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Structure of the human gene and two rat cDNAs encoding the α chain of GTP-binding regulatory protein G_o: Two different mRNAs are generated by alternative splicing

(signal transduction)

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ABSTRACT **G_o is a specific class ("other") of signal-transducing heterotrimeric GTP-binding proteins (G proteins) that is expressed in high levels in mammalian brain. We have cloned two different rat cDNAs encoding the α subunit of G_o ($G_o\alpha$-1 and $G_o\alpha$-2) and a human $G_o\alpha$ chromosomal gene. The human $G_o\alpha$ gene spans more than 100 kilobases and contains 11 exons, including one noncoding exon in the 3′ flanking region. The 5′ flanking region is highly G+C-rich and contains five G·C boxes (Sp1 binding sites) but no TATA box. Exons 7 and 8 coding for amino acid residues 242–354 of $G_o\alpha$ protein are duplicated (referred to as exons 7A, 7B, 8A, and 8B). It was found that exons 7A and 8A code for $G_o\alpha$-1, and 7B and 8B code for $G_o\alpha$-2. This indicates that two different $G_o\alpha$ mRNAs may be generated by alternative splicing of a single $G_o\alpha$ gene. The splice sites of the $G_o\alpha$-1 and $G_o\alpha$-2 genes are completely identical with those encoding human inhibitory G protein α subunits $G_i2\alpha$ and $G_i3\alpha$ [Itoh, H., Toyama, R., Kozasa, T., Tsukamoto, T., Matsuoka, M. & Kaziro, Y. (1988) *J. Biol. Chem.* 263, 6656–6664] and also transducin G protein α subunit $G_t1\alpha$ [Raport, C. J., Dere, B. & Hurley, J. (1989) *J. Biol. Chem.* 264, 7122–7128]. Sequence homology and conservation of the exon–intron organization indicate that the genes coding for $G_o\alpha$, $G_i2\alpha$, $G_i3\alpha$, $G_t1\alpha$, and probably $G_i1\alpha$ may be evolved from a common progenitor. Like $G_o\alpha$-1, $G_o\alpha$-2 is expressed mainly in brain.**

In a variety of transmembrane signaling systems, a family of heterotrimeric GTP-binding proteins (G proteins) are involved as signal transducers (1–4). Recent studies on the cloning of cDNAs and genomic DNAs carried out in several laboratories including our laboratory have revealed that G proteins are a family of GTP-binding proteins with structures closely related but distinct (5). In mammalian cells, the presence of at least nine genes coding for G protein α subunits has so far been shown. The proteins include the stimulatory G protein (G_s) α subunits $G_s1\alpha$ and $G_s2\alpha$ (referred to as Golfα in ref. 6); the inhibitory G protein (G_i) α subunits $G_i1\alpha$, $G_i2\alpha$, and $G_i3\alpha$; the "other" G protein α subunit $G_o\alpha$; the tentatively designated G protein α subunit $G_x\alpha$ (also referred to as $G_z\alpha$; see refs. 7 and 8); and the transducin G protein α subunits of retina rod cells ($G_t1\alpha$) and cone cells ($G_t2\alpha$). There are four different kinds of $G_s1\alpha$ cDNAs (9–11) that are generated from a single $G_s1\alpha$ gene by alternative splicing (12).

G_o was originally isolated from bovine brain (13, 14), and its α subunit, $G_o\alpha$, represents a 39-kDa species that is the major pertussis toxin substrate in brain tissue. The cDNA coding for $G_o\alpha$ has been isolated, and its nucleotide sequence has been determined (15–18). The partial sequence of human $G_o\alpha$ genomic DNA containing exons 1 and 2 also has been reported (19). The function of $G_o\alpha$ in signal transduction has not yet been fully clarified. There are reports indicating that it may be coupled with the muscarinic acetylcholine receptor, the γ-aminobutyric acid receptor, and the opioid receptor and may regulate the Ca^{2+} channel and phospholipase C (1, 2). $G_o\alpha$ protein is expressed mainly in brain (20–22) and to a lesser extent in heart and lung (20, 22). Expression of $G_o\alpha$ mRNA is shown to be tissue specific (22, 23). Immunohistochemical studies indicate that $G_o\alpha$ is almost exclusively located in nervous tissue and neuroendocrine cells (24).

Recently, a G_o-like protein distinct from the major species of $G_o\alpha$ was identified from bovine brain (25) and rat myometrium (26). A G_o-like cDNA was obtained from *Xenopus* oocytes (27). More recently, two classes of *Drosophila* $G_o\alpha$-like mRNAs generated by alternative splicing were reported (28–30). To investigate the heterogeneity of $G_o\alpha$ mRNA and the structural organization of the $G_o\alpha$ gene we isolated two distinct rat $G_o\alpha$ cDNAs and a human chromosomal gene encoding $G_o\alpha$ and determined their structures.¶

EXPERIMENTAL PROCEDURES

Screening of Human Genomic Libraries. Human genomic libraries from fetal liver (31) and placenta (32) respectively provided by T. Maniatis (Harvard University) and M. Shibuya (University of Tokyo) were screened by plaque hybridization (33). Hybridization was performed in 5× SSC (1× SSC = 150 mM NaCl/15 mM sodium citrate, pH 7.0) containing 1× Denhardt's solution (0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll), 20 mM sodium phosphate buffer (pH 7.0), 0.1% sodium dodecyl sulfate, 10% dextran sulfate, 100 μg of denatured calf thymus DNA per ml, and 50% formamide at 42°C, and filters were washed at 55°C in 0.1× SSC/0.1% sodium dodecyl sulfate. ^{32}P-labeled probes were prepared by nick translation (34) or random priming (35) of DNA fragments purified from agarose gel.

Southern Blot Analysis of Isolated Phage Clones. DNA was prepared from the isolated phage clones, digested with re-

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Abbreviations: G protein, GTP-binding protein; G_i, G_s, G_o, G_x, and G_t proteins, inhibitory, stimulatory, other, tentative designation, and transducin G proteins; $G_i\alpha$, $G_s\alpha$, $G_o\alpha$, $G_x\alpha$, and $G_t\alpha$ designations, G_i, G_s, G_o, G_x, and G_t α-subunit designations.

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¶The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M60156–M60165 for segments 1–10 (human $G_o\alpha$ gene).

striction endonucleases, separated on a 1% agarose gel, and transferred to a nitrocellulose membrane. The filters were hybridized with ^{32}P-labeled probes under the same conditions as plaque hybridization. For the analysis of alternate exons, hybridization was performed in 5× SSC containing 1× Denhardt's solution, 20 mM sodium phosphate buffer (pH 7.0), 0.1% sodium dodecyl sulfate, 10% dextran sulfate, 100 μg of denatured calf thymus DNA per ml, and 30% formamide at 30°C, and filters were washed at 37°C in 1× SSC, and 0.1% sodium dodecyl sulfate.

Screening of Rat PC-12 cDNA Libraries. To isolate a G_o-like cDNA, a rat PC-12 cDNA library provided by Y. Sugimoto (Tsukuba Life Science Center) was screened by plaque hybridization. Hybridization was performed under the same conditions as described for the screening of genomic libraries.

DNA Sequence Determination. DNA fragments were subcloned into phage M13 vectors (36), and the DNA sequence was determined by the dideoxy chain-termination methods (37). For analysis of the regions high in G+C content, 2'-deoxy-7-deazaguanosine 5'-triphosphate was used instead of dGTP (38).

Southern Blot Hybridization. High molecular weight DNA was prepared from the human neuroblastoma cell line SK-N-SH and was digested with restriction endonucleases, separated on a 0.8% agarose gel, and transferred to a nitrocellulose membrane. The filters were hybridized with ^{32}P-labeled human $G_o\alpha$ genomic fragments under the same condition as plaque hybridization.

RNA (Northern) Blot Hybridization. Total RNA was prepared from various rat tissues, separated on a 1% agarose gel containing 2.2 M formaldehyde, and transferred to a nitrocellulose membrane. The filter was hybridized with a ^{32}P-labeled rat $G_o\alpha$ cDNA fragment under the same condition as plaque hybridization.

RESULTS

Isolation and Characterization of a Human $G_o\alpha$ Chromosomal Gene. We have screened about 12×10^5 recombinants of two human genomic libraries using rat $G_o\alpha$ (now referred to as $G_o\alpha$-1) cDNA as a probe, and we isolated 11 clones. The libraries were rescreened by using the above clones as probes to yield 9 additional clones. Restriction mapping and nucleotide sequence analysis of these 20 clones revealed that the human $G_o\alpha$ gene spans more than 100 kilobases (kb) (Fig. 1). Originally, we found nine exons (exons 1–6, 7A, 8A, and 9) separated by eight introns, and as will be described below, we isolated two additional exons (exons 7B and 8B), which are alternatives to exons 7A and 8A (Fig. 1).

Isolation of an Alternate Exon to Exon 7A. The G_o-like clone isolated from *Xenopus* oocytes differed from rat $G_o\alpha$-1 cDNA mainly at the C-terminal region (27). Furthermore, Strathmann *et al.* (39) and Hsu *et al.* (40) have recently isolated from mammalian cells two distinct $G_o\alpha$ cDNAs that differ in their C-terminal regions. We attempted to find an alternate exon by screening isolated phage clones using a DNA fragment containing exon 7A as a probe. By screening under low-stringency conditions, we found several clones containing an alternate exon to exon 7A (designated exon 7B) in the region between exons 6 and 7A (intron 6).

Isolation of $G_o\alpha$-2 cDNA. To obtain a different class of $G_o\alpha$ cDNA containing exon 7B, we screened about 4×10^5 recombinants of a rat PC-12 cDNA library under high-stringency conditions with exon 7B as a probe, and we isolated 1 clone (designated λG_o11). This clone contained the sequence corresponding to amino acid residues 151–354 of $G_o\alpha$ protein. The amino acid residues 151–241 are completely identical with those of $G_o\alpha$-1, while the sequence of residues 242–354 (exons 7 and 8) had some differences (see Fig. 5).

Isolation of Exon 8B from Human Genomic DNA. Using the DNA fragment of rat $G_o\alpha$-2 cDNA as a probe, we analyzed human genomic clones further for $G_o\alpha$, yielding a clone containing exon 8B that codes for amino acid residues 293–354 as well as a 3' untranslated region (see Fig. 1).

Organization of Human $G_o\alpha$ Chromosomal Gene. Nucleotide sequence analysis (Fig. 2) indicates that exons 7A and 7B code for amino acid residues 242–292, while exons 8A and 8B code for residues 293–354. Similarities of the deduced amino acid sequences between exon 7B and the corresponding region of rat $G_o\alpha$-1 and $G_o\alpha$-2 are 88.5% and 98.1%, respectively. Those between exon 8B and rat $G_o\alpha$-1 and $G_o\alpha$-2 are 66.7% and 94.4%. Therefore, we conclude that $G_o\alpha$-2 containing exons 7B and 8B are generated by alternative splicing. Analysis of the nucleotide sequences at the splice junctions showed that all introns begin with the sequence G-T at the 5' end and end at the 3' end with A-G.

We have previously determined the organization of the human $G_i2\alpha$ and $G_i3\alpha$ (41) genes. Comparison of the organization of these genes with that of $G_o\alpha$ revealed that the intron junctions are perfectly conserved among the $G_i\alpha$ family and $G_o\alpha$. Also, organization of mouse $G_t1\alpha$ gene (42) is identical.

Characterization of the 5' Flanking Region. The 5' flanking region of the $G_o\alpha$ gene is highly G+C-rich and contains five G·C boxes, which are located at positions −1485, −1128, −1076, −943, and −824, whereas no typical TATA box was found. The cAMP-responsive element TGACGTCA is located at −899. Repeated sequence TCCTCCTCC(TCC) was found at −464 and −443. Similar sequences were found in the promoter region of the human epidermal growth factor receptor gene (43) and the human $G_s\alpha$ gene (12).

Characterization of the 3' Untranslated Region. The 3' untranslated region for $G_o\alpha$-1 is split into exon 8A and exon 9. In exon 9, two putative poly(A) addition signals are found at positions 1115–1120 and 1303–1308. Comparison of the nucleotide sequences of exon 9 with rat $G_o\alpha$-1 cDNA showed two highly conserved regions at positions 1–268 and 810–1277 with sequence identities of 93% and 83%, respectively.

Southern Blot Analysis of Human Genomic DNA. A 0.2-kb *Nco* I–*Sma* I fragment containing exon 1 and a 0.5-kb *Dra*

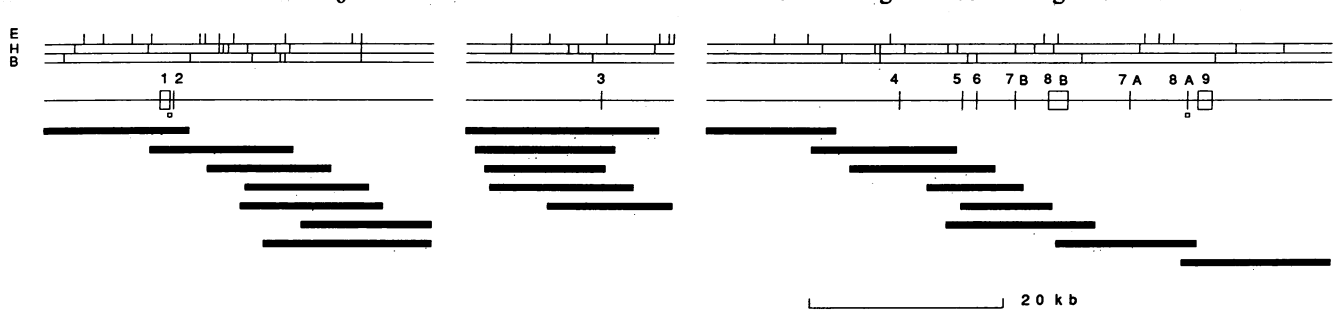

FIG. 1. Restriction map for human $G_o\alpha$ chromosomal gene. Inserts of 20 phage clones are shown by bars. Open boxes under exon 1 and exon 8A represent the DNA fragment used as probes for genomic Southern blotting. E, *Eco*RI; H, *Hin*dIII; and B, *Bam*HI.

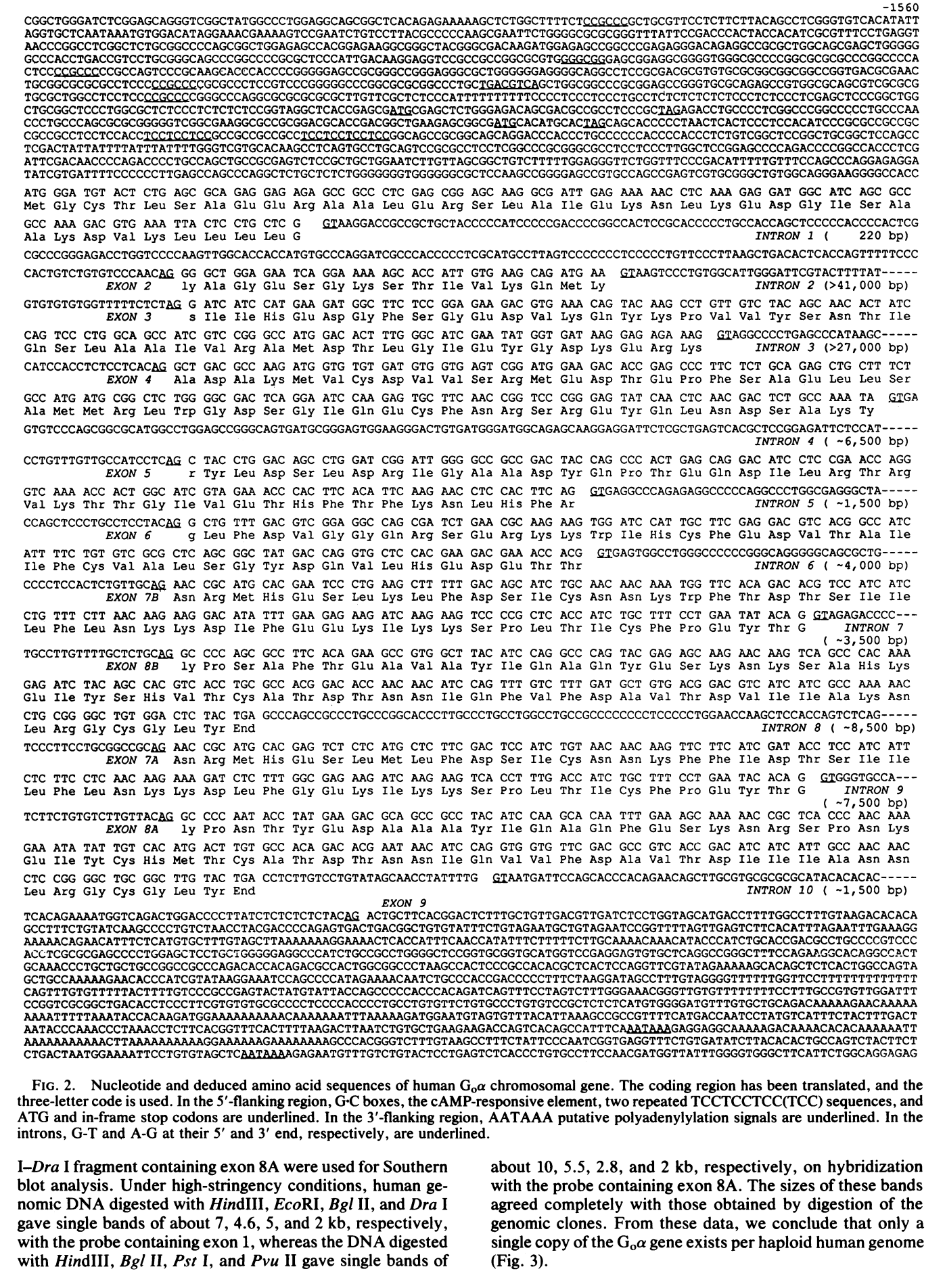

FIG. 2. Nucleotide and deduced amino acid sequences of human $G_o\alpha$ chromosomal gene. The coding region has been translated, and the three-letter code is used. In the 5′-flanking region, G-C boxes, the cAMP-responsive element, two repeated TCCTCCTCC(TCC) sequences, and ATG and in-frame stop codons are underlined. In the 3′-flanking region, AATAAA putative polyadenylylation signals are underlined. In the introns, G-T and A-G at their 5′ and 3′ end, respectively, are underlined.

I–*Dra* I fragment containing exon 8A were used for Southern blot analysis. Under high-stringency conditions, human genomic DNA digested with *Hin*dIII, *Eco*RI, *Bgl* II, and *Dra* I gave single bands of about 7, 4.6, 5, and 2 kb, respectively, with the probe containing exon 1, whereas the DNA digested with *Hin*dIII, *Bgl II*, *Pst* I, and *Pvu* II gave single bands of about 10, 5.5, 2.8, and 2 kb, respectively, on hybridization with the probe containing exon 8A. The sizes of these bands agreed completely with those obtained by digestion of the genomic clones. From these data, we conclude that only a single copy of the $G_o\alpha$ gene exists per haploid human genome (Fig. 3).

Northern Blot Hybridization. The results of RNA blot analysis with rat $G_o\alpha$-2 cDNA as a probe shows two bands of about 4 and 7 kb in various tissues (Fig. 4). The 4-kb transcript is most abundant in cerebrum, while in cerebellum a 7-kb transcript is the major species. The 4-kb band was also detected in heart, while the 7-kb transcript was seen in heart, lung, and spleen. Liver expresses weakly both 4-kb and 7-kb transcripts. No significant band was detected in skeletal muscle, adrenal gland, and adipose tissues.

DISCUSSION

In this study, we isolated two distinct rat $G_o\alpha$ cDNAs and a human genomic DNA for $G_o\alpha$ and determined their sequences. Studies on gene organization indicate that the human $G_o\alpha$ gene consists of at least 11 exons. The entire $G_o\alpha$ gene spans more than 100 kb. The positions of the splice junctions of the human $G_o\alpha$ gene were identical to those of the human $G_i2\alpha$, $G_i3\alpha$, and, possibly, $G_i1\alpha$ gene as well as to the mouse $G_t1\alpha$ gene. In *Drosophila*, the gene coding for a G_o-like protein has eight exons of which six junctions are identical with those of the human $G_o\alpha$ gene. However, the *Drosophila* $G_o\alpha$ gene lacks an intron corresponding to intron 2 of mammalian G_i/G_o genes. Also, there is an alternate exon 1 in the 5′-upstream region that results in two $G_o\alpha$-like proteins differing only in the N-terminal sequences.

The 5′ flanking region of $G_o\alpha$ gene is highly G+C-rich and contains several SP1 binding sites but no TATA box. Although these appear to be a characteristic feature of housekeeping genes, the expression of G_o is restricted to neuronal tissues. The promoter region of $G_o\alpha$ also contains the cAMP-responsive element. In neuroblastoma–glioma hybrid cells, the level of $G_o\alpha$ is elevated in the course of differentiation induced by dibutyryl-cAMP (44). Whether the increase in the G_o levels is due to stimulation of transcription mediated by the cAMP-responsive element remains to be studied.

Recently, there is accumulating evidence that indicates that there are at least two forms of $G_o\alpha$ in mammalian tissues. Furthermore, a G_o-like cDNA was cloned also from *Xenopus* oocytes (27). Its sequence is now found to be homologous with $G_o\alpha$-2 (Fig. 5). More recently, Strathmann *et al.* (39) have isolated cDNA clones encoding two forms of $G_o\alpha$ (designated $G_oA\alpha$ and $G_oB\alpha$) from a mouse brain library and

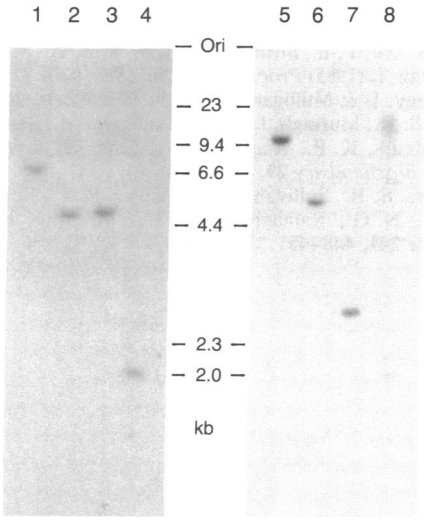

FIG. 3. Southern blot analysis of human genomic DNA. Ten micrograms of human genomic DNA was digested with *Hin*dIII (lanes 1 and 5), *Eco*RI (lane 2), *Bgl* II (lanes 3 and 6). *Dra* I (lane 4), *Pst* I (lane 7), and *Pvu* II (lane 8), separated by electrophoresis on a 0.8% agarose gel, and analyzed by blot hybridization to the human $G_o\alpha$ fragments containing exons 1 and 8A.

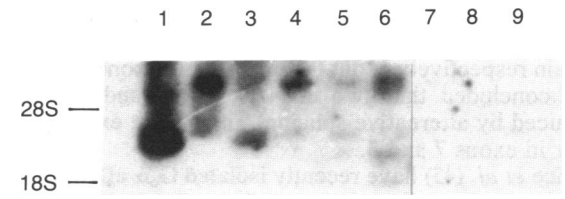

FIG. 4. Northern blot analysis of $G_o\alpha$-2 mRNA. Lanes: 1, cerebrum; 2, cerebellum; 3, heart; 4, lung; 5, liver; 6, spleen; 7, adrenal gland; 8, skeletal muscle; 9, adipose tissue.

suggested that they are probably produced by alternative splicing. Likewise, Hsu *et al.* (40) have reported the isolation of cDNA clones for two forms of $G_o\alpha$ (designated α_{o1} and α_{o2}) from HIT (hamster insulin-secreting tumor) cells.

We also have isolated two distinct rat $G_o\alpha$ cDNAs, one from C_6 glioma cells and the other from PC-12 cells, and have designated them $G_o\alpha$-1 and $G_o\alpha$-2, the former corresponding to $G_oA\alpha$, and latter, to $G_oB\alpha$. The structure of these two $G_o\alpha$ species differs only at the C-terminal regions—i.e., at regions coded by exons 7 and 8 (now referred to as exons 7A or 7B and 8A or 8B). More detailed studies described in the present paper showed that, in intron 6, there are two open reading frames highly homologous to exons 7A and 8A that are now

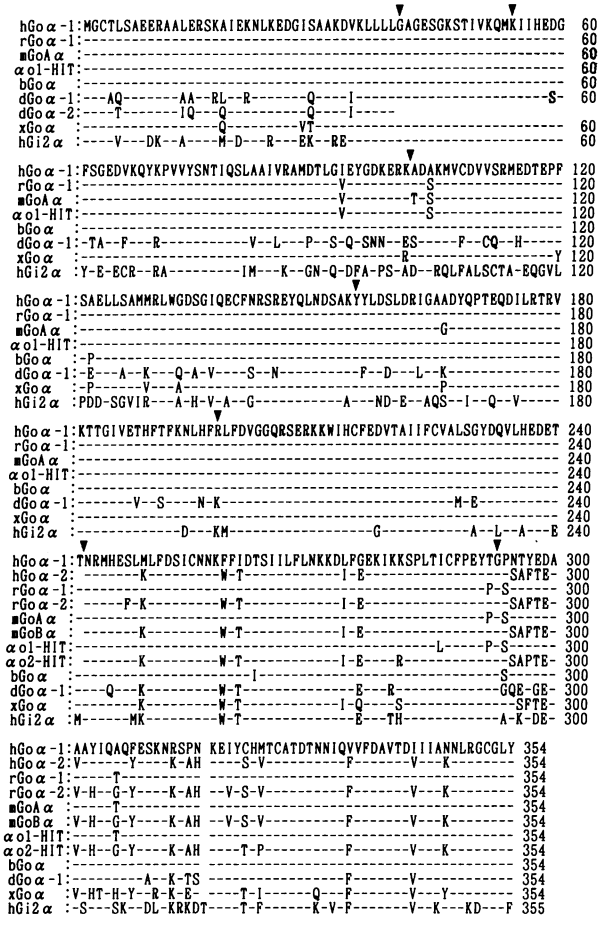

FIG. 5. Comparison of the deduced amino acid sequences of $G_o\alpha$ from various species. The deduced sequences of human (h) $G_o\alpha$-1 and $G_o\alpha$-2 as well as of rat (r) $G_o\alpha$-2 were determined in the present study. Other sequences and their references are: rat $G_o\alpha$-1 (15, 16), mouse (m) $G_oA\alpha$ and $G_oB\alpha$ (39), hamster HIT tumor α_{o1} and α_{o2} (40), bovine (b) $G_o\alpha$ (17, 18), *Drosophila* (d) $G_o\alpha$-1 and $G_o\alpha$-2 (30), *Xenopus* (x) $G_o\alpha$ (27), and human $G_i2\alpha$ (41). Identical amino acid residues are shown by hyphens. Solid arrowheads indicate locations at which introns of human $G_o\alpha$ occur.

referred to as 7B and 8B. Comparison of the sequences of these exons with those of $G_o\alpha$-1 and $G_o\alpha$-2 indicated that they contain respectively exons 7A and 8A and exons 7B and 8B. It is concluded that two cDNAs, $G_o\alpha$-1 and $G_o\alpha$-2, are produced by alternative splicing. They share exons 1–6 but differ in exons 7 and 8.

Price *et al.* (45) have recently isolated $G_o\alpha$ cDNAs having different 3′ noncoding sequences from a bovine retina cDNA library. This may suggest that another noncoding exon may be present downstream of exon 9. Since the regions coded by exons 7 and 8 correspond to the putative effector and receptor binding domains (46), it is possible that two G proteins produced from the single $G_o\alpha$ gene are functionally different.

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