Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 5587–5591, July 1991
Biochemistry

Gα16, a G protein α subunit specifically expressed in hematopoietic cells

(signal transduction)

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Contributed by Melvin I. Simon, March 8, 1991

ABSTRACT **Signal-transduction pathways mediated by guanine nucleotide-binding regulatory proteins (G proteins) determine many of the responses of hematopoietic cells. A recently identified gene encoding a G protein α subunit, Gα16, is specifically expressed in human cells of the hematopoietic lineage. The Gα16 cDNA encodes a protein with predicted M_r of 43,500, which resembles the G_q class of α subunits and does not include a pertussis toxin ADP-ribosylation site. In comparison with other G protein α subunits, the Gα16 predicted protein has distinctive amino acid sequences in the amino terminus, the region A guanine nucleotide-binding domain, and in the carboxyl-terminal third of the protein. Cell lines of myelomonocytic and T-cell phenotype express the Gα16 gene, but no expression is detectable in two B-cell lines or in nonhematopoietic cell lines. Gα16 gene expression is down-regulated in HL-60 cells induced to differentiate to neutrophils with dimethyl sulfoxide. Antisera generated from synthetic peptides that correspond to two regions of Gα16 specifically react with a protein of 42- to 43-kDa in bacterial strains that overexpress Gα16 and in HL-60 membranes. This protein is decreased in membranes from dimethyl sulfoxide-differentiated HL-60 cells and is not detectable in COS cell membranes. The restricted expression of this gene suggests that Gα16 regulates cell-type-specific signal-transduction pathways, which are not inhibited by pertussis toxin.**

Hematopoietic cells have the capacity to sense environmental stimuli and generate specific responses to infection and hemorrhage. Many of these responses are regulated by guanine nucleotide-binding regulatory proteins (G proteins), membrane-associated proteins that transduce signals from cell-surface receptors to intracellular effectors. The nucleotide-binding proteins within this class are heterotrimers, consisting of an α subunit that binds guanine nucleotides, a β subunit, and a γ subunit. A wide range of biochemical responses are regulated by G proteins, and a specific family of transmembrane receptors is proposed to interact with G proteins (for review, see refs. 1–3). The versatility of G protein-mediated signal transduction is reflected in the diversity of genes encoding the G protein subunits (4–5).

Hematopoietic cells are known to express several different G proteins. In neutrophils and monocytes, G proteins mediate the stimulation of phospholipid hydrolysis and the subsequent inflammatory response induced by chemotactic ligands (6, 7). These ligands trigger intracellular events that show different degrees of sensitivity to pertussis toxin (*Bordetella pertussis* islet-activating protein), a bacterial toxin that inhibits G protein function by ADP-ribosylating the α subunit of the heterotrimer (1, 8, 9). The complex patterns of signal transduction manifested by these cells suggest that they could result from the actions of multiple G proteins (5, 10, 11).

We have identified a G protein α subunit, Gα16, that is specifically expressed in human cells of hematopoietic lineage.*

MATERIALS AND METHODS

Molecular Cloning. Total RNA from HL-60 human promyelocytic leukemia cells was reverse-transcribed to cDNA, then amplified by means of PCR. Primers for the PCR were degenerate oligonucleotides corresponding to two regions highly conserved among G protein α subunits (12). The PCR products were cloned into the pGEM4z plasmid (Promega) and analyzed by DNA sequencing (13). The PCR fragment for Gα16 was radiolabeled and used to probe λ gt10 cDNA libraries by plaque hybridization (14). Gα16 cDNA clones were analyzed by DNA sequencing of both strands by using synthetic oligonucleotides as primers. The Gα16 predicted protein was aligned with other mammalian G protein α subunits by using the programs GAP, BESTFIT, and FASTA in the GCG sequence analysis package.

Cell Culture. Hematopoietic cell lines HL-60 (15, 16), KG-1 (17), KG-1a (18), KCL22 (19), THP-1 (20), and Raji (21), provided by H. P. Koeffler and M. C. Territo (University of California, Los Angeles, Department of Medicine), were maintained at a density of 5×10^4 to 10^6 cells per ml in RPMI 1640 medium/10% fetal bovine serum (GIBCO)/penicillin at 100 units/ml/streptomycin at 100 μg/ml/2 mM L-glutamine. Other cell lines were obtained from the American Type Culture Collection (Rockville, MD) and maintained as recommended (21–23). HL-60 cells (approximate passage 60) at a density of 3×10^5 were induced to differentiate by incubation with 1.3% dimethyl sulfoxide for 2–5 days (24). After 5 days of exposure, 80% of the cells reduced nitroblue tetrazolium (NBT) when stimulated with phorbol ester, and 50% of the cells reduced NBT after exposure to the chemotactic peptide fMet-Leu-Phe.

RNA Analysis. RNA was extracted using phenol and guanidium thiocyanate at 60°C (refs. 14 and 25, as modified by M. B. Raines, University of California, Los Angeles, Department of Medicine) and then purified by banding with cesium trifluoroacetate (Pharmacia). Samples containing 10–30 μg of total RNA were prepared as in ref. 26, separated by electrophoresis in a formaldehyde/agarose gel, transferred to Nytran filters (Bethesda Research Laboratories) by capillary blotting, and crosslinked by UV irradiation (Stratalinker, Stratagene). Filters were prehybridized and then hybridized at 42°C with ^{32}P-labeled cDNA probes ($1-2 \times 10^6$ cpm/ml) for 12–48 hr, in 50% (vol/vol) formamide/500

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Abbreviations: G protein, guanine nucleotide-binding regulatory protein; G_s and G_i, stimulatory and inhibitory G protein, respectively.
*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M63904).

mM sodium phosphate, pH 7.2/5% SDS/1% bovine serum albumin/1 mM EDTA (ref. 27, as modified by J. Lem, California Institute of Technology, Pasadena, CA). Probes were labeled by primer extension from random hexanucleotides to a specific activity of 5×10^8 to 10^9 cpm/μg (28). Filters were rinsed in 0.1–0.2× standard saline citrate (SSC)/0.1% SDS at 65°C and then exposed to x-ray film at −70°C with an intensifying screen. The level of RNA expression was quantitated by laser densitometry (LKB 2202 Ultroscan transmission laser densitometer) on autoradiograms in a linear range of exposure. RNA blots were stripped of probe by incubation in 0.01× SSC/0.1% SDS at 100°C for 20 min (1× SSC is 150 mM NaCl/15 mM sodium citrate, pH 7).

The Gα16 3′ untranslated region probe was derived from nucleotides 1180–1805 of the Gα16 cDNA; β-actin probe was derived as described in ref. 29; G protein β1 probe was derived from HL-60 cells (30). The G_{i2} probe was derived from the 3′ untranslated region of a human G_{i2} cDNA clone isolated from HL-60 cells and corresponded to nucleotides 1328–1664 (31).

Protein Analysis. HL-60 cells and COS cells were rinsed in phosphate-buffered saline and harvested by centrifugation at $1000 \times g$. Cells were suspended in TED-AP (10 mM Tris, pH 8/1 mM EDTA/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride/aprotinin at 50 kallikrein inhibitory units/ml/pepstatin at 0.7 μg/ml/leupeptin at 0.5 μg/ml) at 4°C and then lysed by five passes with a hand Dounce homogenizer (Kontes). Nuclei were pelleted by centrifugation at $500 \times g$ for 10 min; then the supernatant was centrifuged at $100,000 \times g$ for 30 min. The pellet (crude membranes) was resuspended in TED-AP, and protein content was determined (Bio-Rad protein assay). Samples containing 30–100 μg of membrane protein were resolved on discontinuous buffer SDS/polyacrylamide gels, electroblotted to nitrocellulose membranes, and probed with rabbit antisera (32).

Antisera to synthetic peptides were prepared as in ref. 32 and then affinity-purified by binding to an Affi-Gel 10 column (Bio-Rad) conjugated with the synthetic peptides, eluting with 3 M sodium thiocyanate, and dialyzing in 10 mM phosphate buffer.

Expression of Gα16 cDNA in *Escherichia coli*. The Gα16 cDNA clones were ligated and cloned into the vector pBluescript KS (Stratagene), and the construction was verified by sequencing of the ligation sites. A fragment that contained the complete coding sequence was subcloned into vector NpT7-5 (33), and the resulting plasmid was used for transformation of *E. coli* strain BL-21/DE3, a T7 lysogen in which the T7 polymerase is driven by the *lac* UV-5 promoter (34). For expression of the recombinant Gα16 protein, this bacterial strain (BL-21/Gα16) was grown at 30°C to an OD of 1.5, then isopropyl β-D-thiogalactoside was added to a final concentration of 0.3 mM, and incubation was continued for another 2 hr. Cells were harvested by centrifugation and stored at −70°C, then suspended in 10 mM Tris, pH 8/1 mM EDTA/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride, lysed with a French pressure cell, and centrifuged at $30,000 \times g$ for 1 hr. Bacterial proteins were analyzed by immunoblotting as above. More than 50% of the recombinant Gα16 protein remained in the supernatant fraction of the bacterial lysate.

RESULTS

Molecular Cloning of Gα16 cDNA. Amplification of HL-60 RNA by the PCR produced PCR fragments corresponding to the previously identified inhibitory (G_i) and stimulatory (G_s) α subunits G_{i2}, G_{i3}, G_s, and the human homologue of G_q (1, 31, 35, 36). A PCR fragment encoding another G protein α subunit, Gα16, was also isolated and subsequently used to screen HL-60 cDNA libraries. Plaques (6×10^5) from a cDNA library derived from HL-60 cells treated with cAMP were screened to isolate 25 cDNA clones including 22H (Fig. 1*B*). A 5′ region of 22H was used to isolate clone 5B from an undifferentiated HL-60 cDNA library. Oligonucleotide and cDNA probes from 5B were then used to screen 5×10^6 plaques to isolate cDNA clone 19A, which extends from the 5′ untranslated region of Gα16 to an *Eco*RI site at position 676.

The sequence of the Gα16 cDNA constructed from overlapping cDNA clones is shown in Fig. 1*A*, and significant features of the primary structure are highlighted in Fig. 1 *C* and *D*. The Gα16 cDNA encodes a protein with a predicted molecular mass of 43,500, which shows 40% amino acid identity with G_s and 45% identity with members of the G_i class of G proteins. Gα16 can be grouped with the G_q class of G proteins because it shares 57% amino acid sequence identity with Gα11 and G_q (36). The amino acid sequences that are conserved in the GTPase class of proteins and that have been implicated in guanine nucleotide binding are found in Gα16 (Fig. 1*C*, sequences denoted A, C, E, G, and I) (1, 37, 38). Also present is a conserved arginine (R) residue at position 186, which is homologous to the cholera toxin ADP-ribosylation site in G_s. Gα16 is not a predicted substrate for ADP-ribosylation by pertussis toxin because it lacks a cysteine residue four amino acids from the carboxyl terminus (1–5).

Gα16 is a G protein α subunit with distinctive features. The sequence in region A differs from other reported G proteins by the presence of a proline, rather than alanine or threonine, at amino acid 50 (Fig. 1 *C* and *D*; refs. 4 and 5). The sequence in this region, GPGESGK, has also been found in a murine G protein α subunit (Gα15), which is structurally related to Gα16 (T. Wilkie and M.I.S., unpublished data). The region A domain is important for GTP binding and for the endogenous GTPase activity of G proteins. Two G proteins with a divergent sequence in this region, G_z and G_q, have decreased ability to hydrolyze GTP and decreased guanine nucleotide binding in comparison with G_s and G proteins of the G_i class (39, 40). Other domains known to be important to GTPase activity, region C and the region adjacent to Arg-186, are conserved in Gα16 (1, 37).

The Gα16 predicted protein includes two inserts of seven amino acids in the carboxyl terminal region, when optimally aligned with other G protein α subunits (Fig. 1*D*). The first insert (amino acids 317–323) is similar to the corresponding region of G_s. The second insert region (amino acids 331–337) includes several charged amino acids and is not homologous with other G protein α subunits. These inserts are in a domain that has been proposed to determine the interaction of G proteins with effectors (38, 41). The primary structure of G protein α subunits varies widely in this region, which is the site of alternative splicing in G_o, and in G_s includes an exon that is not homologous with other G protein genes (42–44).

The amino-terminal region of the Gα16 predicted protein diverges from the sequence of other reported G protein α subunits (Fig. 1*D*). The initiation methionine is in the context of the consensus sequence for translational initiation proposed by Kozak (45) and is preceded by a termination codon in the same reading frame (bases −13 to −15). In contrast with G proteins of the G_i class, Gα16 is not a predicted substrate for modification by N-myristoylation, as it lacks a glycine at position 2 (46).

These features of the Gα16 sequence imply that the Gα16 protein could manifest specific properties in signal transduction. To further explore the function of Gα16, we examined the pattern of expression of the Gα16 gene in a variety of human cells.

Expression of Gα16 in Human Cell Lines. The Gα16 gene is expressed in a lineage-specific pattern in human cell lines with the phenotype of hematopoietic cell precursors (Fig. 2).

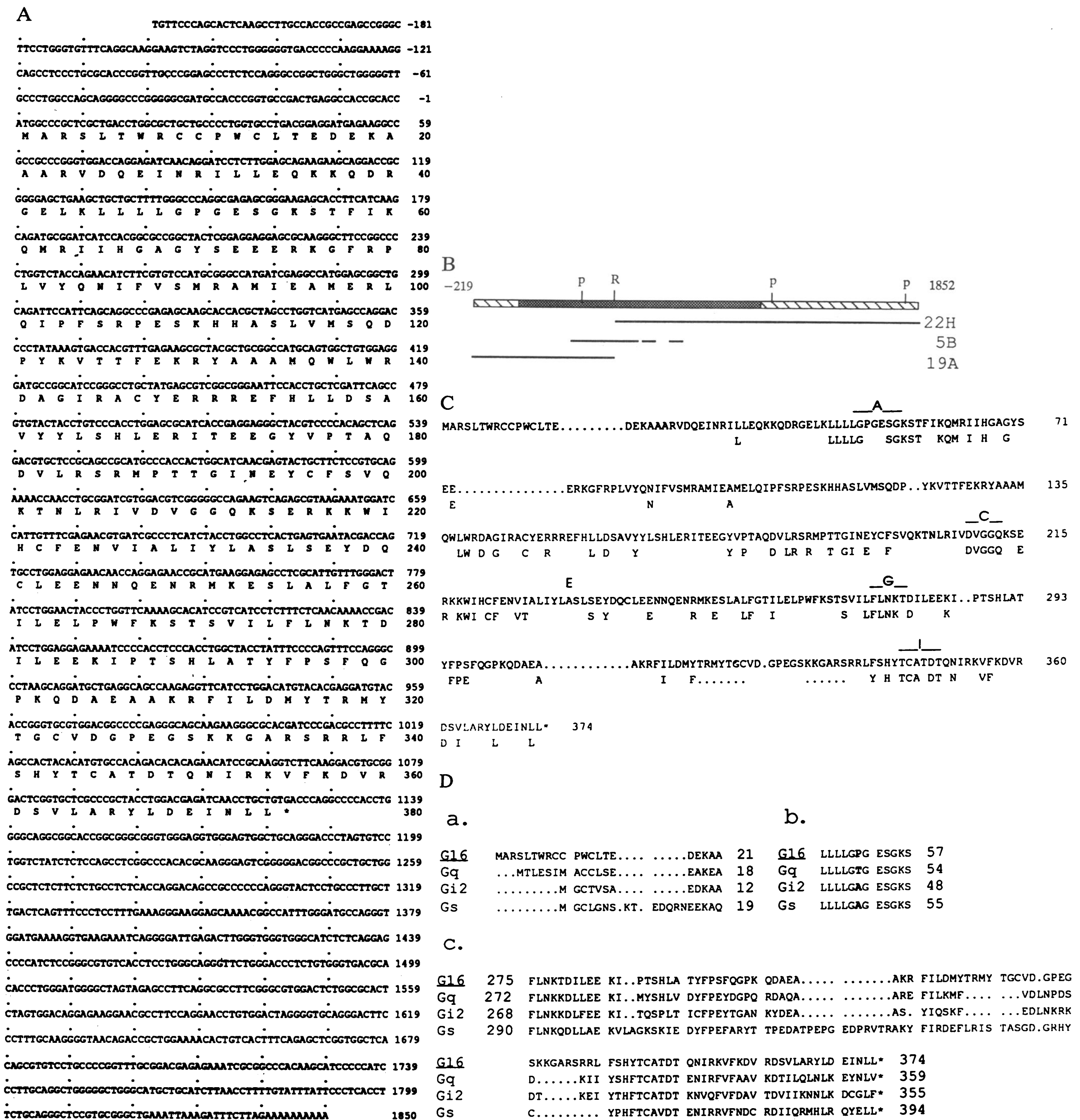

FIG. 1. Sequence of Gα16 cDNA and features of Gα16 predicted protein. (*A*) Nucleotide sequence of Gα16 cDNA showing predicted protein sequence. (*B*) Gα16 cDNA clones aligned with the complete Gα16 cDNA. Stippled region is coding sequence; hatched bars are 5′ and 3′ untranslated regions. P, *Pst* I; R, *Eco*RI. (*C*) Sequence of the Gα16 predicted protein. This sequence is compared with the consensus amino acid sequence derived from other reported mammalian G protein α subunits (second row). Guanine nucleotide-binding domains A, C, E, G, and I are marked (4, 37). (*D*) Significant structural features of Gα16 predicted protein. Gα16 is compared with the amino-terminal region (*a*); region A guanine nucleotide-binding domain (*b*); and carboxyl-terminal region (*c*) of G proteins representative of each G protein class.

A 2.2-kilobase (kb) Gα16 transcript is evident on Northern (RNA transfer) blots of RNA from human myeloid leukemic cell lines HL-60 (16), KG-1 (17), KG1a (18), and KCL22 (19). Gα16 expression was also seen in T-cell lines CEM and Molt-4 (21) and at lower levels in human erythroleukemia cell line HEL (23) and in the monocytic cell line THP-1 (20). In contrast, no Gα16 expression was detected in RNA from a human fibrosarcoma cell line (HT1080), adrenal carcinoma cell line (SW13), or in 13 other nonhematopoietic cell lines examined by Northern blotting (Fig. 2). Human B-cell lines Raji and IM-9 also do not express Gα16 (21). K-562, a chronic myelogenous leukemia cell line with erythroid and megakaryocytic differentiation potential similar to that of HEL, does not express Gα16 (22).

The highest levels of expression of Gα16 were found in committed myeloid precursor cells of promyelocytic (HL-60) or myeloblast (KG-1) phenotype. To further define Gα16 expression in the myeloid lineage, we prepared RNA from

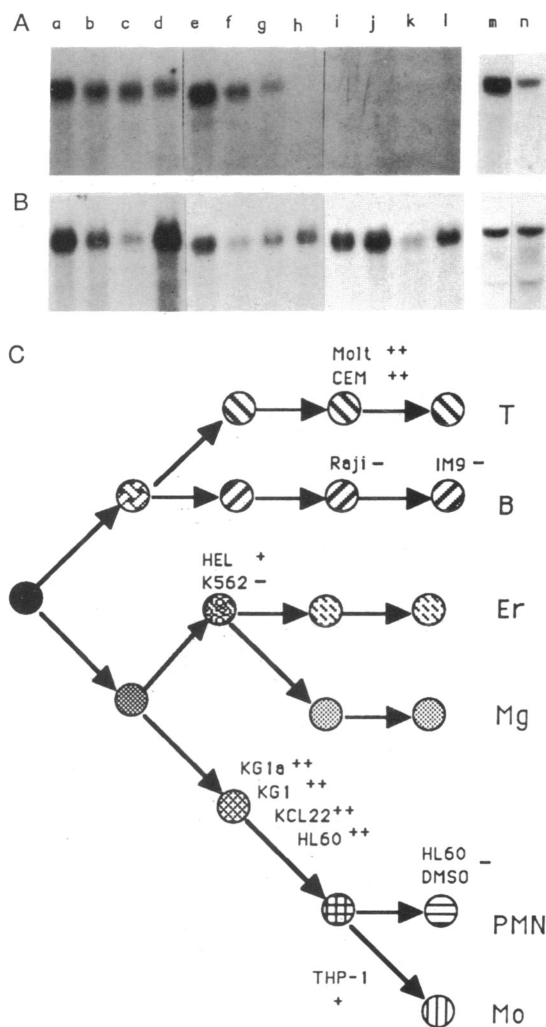

FIG. 2. Gα16 RNA expression in human cell lines. (A) Northern blot generated with 10 μg of RNA from cells as listed for the following lanes: a, KG-1a; b, KG-1; c, HL-60; d, KCL22; e, CEM; f, Molt-4; g, HEL; h, K562; i, HT1080; j, SW13; k, Raji; and l, IM-9. Lanes m and n are from a separate Northern blot generated with 30 μg of RNA from cells HL-60 and THP-1, respectively. The blots were hybridized with a cDNA probe derived from the 3′ untranslated region of Gα16 (A), then stripped and hybridized with human β actin probe (B, lanes a–l) or a probe derived from the human β1 G protein subunit (B, lanes m and n). No expression of Gα16 was seen in Northern blots generated with 15–30 μg of RNA from WiDR or Co115 colon adenocarcinoma cells, RD or HT117 rhabdomyosarcoma cells, HT161 osteosarcoma cells, Y79 retinoblastoma cells, LAN-1 or HT230 neuroblastoma cells, A875 or HS294 melanoma cells, A172 glioblastoma cells, Ad12-transformed fetal retinal cells, or MRC-5 diploid human fibroblasts. These RNA samples all showed abundant expression of β-actin. (C) Expression of Gα16 in human hematopoietic cell lines. Phenotypes of cell lines representative of each lineage are described in refs. 15–23. T, T lymphocyte; B, B lymphocyte; Er, erythroid; Mg, megakaryocytic; PMN, neutrophil; Mo, monocyte (14). Cell lines were graded for degree of expression of Gα16 as −, +, or ++. DMSO, dimethyl sulfoxide.

HL-60 cells induced towards neutrophil differentiation with dimethyl sulfoxide (24). In these terminally differentiated cells, the expression of Gα16 RNA was decreased to 10.5% (± 3.5% SEM for four separate differentiation experiments) of the level seen in undifferentiated cells. In contrast, expression of the α subunit of G_{i2} increased with differentiation by 160% (± 8% SEM for three experiments) relative to levels in undifferentiated cells (Fig. 3). These results suggest that the G_{i2} and Gα16 genes are divergently regulated in individual HL-60 cells when this clonal population of cells is induced to differentiate.

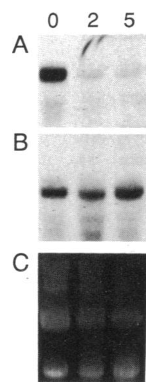

FIG. 3. G protein expression in HL-60 cells differentiated towards neutrophils. Autoradiogram of Northern blot of 30 μg of total RNA from undifferentiated HL-60 cells (lane 0), HL-60 cells exposed to 1.3% dimethyl sulfoxide for 2 days (lane 2), HL-60 cells exposed to 1.3% dimethyl sulfoxide for 5 days (lane 5). Blot was probed with Gα16 (A), then stripped, and reprobed with G_{i2} 3′ untranslated probe (B). (C) Photograph of ethidium bromide-stained RNA gel before transfer.

Gα16 Protein. To study expression of the Gα16 protein, we generated antisera against synthetic peptides identical to regions of the Gα16 predicted protein. Two peptides were studied: peptide D3, corresponding to residues 327–344 of Gα16 (GSKKGARSRRLFSHYTC), and peptide CT55 (C-RDSVLARYLDEINLL), which corresponds to the carboxyl terminus of Gα16. Rabbit antisera to these peptides were affinity purified and shown to react with recombinant Gα16 overexpressed in the bacterial strain BL21/Gα16, specifically recognizing a protein of 42–43 kDa on immunoblots (Fig. 4). Both antisera also recognized a protein band of 42–43 kDa in immunoblots of membrane fractions from undifferentiated HL-60 cells. The levels of this protein were decreased in membrane fractions from HL-60 cells induced to differentiate by exposure to dimethyl sulfoxide for 5 days and were undetectable in COS cell membrane fractions (Fig. 4). Reactivity of each antiserum with the 42- to 43-kDa protein was inhibited by preincubation of antisera with the immunizing peptide. The fact that two antisera derived from distinct regions of the Gα16 protein specifically reacted with a common protein in HL-60 membranes, which was identical in size with recombinant Gα16, suggests that this 42- to 43-kDa protein is Gα16.

DISCUSSION

The restricted expression of the Gα16 gene suggests that Gα16 plays a role in signal-transduction pathways that are specific to myelomonocytic cells and T cells. The Gα16 expression pattern is unique among G proteins. Other G

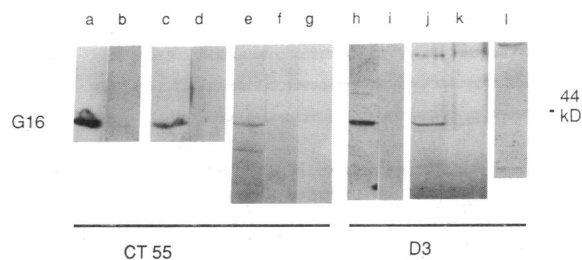

FIG. 4. Expression of Gα16 protein. Immunoblots were probed with affinity-purified antisera to synthetic peptides. Lanes: a, b, h, and i, bacterial strain BL21/Gα16 induced isopropyl with β-D-thiogalactoside (50 μg of protein); c, d, e, and j, HL-60 membranes (100 μg of protein); f and k, membranes from dimethyl sulfoxide-differentiated HL-60 cells (100 μg of protein); g and l, COS cell membranes, 100 μg. Lanes a–g were probed with a 1:200 dilution of antiserum CT55; lanes h–l were probed with a 1:100 dilution of antiserum D3. Lanes b and d, antibody reaction was blocked by preincubation of antiserum with peptide CT55 at 10 μg/ml. Lane i, antibody reaction was blocked by preincubation of antiserum with peptide D3 at 5 μg/ml.

protein α subunits that are present in HL-60 cells and in mature myeloid cells, G_{i2}, G_{i3}, G_s, and G_q, are also found in nonhematopoietic cells and a variety of tissues (1, 31, 35, 36). Several G protein α subunits are known to change in expression with differentiation of mammalian cells (47–49). However, the Gα16 gene must be subject to additional regulatory processes. Gα16 is expressed only in hematopoietic cells. Within the myeloid lineage, Gα16 expression is further specified, as it is down-regulated during terminal differentiation of HL-60 cells induced by dimethyl sulfoxide. The pattern of Gα16 expression in other myeloid differentiation pathways has not yet been defined. In lymphoid cells, Gα16 expression is again lineage-specific. T-cell lines express Gα16 RNA at high levels, whereas Gα16 expression was not detected in two B-cell lines with lymphoblast (Raji) or plasmacytoma (IM9) phenotypes.

In HL-60 cells, a change in G protein gene expression and in G protein repertoire parallels a coordinated developmental change. Induction of terminal differentation by incubating these cells with dimethyl sulfoxide is associated with an increase in expression of the G_{i2} α subunit and a decrease in expression of Gα16. This occurs as cells acquire specific signal-transduction systems and morphological features characteristic of neutrophils while losing the capacity to proliferate and to differentiate to monocytes or eosinophils (15, 24). The pattern of G protein gene expression in this cell line may indicate that Gα16 is involved in regulating signal-transduction processes that are not active in neutrophils but are manifested in other classes of leukocytes or in hematopoietic precursor cells.

The protein encoded by the Gα16 gene lacks a pertussis toxin ADP-ribosylation site. This fact is of special interest because of the increasing appreciation of G protein-mediated phenomena that are not inactivated by pertussis toxin (1–5). Mature cells of the lineages that express Gα16 manifest specific signal-transduction pathways that have been the subject of intense study (6–11, 50, 51). Gα16 could regulate pertussis toxin-resistant signaling processes in these cells (8, 9, 52–54). The pattern of Gα16 expression is also consistent with a role in hematopoietic cell proliferation or differentiation. The cell lines that express Gα16 are capable of indefinite proliferation and have the potential to develop along several pathways. These cell lines are similar in phenotype to normal hematopoietic progenitor cells and in certain cases can be induced to differentiate *in vitro* into cells with the properties of normal leukocytes (15–23). These attributes of the hematopoietic system will allow definitive studies on the regulation of the Gα16 gene and the role of the Gα16 protein in the physiology of myeloid cells and T cells.

We are grateful to J. Didsbury, Duke University Medical Center, for providing a cDNA library; to H. P. Koeffler and to M. C. Territo, University of California, Los Angeles, Department of Medicine, for providing cell lines; and to M. Linder, University of Texas Southwestern Medical Center, for providing plasmid NpT7-5. We thank T. Wilkie and M. Strathmann for communicating unpublished data, and we thank B. Birren, T. Wilkie, and N. Gautam for a critical review of the manuscript. This work was supported by a Clinical Investigator Award from the National Cancer Institute (to T.T.A.) and by grants from the National Institutes of Health to M.I.S.

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