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A small multigene family encodes G_i signal-transduction proteins

(guanine nucleotide-binding regulatory proteins/T-cell activation/molecular evolution)

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ABSTRACT **The guanine nucleotide-binding regulatory proteins known as G proteins are receptor-associated signal-transduction molecules that are implicated in the control of a variety of metabolic processes. Recent evidence suggests that G proteins may mediate B-lymphocyte responses to bacterial lipopolysaccharide and may also transduce signals from the T-cell antigen receptor. Since these receptors are uniquely expressed on lymphoid cells, we used molecular cloning strategies to ask whether lymphocytes contain specialized G-protein α subunits to assist in signal transduction. Comparison of our two deduced human α_i amino acid sequences with those previously determined for bovine and rodent G proteins permits the identification of three closely related but distinct types of α_i molecules that comprise a small multigene family. Using gene-specific probes, we found that both of our α_i genes are expressed in most cell types but in differing ratios. Our data support the view that a modest repertoire of extremely closely related G proteins mediates the transduction of signals derived from multiple different receptor molecules.**

Guanine nucleotide-binding regulatory proteins (G proteins) are receptor-associated $\alpha\beta\gamma$ oligomeric complexes that assist in signal transduction. In response to receptor–ligand interaction, the α chain of the G protein exchanges GTP for GDP, dissociates from the $\beta\gamma$ dimer, and is activated to transduce signals (1, 2). Subsequent GTP hydrolysis by the dissociated α chain permits regeneration of the inactive ternary complex (3). Some G proteins are expressed in a tissue-specific fashion; for example, retinal rods and cones each utilize unique G proteins (transducins) to regulate cGMP phosphodiesterase activity (4). Additional G proteins are widely distributed and are believed to regulate diverse physiologic processes including hydrolysis of phosphotidylinositol 4,5-bisphosphate by phospholipase C (5), secretion and chemotaxis in neutrophils (6, 7), and secretion in mast cells (8). Structural analyses indicate that the heterogeneity of G proteins is concentrated in the α chains (1, 2) and that the $\beta\gamma$ subunits are functionally interchangeable (9).

In lymphocytes, recent experiments suggest that G proteins may mediate signal transduction from the T-cell antigen receptor (10). Since these receptors are exclusively expressed in lymphoid cells, and since the known G-protein repertoire includes some molecules that are cell-type-specific, it seemed plausible that lymphocytes might utilize a unique set of G proteins.

To search for lymphocyte-specific G proteins we concentrated on defining the repertoire of α chains. By exhaustively screening human lymphocyte cDNA libraries with a probe derived from the bovine retinal G protein transducin (11), we obtained two types of α-chain clones.‖ One clone encodes the human homologue of previously described rodent inhibitory α-chain (α_i) molecules, whereas the second defines a novel α_i chain. Neither clone identifies a lymphocyte-specific transcript; however, the ratio of these different α_i transcripts varies in a tissue-specific fashion. Our data define a small multigene family of G_i proteins and suggest that multiple, distinct receptors may interact with a relatively small number of G-protein signal-transduction elements.

MATERIALS AND METHODS

cDNA Libraries and Library Screening. A human peripheral blood T-lymphocyte cDNA library (12), a library generated using RNA purified from the PEER T-cell line (13), a murine thymus cDNA library (14), and a human cDNA library made using fetal liver mononuclear cells (H. W. Schroeder, Jr., J. L. Hillson, and R.M.P., unpublished work) were screened by standard methods (15) using hybridization at 50°C for 24 hr to nick-translated (16) probes at specific activities of 10^8 cpm/μg and concentrations of 10^6 cpm/ml. Filters were washed in 0.3 M sodium chloride/0.03 M sodium citrate at 42°C for 4 hr prior to autoradiography using intensifying screens.

DNA Sequencing. All coding sequences were determined on both strands of pUC subclones (17) by use of oligonucleotide primers (18, 19).

RNA Blot Analysis. Total (20) or poly(A)$^+$ (21) RNA was fractionated by electrophoresis in denaturing formaldehyde/agarose gels, transferred to Nytran or nitrocellulose membranes (Schleicher & Schull), and analyzed as described (22).

RESULTS

Strategy for Identifying Human cDNA Clones Encoding α_i-Like Proteins. We initially screened a human fetal liver mononuclear cell cDNA library (1.3×10^5 primary recombinants) and a mouse thymus cDNA library (2×10^5 recombinants after one round of amplification) with a bovine transducin probe (11). From each of these libraries, we isolated clones encoding α_i-like proteins as determined by DNA sequencing of the subcloned inserts. A mixed probe consisting of coding-region fragments from one murine and one human α_i-like cDNA clone was used to screen both the PEER library and the human peripheral blood T-cell library in the manner described above (10^5 recombinants in each case), except that the filters were washed in 0.15 M sodium chloride/0.015 M sodium citrate at 50°C. With this protocol, we identified 24 human cDNA clones that were grouped into two classes.

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Abbreviation: G protein, guanine nucleotide-binding regulatory protein.

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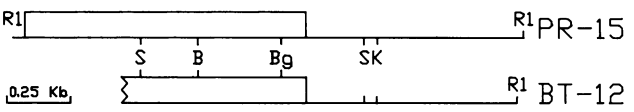
‖These sequences are being deposited in the EMBL/GenBank data base (Bolt, Barenek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession nos. J03004 and J03005).

Structure of α_i Proteins Expressed in Human Lymphocytes as Deduced from cDNA Clones. Fifteen of our cDNA clones were assigned to a single class represented by clones BT-12 (derived from the human fetal mononuclear cell library) and PR-15 (derived from the PEER cell line library). The composite sequence typified by the PR-15 clone contains 71 base pairs (bp) of 5′ untranslated region, a 355-codon open reading frame, and 550 bp of 3′ untranslated region terminating in a series of adenosine residues (Fig. 1). This poly(A) tract appears to result from internal priming of the cDNA by oligo(dT) and does not represent the 3′ extent of the mature mRNA (data not shown). The PR-15 nucleotide sequence is 73% identical to the bovine transducin probe over a 625-bp region (data not shown). Thus our low-stringency screening protocol was adequate to identify molecules sharing about 75% nucleotide identity.

Five cDNA clones were clearly derived from a second class of α_i-encoding transcripts in lymphocytes. Fig. 2 presents a partial restriction map of two representative clones of this class, PR-10 and PL-26, and the complete sequence compiled from overlapping phage inserts. The sequence includes 2 bp of 5′ untranslated region, an open reading frame

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  D G E K A A R E V K L L L L G A G E S G K S T I V  50
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  K Q M K I I H E D G Y S E E E C R Q Y R A V V Y S  75
AAGCAGATGAAGATCATCCACGAGGATGGCTACTCCGAGGAGGAATGCCGGCAGTACCGGGCGGTTGTCTACAGC
  N T I Q S I M A I V K A M G N L Q I D F A D P S R 100
AACACCATCCAGTCCATCATGGCCATTGTCAAAGCCATGGGCAACCTGCAGATCGACTTTGCCGACCCCTCCAGA
  A D D A R Q L F A L S C T A E E Q G V L P D D L S 125
GCGGACGACGCCAGGCAGCTATTTGCACTGTCCTGCACCGCCGAGGAGCAAGGCGTGCTCCCTGATGACCTGTCC
  G V I R R L W A D H G V Q A C F G R S R E Y Q L N 150
GGCGTCATCCGGAGGCTCTGGGCTGACCATGGTGTGCAGGCCTGCTTTGGCCGCTCAAGGGAATACCAGCTCAAC
  D S A A Y Y L N D L E R I A Q S D Y I P T Q Q D V 175
GACTCAGCTGCCTACTACCTGAACGACCTGGAGCGTATTGCACAGAGTGACTACATCCCCACACAGCAAGATGTG
  L R T R V K T T G I V E T H F T F K D L H F K M F 200
CTACGGACCCGCGTAAAGACCACGGGGATCGTGGAGACACACTTCACCTTCAAGGACCTACACTTCAAGATGTTT
  D V G G Q R S E R K K W I H C F E G V T A I I F C 225
GATGTGGGTGGTCAGCGGTCTGAGCGGAAGAAGTGGATCCACTGCTTTGAGGGCGTCACAGCCATCATCTTCTGC
  V A L S A Y D L V L A E D E E M N R M H E S M K L 250
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  F D S I C N N K W F T D T S I I L F L N K K D L F 275
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  E E K I T H S P L T I C F P E Y T G A N K Y D E A 300
GAGGAGAAGATCACACACAGTCCCCTGACCATCTGCTTCCCTGAGTACACAGGGGCCAACAAATATGATGAGGCA
  A S Y I Q S K F E D L N K R K D T K E I Y T H F T 325
GCCAGCTACATCCAGAGTAAGTTTGAGGACCTGAATAAGCGCAAAGACACCAAGGAGATCTACACGCACTTCACG
  C A T D T K N V Q F V F D A V T D V I I K N N L K 350
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  D C G L F Z                                     355
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GGAAGATGGGCAAGAAGATCACGCTCCCCGCCTGTTCCCCCGCCGCTTTTCTCCTCTTTCCTCTCTTTGTTCTCA
GCTCCCCCTGTCCCCTCAGCTCCAGACGTAGGGGAGGGGTTCGCACAGGCCTCCCTGTTTGAAGCCTGCCCTTGT
CTGAGATGCTGGTAATGGCCATGGTACCCTTCTGGCTTATGTTCTGGTTTTTAACATTGTCTTGTCTGTGTATGA
GGGAGGGGCACATGCTGTGATCTCCAAGGCTGCGTCTGGAGGGGCCCCTGCTTCTCCAGCCTGGACCCCCAGCTT
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GCGCGGGCCCCAAGGAAAAAGCACAAGAACGTGAGACGCCACCATTCCTGGAAACCACAGTCCACCTGCTCATTC
TCGTAGCTTTTTAAAAAAATAAAAAAAAAAAAAAAAAGAATTC
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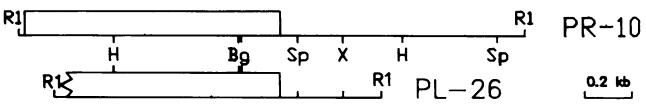
FIG. 1. Restriction map and nucleotide sequence of a human α_i-encoding cDNA. Partial restriction maps are shown for two representative cDNA clones derived from libraries generated using human fetal liver mononuclear cells (BT-12) or the PEER human T-cell line (PR-15). The presumed coding region is represented by an open bar. Restriction enzymes are B, *Bam*HI; Bg, *Bgl* II; K, *Kpn* I; Rl, *Eco*RI; S, *Sph* I. The conceptual translation product is presented above the nucleotide sequence in single-letter code (23). The arbitrary nucleotide sequence positions are numbered at the left; the codon positions are numbered at the right.

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GACGGGGAAAAAGCGGCCAAAGAAGTGAAGCTGCTGCTACTCGGTGCTGGAGAATCTGGTAAAAGCACCATTGTG
  K Q M K I I H E D G Y S E D E C K Q Y K V V V Y S  75
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  N T I Q S I I A I I R A M G R L K I D F G E A A R 100
AATACTATACAGTCCATCATTGCAATCATAAGAGCCATGGGACGGCTAAAGATTGACTTTGGGGAAGCTGCCAGG
  A D D A R Q L F V L A G S A E E G V M T P E L A G 125
GCAGATGATGCCCGGCAATTATTTGTTTTAGCTGGCAGTGCTGAAGAAGGAGTCATGACTCCAGAACTAGCAGGA
  V I K R L W R D G G V Q A C F S R S R E Y Q L N D 150
GTGATTAAACGGTTATGGCGAGATGGTGGGGTACAAGCTTGCTTCAGCAGATCCAGGGAATATCAGCTCAATGAT
  S A S Y Y L N D L D R I S Q S N Y I P T Q Q D V L 175
TCTGCTTCATATTATCTAAATGATCTGGATAGAATATCCCAGTCTAACTACATTCCAACTCAGCAAGATGTTCTT
  R T R V K T T G I V E T H F T F K D L Y F K M F D 200
CGGACGAGAGTGAAGACCACAGGCATTGTAGAAACACATTTCACCTTCAAAGACCTATACTTCAAGATGTTTGAT
  V G G Q R S E R K K W I H C F E G V T A I I F C V 225
GTAGGTGGCCAAAGATCAGAACGAAAAAAGTGGATTCACTGTTTTGAGGGAGTGACAGCAATTATCTTCTGTGTG
  A L S D Y D L V L A E D E E M N R M H E S M K L F 250
GCCCTCAGTGATTATGACCTTGTTCTGGCTGAGGACGAGGAGATGAACCGAATGCATGAAAGCATGAAACTGTTT
  D S I C N N K W F T E T S I I L F L N K K D L F E 275
GACAGCATTTGTAATAACAAATGGTTTACAGAAACTTCAATCATTCTCTTCCTTAACAAGAAAGACCTTTTTGAG
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  A Y I Q C Q F E D L N R R K D T K E I Y T H F T C 325
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  A T D T K N V Q F V F D A V T D V I I K N N L K E 350
GCCACAGACACGAAGAATGTGCAGTTTGTTTTTGATGCTGTTACAGATGTCATCATTAAAAACAACTTAAAGGAA
  C G L Y Z                                       354
TGTGGACTTTATTGAGAAGCATGGATGTTAGTGAAAGTTACTACAGTGTGGAGTGTTGAGACCAGACACCTTTTG
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AAGGTTTTTTCATCAAGAGAAGAATAACTTTACTAAATTTTATTTCTTTATTTGCAAAAGAATCTTTATTAAAAC
AAACAATCTTAACTATGAAAAAAAAAGCCGAATTC
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FIG. 2. Restriction map and nucleotide sequence of a second class of human α_i-encoding cDNA clones. Partial restriction maps are shown for two representative cDNAs derived from libraries generated using the human PEER T-cell line (PR-10) or human peripheral blood T lymphocytes (PL-26). Restriction sites are: Bg, *Bgl* II; H, *Hin*dIII; N, *Nco* I; Rl, *Eco*RI; Sp, *Sph* I; X, *Xba* I. Deduced amino acid sequence is shown in one-letter code above the nucleotide sequence.

of 354 codons, and 464 bp of 3′ untranslated region. A polyadenylylation site was not identified in PL-26.

Four additional short clones were obtained that did not contain any open reading frames (data not shown). We believe that all four of these clones were selected as a result of weak, fortuitous hybridization.

Expression of PL-26- and BT-12-Like Transcripts Is Not Restricted to Lymphoid Cells. To examine the tissue specificity of expression of our α_i-encoding cDNA clones, we isolated fragments from the distinct 3′ untranslated regions of each to serve as gene-specific probes. A 315-bp *Kpn* I–*Eco*RI fragment from BT-12 and a 357-bp *Sph* I–*Eco*RI fragment from PL-26 were each subcloned and used to probe RNA blots. Fig. 3 shows that 2.6-kilobase (kb) transcripts homologous with BT-12 are easily identified in RNA derived from human brain, kidney, peripheral blood mononuclear cells, T lymphocytes, monocytes, and neutrophils. Residual BT-12 probe was then stripped and the blots were reanalyzed using a PL-26-specific probe. As shown in Fig. 3, PL-26 detected transcripts in brain and kidney, though at slightly higher levels in kidney as compared with the BT-12 probe. In peripheral blood cells, PL-26-related transcripts are present in two size classes, 2.6 and 2.4 kb.

The Two Types of Human α_i Transcripts Are Differentially Expressed. Although most cell types contain transcripts of both the BT-12 and PL-26 types, the relative ratio of these transcripts varies substantially from tissue to tissue. In Fig.

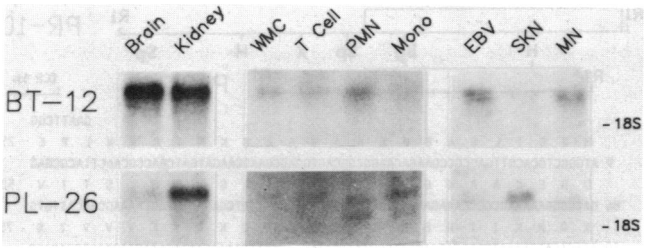

FIG. 3. Expression of α_i-encoding mRNA in human tissues and cell lines. Shown is an RNA blot analysis obtained using 5 μg of poly(A)$^+$ human brain and kidney RNA; 10 μg of total RNA from human whole mononuclear cells (WMC), purified T cells, neutrophils (PMN), or monocytes (Mono); and 6 μg of total RNA from an Epstein–Barr virus-transformed polyclonal B-cell population (EBV), the neuroblastoma SKN-BE2, or the Thayer human melanoma cell line (MN). Each blot was probed with a ^{32}P-labeled gene-specific sequence from BT-12 or PL-26 (see text for details). The positions of 18S ribosomal RNA markers are shown at right.

3, an Epstein–Barr virus-transformed polyclonal B-cell line, a neuroblastoma cell line (SKN-BE2), and a melanoma cell line are compared. Both the transformed B-cell line and the melanoma line contain relatively high levels of BT-12-like transcripts. For the neuroblastoma line, the situation is reversed, with PL-26-like transcripts being comparatively more abundant. Presumably this differential regulation of α_i-encoding mRNA levels reflects the different signal-transduction processes that occur in each cell type.

DISCUSSION

Considerable recent evidence implicates G proteins in the transduction of activating signals in lymphocytes. Nonhydrolyzable GTP analogues have been shown to rapidly activate ornithine decarboxylase in human peripheral blood T lymphocytes, thus mimicking an early feature of the activation sequence induced by T-cell receptor stimulation (24). In related experiments, Imboden *et al.* (10) demonstrated that exposure of a human T-cell line (Jurkat) to α_s-modifying cholera toxin abolished the increase in intracellular calcium concentration associated with stimulation of the T-cell antigen receptor. Similarly, a potential α_i-like target for pertussis toxin that coprecipitates with the T3 (CD3) component of the T-cell receptor has been preliminarily described (25). Pertussis toxin has also been shown to block the response of a B-cell line to lipopolysaccharide (26). In this report we have examined the G-protein repertoire in human lymphocytes by cloning cDNAs encoding G-protein α_i chains and have defined a small multigene family of α_i proteins.

To date, six distinct α chains have been described as components of five types of G proteins. These are G_s, the stimulatory G protein of adenylate cyclase (27); G_o, a G protein that may regulate calcium channels in the brain (28, 29); the rod and cone transducins (4); and two forms of G_i, previously believed to represent analogous adenylate cyclase inhibitory proteins defined in two different mammals (see below).

PR-15 cDNA Encodes Human α_{i2}. As shown in Fig. 4, the amino acid sequence deduced by conceptual translation of the PR-15 clone is nearly identical to those previously deduced from cDNAs derived from rat C6 glioma cells (28) and a mouse macrophage cell line (31), differing by six and eight residues, respectively, with only three nonconservative (23) substitutions. This extraordinarily high level of conservation strongly suggests that the three sequences are encoded by analogous genes. In accord with the nomenclature introduced by Gilman (1), we have designated these sequences α_{i2}. Our sequence is essentially identical to that recently determined for an α-chain transcript expressed in the human monocytoid line U937 (33), differing by a single silent transversion at the third position of codon 89.

Conservation of 3′ Untranslated Regions in α-Chain Sequences. Although most noncoding sequences in genes diverge at the rate of unselected DNA (34), it is striking that the 3′ untranslated regions of the α_{i2} transcripts from human and rodent sources are 74% identical (comparison not shown). Similarly, the human and rodent α_s 3′ untranslated regions are more than 90% identical though unrelated to those associated with other α-chain transcripts (35). This high degree of conservation of untranslated sequences implies that each confers some essential property upon the specific G-protein transcript, perhaps regulating mRNA stability. Similar conservation has been reported for the 3′ untranslated regions of a few other genes, notably the actin genes (36).

PR-10 cDNA Defines a Third Member of the α_i Gene Family. Another α_i sequence, termed α_{i1}, has been derived from a cDNA generated using bovine brain mRNA (32). Although this α_i sequence and the rodent α_i sequences were previously classified as products of analogous genes in different species, discordance between the rat protein sequence and the predicted sequence from the rat cDNA suggested that the rat genome contains at least two closely related α_i genes (1, 28). We therefore compared our PR-10-encoded sequence to that deduced for bovine α_{i1}.

Overall, the deduced amino acid sequence of PR-10 is 94% identical to the bovine α_{i1} sequence, with six nonconservative substitutions (Fig. 4). Although these sequences are therefore quite closely related, they are not nearly as similar as are the α_{i2} sequences just discussed. The human and rat α_{i2} sequences are 93% identical at the nucleotide level throughout their coding regions. In contrast, the PR-10 sequence is 75% identical with its human α_{i2} counterpart and only 79% identical with bovine α_{i1} (Table 1).

We used the method of Kimura (37) to estimate evolutionary distances between these homologous nucleotide sequences. In general, synonymous substitutions accumulate at a fairly constant rate irrespective of the genes compared (37). Thus estimation of the synonymous component of the frequency of third-codon-position substitutions (K_s) provides a measure of evolutionary distance (37). As shown in Table 1, the evolutionary interval between the human and rat α_{i2} sequences (K_s = 0.22) is much less than that separating the human α_{i2} and PR-10 sequences, indicating that the genes encoding PR-10 and α_{i2} had already appeared prior to the divergence of man and rat. In addition, pairwise comparison of the human α_{i2} or PR-10 sequences with bovine α_{i1} suggests that those genes are separated by comparable evolutionary intervals (K_s = 1.08 and 0.97, respectively; Table 1). From this analysis we conclude that the PR-10 transcript is almost certainly encoded by a gene distinct from both α_{i1} and α_{i2}, and here designated α_{i3}. Not surprisingly, in light of these comparisons, the 3′ untranslated regions of the bovine α_{i2} and the PR-10 clone are completely unrelated. Recently, Jones and Reed (38) have identified α_{i1}, α_{i2}, and α_{i3} transcripts in rat olfactory epithelium.

All three α_i molecules contain a site that is the likely target of pertussis toxin-mediated ADP-ribosylation, at Cys-352 (39, 40). An arginine at position 179 is in an appropriate sequence context for ADP-ribosylation by cholera toxin (41). Since the sequence requirements for sensitivity to cholera toxin are incompletely defined (1), we cannot predict which, if any, of these molecules would be affected by toxin treatment.

Sequence Heterogeneity Within the α_i Family. The deduced amino acid sequences of human α_{i2} and human α_{i3} are 86% identical, with 16 nonconservative changes. These differences are clustered in two regions spanning residues 82–143

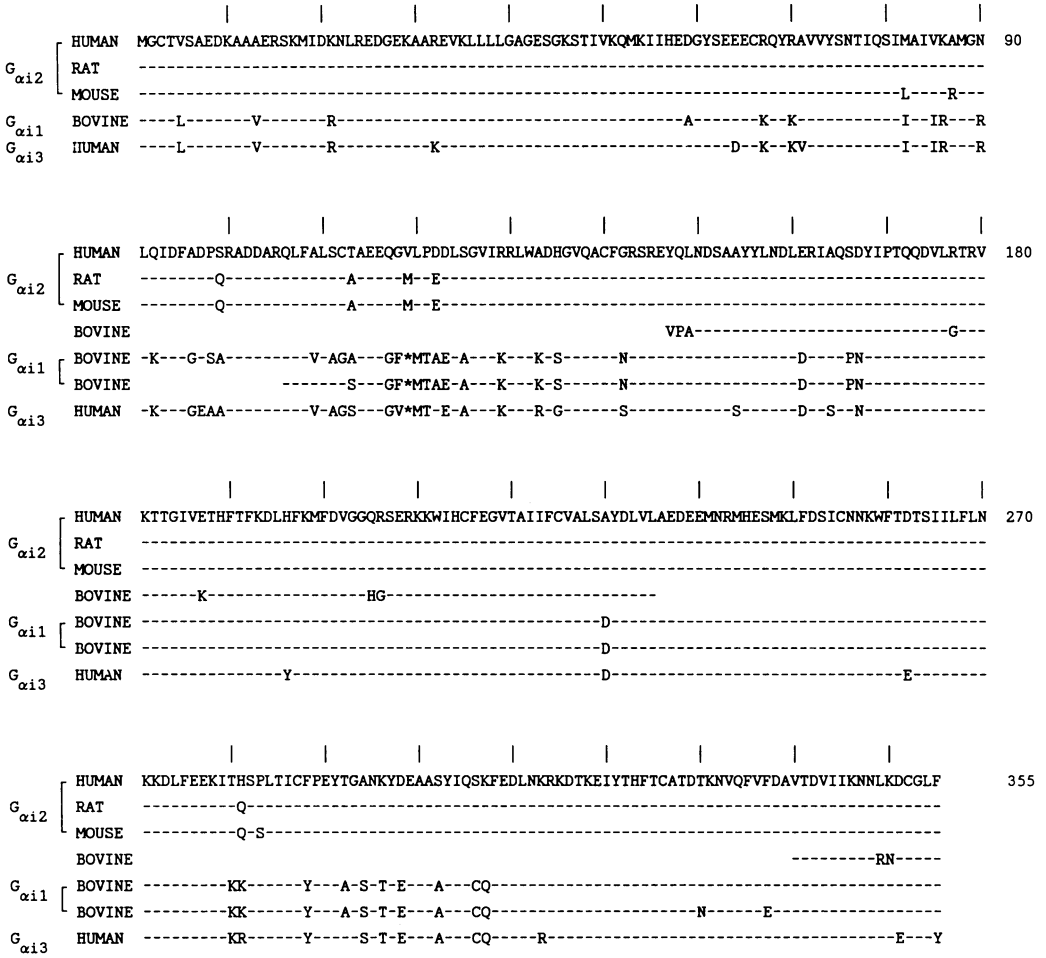

FIG. 4. Comparison of published α_i-chain sequences. Shown are the conceptual translation products deduced from cDNA clones for a series of mammalian α_i chains aligned with the human PR-15 (α_{i2}) sequence and presented in single-letter code (23). Positions identical with PR-15 are indicated by a dash; a single gap is marked with an asterisk. Analogous genes from different species are bracketed. Two partial α_i-like sequences from bovine pituitary have been described (30). One sequence is closely related to α_{i1}; the second partial sequence cannot be classified. Tissue sources and references are as follows: human T-cell α_{i2} (PR-15, this paper), rat glioma α_{i2} (28), mouse macrophage α_{i2} (31), bovine pituitary partial unclassified sequence (30), bovine brain α_{i1} (32), bovine pituitary partial α_{i1} (30), and human T-cell α_{i3} (PR-10, this paper).

and 281–308 (Fig. 4). By modeling the structures of the G-protein α chains on a template derived from the crystal structure of the distantly related bacterial elongation factor EF-Tu, these two regions of heterogeneity have been proposed to define, respectively, effector- and receptor-interaction "domains" (42). Interestingly, these "domains," thus defined, also encompass the positions that vary within a single α-chain class but between different species. If the α_{i2} chains in mouse, man, and rat all subserve the same function(s), it would appear that these domains are the only portions of the molecule that tolerate any substitutions. Since the different α_i classes clearly diverged before the mammalian radiation (Table 1), many of the sequence substitutions in these putative receptor and effector domains may have resulted from genetic drift in the relative absence of environmental selection. Hence α_i family members may be functionally interchangeable isoforms, products of gene-duplication events that have since diverged slowly.

At the same time, strong evolutionary conservation of the α_{i2} sequence between human and rat implies that all regions of these molecules are subjected to intense selection pressure. Thus we suspect that the α_{i1} and α_{i3} sequences will be similarly conserved and that the subtle differences between these molecules permit them to participate in distinct signal-transduction pathways. It follows that differences in α_i mRNA ratios may reflect the predominance of particular signal pathways in particular cell types.

Expression Pattern of α_i Transcripts. Although our α_{i2} and

Table 1. Evolutionary distances separating α_i sequences

Comparison	% homology	K_1	K_2	K_3	K_s
Human vs. rat α_{i2}	93	0.013 ± 0.007	0.003 ± 0.003	0.25 ± 0.03	0.22 ± 0.03
Human α_{i2} vs. bovine α_{i1}	70	0.13 ± 0.02	0.06 ± 0.01	1.23 ± 0.20	1.08 ± 0.16
Human α_{i2} vs. human PR-10	75	0.16 ± 0.02	0.08 ± 0.02	1.26 ± 0.24	1.12 ± 0.17
Human PR-10 vs. bovine α_{i1}	79	0.08 ± 0.02	0.03 ± 0.01	1.11 ± 0.17	0.97 ± 0.14
Human vs. rat presomatotropin		0.26 ± 0.04	0.18 ± 0.03	0.53 ± 0.07	0.44 ± 0.07

Percent homology was determined by directly aligning nucleotide sequences. A one-codon deletion at position 119 was presumed to have resulted from a single genetic event. K_1, K_2, and K_3 are evolutionary distances per nucleotide site for codon positions 1, 2, and 3, respectively, calculated according to the method of Kimura (37). The calculated divergence of the human and rat presomatotropins was taken directly from ref. 37. Values are means ± SD.

α_{i3} cDNA clones were derived from lymphocyte cDNA libraries, neither defines a lymphocyte-specific transcript. From RNA blot hybridization using the gene-specific BT-12 and PL-26 3′-untranslated-region probes, it is clear that the ratio of α_{i2} to α_{i3} transcripts does indeed vary from cell to cell (Fig. 3). In addition, the α_{i3} transcripts exist in two forms, 2.6 and 2.4 kb. Unfortunately, none of our α_{i3} cDNA clones are informative with regard to the difference between these two transcripts. T lymphocytes contain mainly the 2.6-kb α_{i3} mRNA, and hence our clones were probably derived from this larger transcript (Fig. 3 and data not shown). Sequence analysis of two bovine α_s cDNAs (43) and subsequently four human α_s cDNAs (35) suggests that alternative splicing may generate transcripts encoding different forms of the α_s protein. It is thus conceivable that the two forms of the α_{i3} mRNA also encode distinct proteins.

Form and Function in Mammalian G Proteins. The existence of tissue-specific G proteins, in particular the rod and cone transducins (4), has prompted speculation that the repertoire of G proteins may match that of the cell surface receptors with which they interact. We have demonstrated that the α_i proteins are encoded by a small multigene family. The hybridization strategy that we used was successful in identifying G-protein cDNAs with approximately 75% homology. Nevertheless, we cannot exclude the possibility that additional α-chain-encoding transcripts are present in lymphocytes. For example, α_s transcripts, though certainly present in T lymphocytes (31), were not detected in our analysis.

Leaving aside the question of the exact number of α-chain-encoding genes in the mammalian genome, our cDNA clones should provide the necessary tools to establish whether the closely related α_{i2} and α_{i3} molecules subserve similar functions or mediate distinct signal-transduction pathways in T lymphocytes. In addition, since differences among members of the α_i gene family have been conserved throughout mammalian evolution, introduction of defined mutations into these sequences will likely prove informative regarding the regulation of cell physiology by G-protein-mediated signal transduction.

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