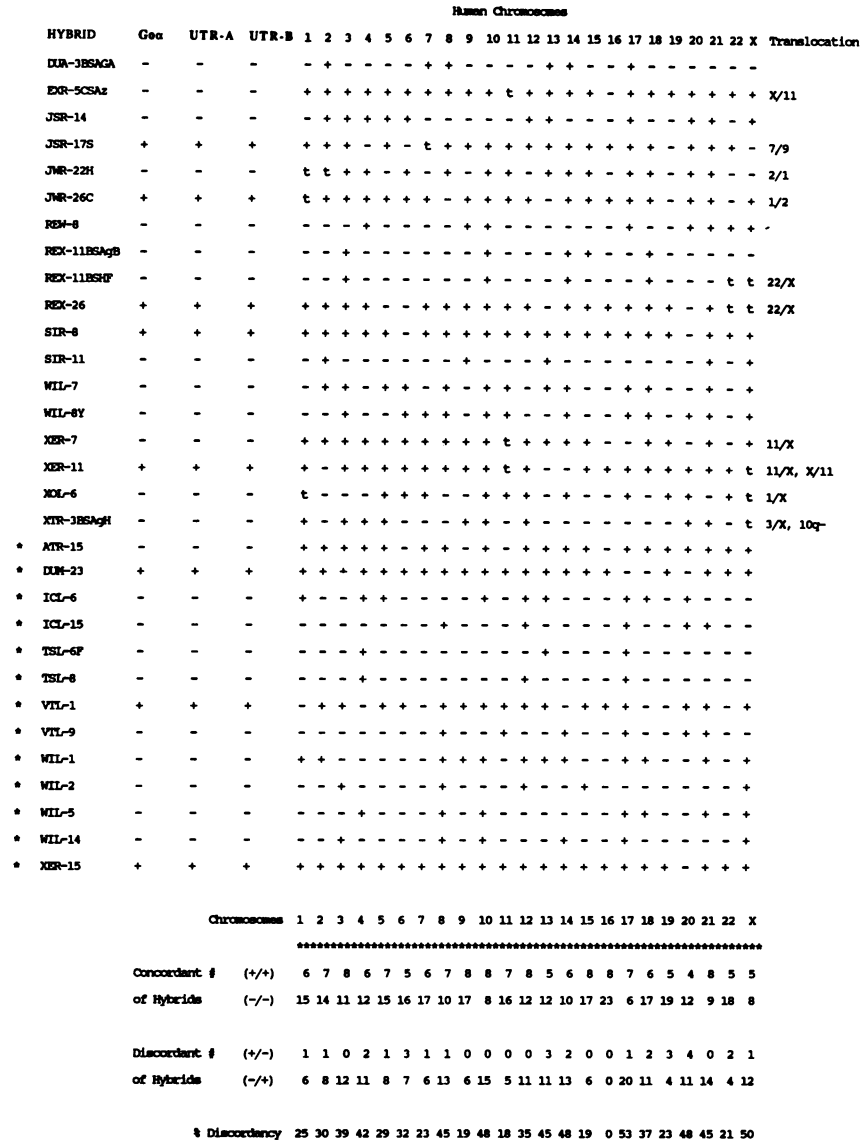

FIG. 7. Detection of the human $G_{o\alpha}$ gene in *Pst*I digests of genomic DNA from human-mouse hybrid cell lines. Blots of *Pst*I-digested DNA from the indicated cell lines were hybridized with the λG09 cDNA and oligonucleotide probes UTR-A and UTR-B. The presence (+) or absence (−) of hybridization with a human restriction fragment is recorded. Concordant hybrids hybridized with the probes and retained the chromosome or did neither. Discordant hybrids either hybridized with the probes but lacked the chromosome (+/−) or vice versa (−/+). DNA that hybridized with each of the three probes mapped to human chromosome 16. The 31 human-mouse hybrids represent 14 unrelated human cell lines and four mouse cell lines (58–60). Hybrids marked with an asterisk were characterized by mapped enzyme markers only. The rest were characterized by mapped enzyme markers and karyotypic analysis and partly by mapped DNA probes (57, 59). "t" indicates a chromosome translocation (described in the last column) and the absence of an intact chromosome.

of mammalian DNAs, $G_{o\alpha}2$ also arises by alternative splicing of transcripts from the single $G_{o\alpha}$ gene that we have mapped to chromosome 16. As the $G_{o\alpha}$ gene appears to be large (>60 kb) (29), it is possible that other splice variants of $G_{o\alpha}$ remain to be discovered. If these share nucleotide identities with the sequence-specific oligonucleotide probes used in this study, it seems probable that they are also products of the same $G_{o\alpha}$ gene.

The 3′ UTRs of $G_{o\alpha}$, like those of other G_α subunits, seem to be extensively conserved over millions of years of species divergence, from which it may be inferred that they are under strict evolutionary selection pressures and are presumably of functional importance. In the published cDNA sequences for each type of G_α subunit, there are extensive interspecies similarities in the 3′ UTRs as well as in coding regions (9). For example, the 300 nucleotides nearest the stop codon in the 3′ UTR of human and rat $G_{s\alpha}$ cDNAs are 92 to 96% identical. On the other hand, the 3′ UTRs of $G_{s\alpha}$ and $G_{i\alpha}$ are not related to each other or to those of any other known G_α subunits. In fact, these UTRs are often the most distinctive sequences of products of separate genes. Alternative splicing of 3′ UTRs has not yet been demonstrated for other G-protein mRNAs. Given the similarities in structure of genes for different G-protein subunits, it would not be surprising to find alternative splicing of UTRs in other G_α mRNAs. As expression of $G_{o\alpha}$ is tissue specific (2, 8, 18, 19, 32, 48, 49, 65) and changes during cellular differentiation (2, 7, 21), it is tempting to speculate that alternative splicing of

these UTRs participates in transcriptional or posttranscriptional regulation of $G_{o\alpha}$ expression.

ACKNOWLEDGMENTS

We thank Carol Kosh for help in preparing the manuscript and S. R. Price and Randy Haun for expert advice on computer analysis.

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