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Characterization of G-protein α subunits in the G_q class: Expression in murine tissues and in stromal and hematopoietic cell lines

(heterotrimeric GTP-binding protein/signal transduction/multigene family/phospholipase C)

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ABSTRACT **Murine Gα14 and Gα15 cDNAs encode distinct α subunits of heterotrimeric guanine nucleotide-binding proteins (G proteins). These α subunits are related to members of the G_q class and share certain sequence characteristics with $G\alpha_q$, Gα11, and Gα16, such as the absence of a pertussis toxin ADP-ribosylation site. Gα11 and $G\alpha_q$ are ubiquitously expressed among murine tissues but Gα14 is predominantly expressed in spleen, lung, kidney, and testis whereas Gα15 is primarily restricted to hematopoietic lineages. Among hematopoietic cell lines, Gα11 mRNA is found in all cell lines tested, $G\alpha_q$ is expressed widely but is not found in most T-cell lines, Gα15 is predominantly expressed in myeloid and B-cell lineages, and Gα14 is expressed in bone marrow adherent (stromal) cells, certain early myeloid cells, and progenitor B cells. Polyclonal antisera produced from synthetic peptides that correspond to two regions of Gα15 react with a protein of 42 kDa expressed in B-cell membranes and in *Escherichia coli* transformed with Gα15 cDNA. The expression patterns that were observed in mouse tissues and cell lines indicate that each of the α subunits in the G_q class may be involved in pertussis toxin-insensitive signal-transduction pathways that are fundamental to hematopoietic cell differentiation and function.**

G proteins mediate signal transduction between ligand-bound seven-transmembrane-segment receptors and intracellular effectors, such as phospholipases, adenylyl cyclase, and ion channels (1–3). G proteins are heterotrimeric, composed of α, β, and γ subunits. The α subunit binds guanine nucleotide and is believed to confer receptor and effector specificity. The β and γ subunits are also required for receptor interaction and can regulate effector function (4). To explore the diversity of G-protein-mediated signal-transduction pathways, we developed an approach utilizing the PCR (5) to identify novel cDNA clones that share certain highly conserved amino acid sequences common to all G-protein α subunits. We identified cDNA clones corresponding to 8 previously uncharacterized α subunits (6–9) among a total of 16 distinct proteins expressed in mammals. Additional screens revealed several α subunits from *Drosophila* (7), *Dictyostelium* (10), *Neurospora* (K. Borkovitch, G. Turner and M.I.S., unpublished observation), and *Arabidopsis* (11). Sequence comparison of all known G proteins indicated that at least four classes of related α subunits were expressed in mammals, termed G_s, G_i, G12, and G_q (3), that were also found in other animal phyla. These classifications appear to have functional relevance with respect to effector specificity; for example, α subunits of the G_s class, such as *Drosophila* and rat $G\alpha_s$ and rat $G\alpha_{olf}$, activate adenylyl cyclase (9, 12), whereas most α subunits of the G_i class are substrates for ADP-ribosylation by pertussis toxin (PTX) and members of the G_q class were found to activate phospholipase C (PLC) β (13, 14).

In this paper we present the cDNA sequence[§] of two murine α subunits, Gα14 and Gα15, that are members of the G_q class of G proteins (7). The G_q class of α subunits probably regulates an array of PTX-insensitive signal-transduction pathways (15). The two previously reported members of this class, $G\alpha_q$ and Gα11, are expressed in all murine tissues, whereas Gα14 expression is more restricted and Gα15 is primarily expressed in hematopoietic cell types.

MATERIALS AND METHODS

PCR. PCR amplification (16) of randomly primed cDNA with the degenerate oligonucleotides oMP19, -20, -21, and -41 (5) was done in a Perkin–Elmer/Cetus thermal cycler (denatured at 94°C for 1 min, annealed at 40°C for 1 min 30 sec, and extended at 72°C for 2 min; 35 cycles). PCR with gene-specific oligonucleotides was done in an Ericomp thermal cycler cooled by water circulated through an ice/water bath [denatured at 93°C for 10 sec, annealed at 65°C (Gα15) or 55°C (Gα11, $G\alpha_q$, and Gα14) for 15 sec, and extended at 72°C for 40 sec; 25 cycles]. The gene-specific oligonucleotides used were CT25 (5′-GGGGTAGGTGATGATTGTGCG-3′), CT106 (5′-CTCGCTTAGTGCCACC-3′), CT107 (Fig. 1*A*), CT116 (Fig. 1*B*), CT117 (Fig. 1*B*), CT118 (Fig. 1*B*), CT121 (Fig. 1*B*), CT133 (Fig. 1*B*), CT144 (5′-GTGGGCCGC-TCTAGGCACCA-3′), CT145 (5′-CTGAAGTACCCCAT-TGAACAT-3′), CT146 (5′-TGGCCTTAGGGTGCAGG-GGG-3′), CT165 (5′-ATCTTCACGGCCATGCAGGC-CATG-3′), CT167 (Fig. 1*A*), CT168 (Fig. 1*A*), GQ4 (5′-ATTCGCTAAGCGCTACTAGA-3′), GQR (5′-AGTGGGGACACAAACTCTAAGCA-3′), and oMP38 (5′-GTAGCCGACCCTTCCTATCT-3′). Each oligonucleotide was used in the PCR at 10 ng/μl with 100 ng of randomly primed cDNA in a 10-μl reaction volume. PCR buffer contained 1.5 mM MgCl, 40 mM NaCl, 10 mM Tris (pH 8.8), and 0.01% gelatin. *Thermus aquaticus* (Taq) DNA polymerase was supplied by Cetus.

RNA Expression Analysis by PCR. Randomly primed cDNA prepared from total RNA of various tissues and cell lines was amplified by PCR using the degenerate oligonucleotides oMP20 and oMP21 combined with either oMP19 or oMP41. In separate reactions, gene-specific oligonucleotides were used to assay Gα11 (CT165 and CT25), $G\alpha_q$ (oMP38 and GQ4), Gα14 (CT167 and CT168), or Gα15 (CT133 and

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Abbreviations: PTX, pertussis toxin; PLC, phospholipase C; ES cells, embryonic stem cells.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M80631 and M80632).

CT116). Conditions of the PCR were held within the range of quantitative amplification to provide a relative measure of RNA content in the various samples. β-Actin primers (CT144 and CT146, 0.2 ng each) were included to provide an internal control of the PCR and did not affect amplification of the Gα cDNAs. The PCR products were blotted to GeneScreen (DuPont) and hybridized with radiolabeled oligonucleotides (17) specific to Gα11 (CT25), $G\alpha_q$ (GQR), Gα14 (CT107), or Gα15 (CT117) in 5× Denhardt's solution/0.75 M NaCl/50 mM sodium phosphate/5 mM EDTA/0.5% SDS at 42°C for ≥4 hr. Blots were washed as described (7). Films were exposed for several hours with intensifying screens at −70°C.

Northern and Western Analysis. Northern blots were hybridized with radiolabeled probes for Gα14 [nucleotides (nt) 1317–poly(A) (Fig. 3*A*)] or Gα15 [nt 68–600 (Fig. 3*B*) or nt 1098–poly(A) (data not shown)], washed three times (30 min each) in 5 mM Tris, pH 7.4/0.1 mM EDTA/0.5% SDS at 60°C. Western blots were prepared with reagents and procedures as described (18).

Cell Culture. All B-cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and 50 μM 2-mercaptoethanol (19–21). NIH 3T3, RS42, and the stromal cell line S17 (22) were maintained in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. All cell lines were grown to a density of 10^6 cells per ml prior to isolation of RNA and protein.

RESULTS

Nucleotide Sequence of Gα14 and Gα15. Gα14 and Gα15 were identified as unique Gα subunits based on the DNA and corresponding amino acid sequence of the cloned PCR fragments that were amplified from mouse spermatid cDNA between the degenerate oligonucleotide primers oMP19, -20, and -21 (Fig. 1; ref. 5). PCR fragments of Gα14 and Gα15 were subsequently used to screen lung and spleen cDNA libraries, respectively. The cDNA sequence of Gα14 (Fig. 1*A*) was obtained from three independent and overlapping clones. The sequence of Gα15 (Fig. 1*B*) was obtained from two overlapping cDNA clones that were isolated from independent libraries and several independently derived PCR products. The sequence of the two cDNA clones was identical in the overlapping region, except for a 162-nt insert in clone 781-1 at nt 745 that was produced by alternative splicing (data not

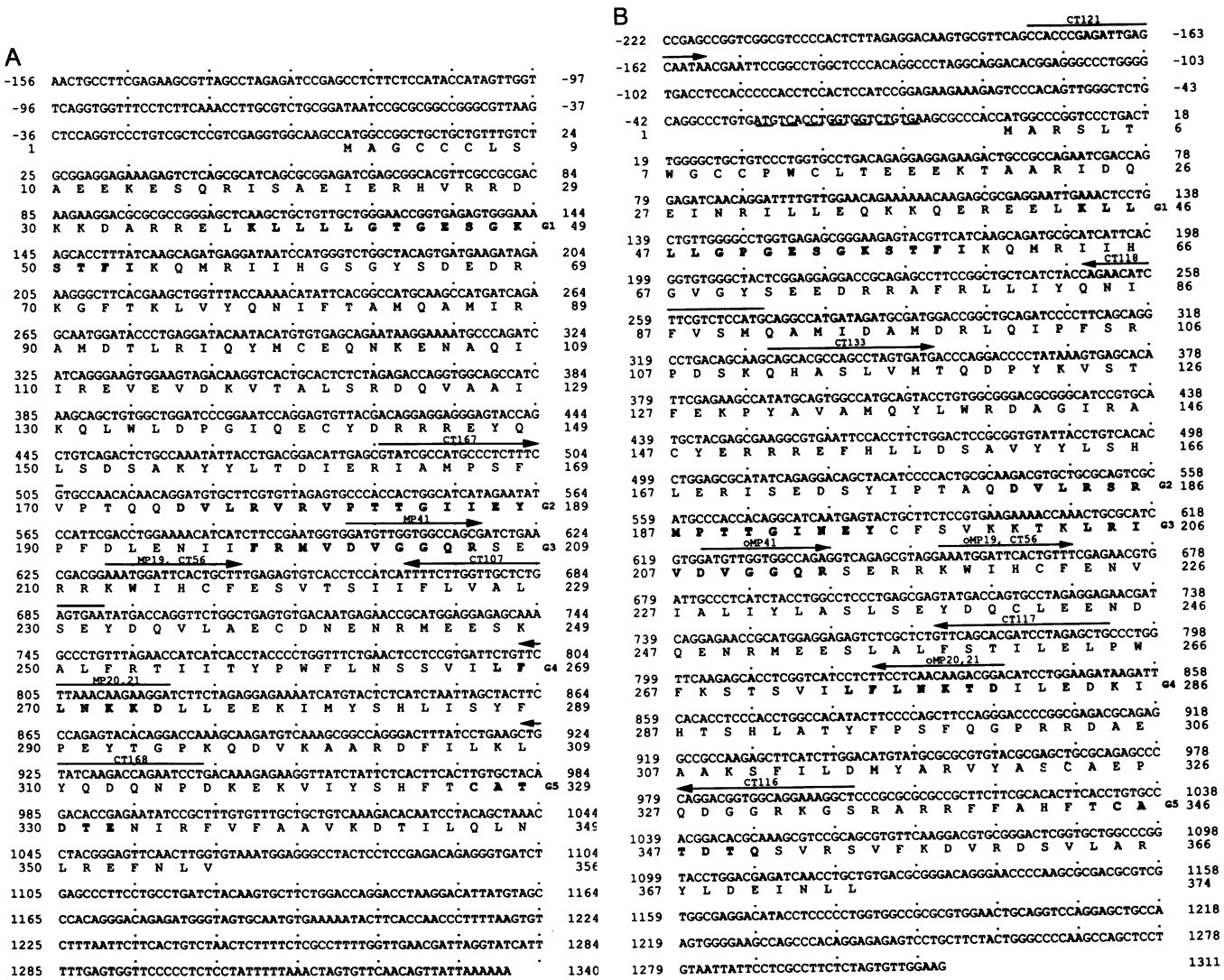

FIG. 1. cDNA sequence of Gα14 and Gα15. The sequence of Gα14 (*A*) was obtained from three cDNA clones, the following nucleotides inclusive: clone 889-1 (876 to 1340); 889-2 (−156 to 1340); 889-5 (−156 to 496). The sequence of Gα15 (*B*) was isolated from two independent cDNA clones, the following nucleotides inclusive: 763-5 [563 to the poly(A) tail]; 781-1 (−222 to 1311). Oligonucleotides that were used for cloning and expression analysis are located below the arrows; left-pointing arrowheads indicate antisense oligonucleotides. The amino acids symbolized in boldface type are constituents of the conserved motifs that contribute to guanine nucleotide-binding and the conformational switch hinge, identified in the right margin as G1 through G5 (23, 24).

shown). This insert was not observed in the original PCR clones of Gα15 obtained by PCR amplification between the degenerate oligonucleotides oMP19 and oMP21, nor was it observed in 15 independent PCR products that were amplified between CT133 and CT117 from cDNA of spleen and a variety of mouse hematopoietic cell lines. The sequence flanking the translation initiation site was also corroborated by directly sequencing the DNA products PCR-amplified from spleen cDNA in three independent reactions between the oligonucleotides CT121 and CT118 (Fig. 1*B*).

Gα14 and Gα15 encode proteins of 360 and 374 amino acids, respectively, that are predicted to be α subunits of heterotrimeric G proteins by virtue of high sequence identity to the conserved amino acids in other Gα subunits. Gα14 and Gα15 are 81% and 58% identical to $G\alpha_q$, respectively, but only about 40% identical to all other Gα subunits outside of the G_q class. We presume that translation of Gα14 and Gα15 initiates where indicated because these are the first methionine codons that are encountered in the coding sequence and they reside within the consensus sequence (25) just upstream of a region that is conserved among all Gα subunits. Gα15 contains a short open reading frame upstream of the initiation codon; this feature is shared only with Gα12 (8) and Gα16 (18) among the other α subunits and may provide a mechanism for translational regulation of these α subunits in some cell types.

PTX can block signal transduction generated from stimulated receptors by ADP-ribosylation of the GDP-bound form of certain α subunits. Members of the G_i class of α subunits that are sensitive to PTX each have a cysteine residue 4 amino acids from the carboxyl terminus. In contrast, α subunits that do not have cysteine at this position, including $G\alpha_s$, $G\alpha_{olf}$, $G\alpha_z$, and $G\alpha_q$, are not substrates for PTX (26, 27). Likewise, Gα14 and Gα15 lack a cysteine residue at this position, and consistent with this observation, protein extracts containing bacterially expressed Gα15 are not ADP-ribosylated by PTX (V.Z.S., unpublished data). These α subunits are expected to mediate signal-transduction pathways that are refractory to PTX.

Tissue Distribution of α-Subunit mRNA from the G_q Class. The expression of Gα14 and Gα15 mRNA was assayed and compared with that of Gα11 and $G\alpha_q$ mRNA in a variety of mouse tissues. The patterns of Gα14 and Gα15 expression were unique among the known mammalian α subunits. Tissue distribution was assayed by PCR amplification of randomly primed cDNA with oligonucleotides specific for each α subunit, and Southern blots of the PCR products were hybridized with oligonucleotide probes specific to $G\alpha_q$, Gα11, Gα14, or Gα15 (Fig. 2). Coamplification with oligonucleotide primers specific to β-actin demonstrated that each sample contained comparable amounts of cDNA template. The patterns of $G\alpha_q$ and Gα11 expression agreed with previous PCR and Northern analysis (7) and served as internal controls. Whereas Gα11 was ubiquitous and $G\alpha_q$ was widely expressed, Gα14 and Gα15 were restricted in their tissue distribution.

The pattern of Gα15 expression was of particular interest because it was apparently restricted to certain tissues, such as spleen, thymus, lung, and bone marrow (Fig. 2), that are rich in hematopoietic cell types. Macrophages are relatively abundant in the adult mouse lung, but spleen harbors hematopoietic cells of the myeloid, B, and T lymphoid lineages. Gα15 mRNA was expressed in all seven samples that were assayed from normal mouse spleen and in the spleen of nude mice, which are deficient in T-cell production. Expression in thymus was lower than in spleen; Gα15 may be relatively less abundant in T lymphocytes, developmentally restricted within this lineage, or predominantly expressed in other thymic cell types. Gα15 mRNA was relatively abundant in embryonic liver (day 14) but undetectable in adult liver, suggesting that Gα15 is also expressed early in hematopoietic

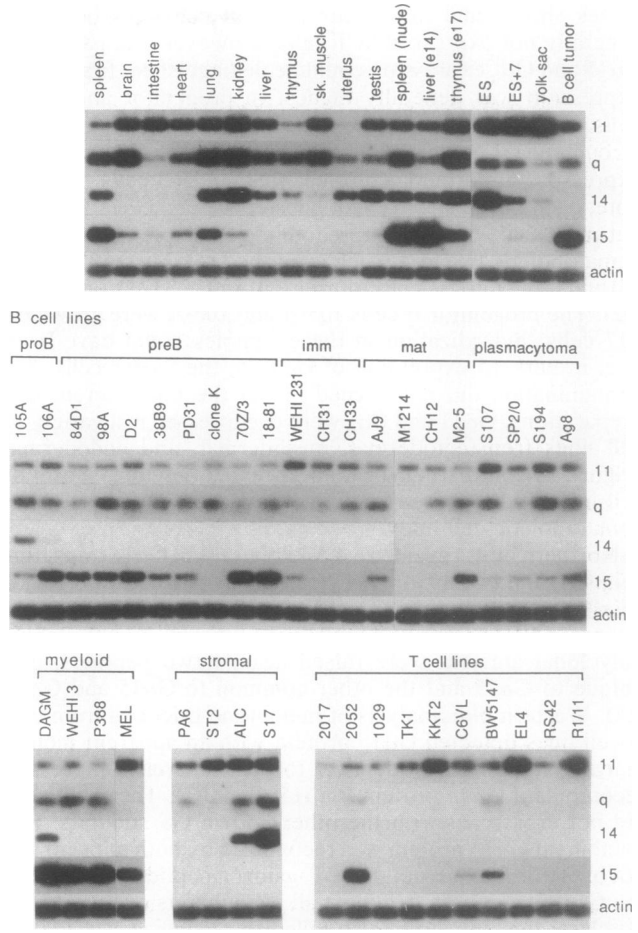

FIG. 2. PCR analysis of RNA expression in mouse tissues and cell lines. Randomly primed cDNA was amplified by PCR using oligonucleotide primers specific for each Gα subunit in separate reactions. Coamplification with β-actin primers served as an internal control. The amplified products were blotted onto nylon filters and hybridized with a radiolabeled oligonucleotide specific to each gene. sk., Skeletal; e14 and e17, embryonic days 14 and 17; ES, embryonic stem cells; ES+7, embryonic stem cells after 7 days of differentiation; imm, immature; mat, mature.

development. Gα15 was weakly expressed in yolk sac of day 8 embryos and in both proliferating embryonic stem (ES) cells and ES cells that had been differentiated to form blood islands in culture. Gα14 mRNA was most abundant in spleen, lung, kidney, and testis and, interestingly, in ES cells but not in differentiated ES cells or yolk sac (Fig. 2). These same patterns of mRNA accumulation among the G_q class of α subunits were observed in separate PCR analysis using the degenerate oligonucleotide oMP19 or oMP41 paired with oMP20 or oMP21 (data not shown).

Expression of the G_q Class in Cultured Hematopoietic Cells. We analyzed RNA from cloned hematopoietic cell lines to determine the restrictions in hematopoietic expression among the α subunits of the G_q class. The PCR analysis was performed as described above using the two sets of degenerate oligonucleotides and, in separate reactions, each set of the specific oligonucleotides (Fig. 2). The analysis included RNA isolated from cell lines that were arrested at various maturational stages of B- and T-lymphocyte differentiation, cells from diverse myeloid lineages, four bone marrow adherent (stromal) cell lines, and many other nonhematopoietic cell types.

Gα11 was expressed in all murine cell lines assayed. Hematopoietic expression of $G\alpha_q$ and Gα15 was nearly parallel; both were expressed during all B-cell maturational

stages shown and in various myeloid cell lines but were generally not expressed in T cells. However, the pattern of Gα15 and $G\alpha_q$ expression could be distinguished; first, Gα15 expression was generally highest in progenitor and pre-B cells whereas $G\alpha_q$ expression was essentially constant throughout B-cell maturation, and second, $G\alpha_q$ was not expressed in murine erythroleukemia (MEL) cells. Of final note, each of the stromal cell lines expressed Gα11 and $G\alpha_q$ but not Gα15. Gα14 was predominantly expressed early in hematopoietic lineages, such as in the DAGM (DA3.15; ref. 28) and progenitor B-cell cultures (29) and in S17 stromal cells (22). The progenitor B cells 105-A and 106-A were grown on S17 cells; hybridization in these samples could have been due, in part, to expression of Gα14 in the feeder cells, but contamination did not exceed 5% of the nonadherent cell preparations. Gα11 and $G\alpha_q$ were coexpressed in a variety of cell lines from ectodermal, mesodermal, and endodermal origins. In contrast, Gα14 was expressed only in a subset of cell lines and Gα15 expression was most significant within hematopoietic cell lines.

Northern blots revealed a 4.5-kilobase (kb) Gα14 transcript (Fig. 3*A*) and two major Gα15 transcripts of 2.4 kb and 2.2 kb (Fig. 3*B*). Northern blots also detected Gα15 mRNA in 70Z/3 but not in NIH 3T3 or RS42 cell lines (data not shown). Rabbit polyclonal antisera were raised against two peptides, one unique to Gα15 and the other common to Gα15 and Gα16 (18). Western blot analysis of membrane fractions from two B-cell lines detected Gα15 protein with an apparent molecular mass of 42 kDa, identical to the bacterially expressed recombinant Gα15 protein (rGα15; Fig. 3*C*). These antisera did not crossreact with the other known Gα subunits. Recombinant Gα15 protein was recognized by both antisera but not by a third antiserum, raised against a peptide sequence in the G1 box common to almost all Gα subunits outside of the G_q class; this antiserum also failed to recognize $G\alpha_q$ (27).

The cell lines used to assay Gα14 and Gα15 expression grow indefinitely in culture and it is possible that expression of Gα genes in a cell line is essential for maintenance of proliferation. Discrete amino acid substitutions at Arg^{189} and Gln^{227} in $G\alpha_s$ (and the corresponding residues in $G\alpha_i$), which maintain the GTP-bound active state of these α subunits, have been correlated with hypertrophy and oncogenesis in certain tissues (30, 31). We examined the cDNA sequence of Gα14 and Gα15 in 11 and 15 of the highest expressing cell lines, respectively, to determine whether analogous mutations in Gα14 and Gα15 were correlated with cell proliferation. Direct DNA sequencing of the PCR products that were amplified between specific primers for Gα14 (CT167 and CT168, Fig. 1*A*) or Gα15 (CT133 and CT117, Fig. 1*B*) revealed that each cell line assayed had the same amino acid sequence as expressed in normal mouse tissues. Silent substitutions were found only in the first nucleotide of the Arg^{180} codon in Gα14 in two of the cell lines assayed.

DISCUSSION

The G-protein α subunits that are expressed in mammals can be grouped into four classes based on comparison of the primary structure of 16 distinct sequences (3). The α subunits share considerable amino acid identity within the conserved motifs that contribute to the GTP-binding pocket and the conformational switch domain. The four classes, termed G_s, G_i, G12, and G_q, are primarily distinguished by their amino acid sequence outside of these regions. The α subunits reported here, Gα14 and Gα15, are most closely related to members of the G_q class.

Sequence comparisons suggest that Gα14 may be more similar biochemically to the other members of the G_q class than is Gα15. For example, the sequence of Gα14 is identical to those of $G\alpha_q$ and Gα11 within the GTP-binding domains and the conformational switch region, labeled G1 to G5 in Fig. 1, whereas Gα15 has a number of amino acid substitutions. Sequence differences in these domains may influence the relative rates of GDP/GTP exchange or GTP hydrolysis (26). The Gα14 and Gα15 proteins are predicted to be similar in at least two important aspects held commonly with other members of the G_q class. The amino terminus of both proteins lacks the consensus site for myristoylation (32) that facilitates this posttranslational modification of several α subunits in the G_i class (33). Additionally, Gα14 and Gα15 lack the cysteine residue four amino acids from the carboxyl terminus that is ADP-ribosylated in the α subunits of the G_i class (1) and thus are predicted to be insensitive to PTX.

G proteins that are refractory to PTX have been shown to regulate signaling pathways in a variety of mammalian tissues and cell types (15). Therefore, we determined the mRNA expression pattern of the α subunits in the G_q class in various mouse tissues. Gα11 and $G\alpha_q$ were expressed ubiquitously (7); in comparison, Gα14 was expressed in a few tissues and Gα15 was predominantly expressed in tissues that are rich sources of hematopoietic cells (Fig. 2). To further characterize expression of the G_q class within the hematopoietic compartment, we assayed expression of the four α subunits isolated from mouse in a variety of murine hematopoietic and stromal cell lines, in addition to cell lines derived from nonhematopoietic tissues. Gα11 mRNA was expressed in every cell line tested. In contrast, Gα14 mRNA exhibited the most restricted pattern within the hematopoietic compartment; it was expressed only in certain bone marrow adherent (stromal) cell lines, probably in progenitor B cells, and in some immature myeloid cell lines. $G\alpha_q$ was expressed in all nonhematopoietic tissues and cell lines that were assayed; it was also expressed in B cells and

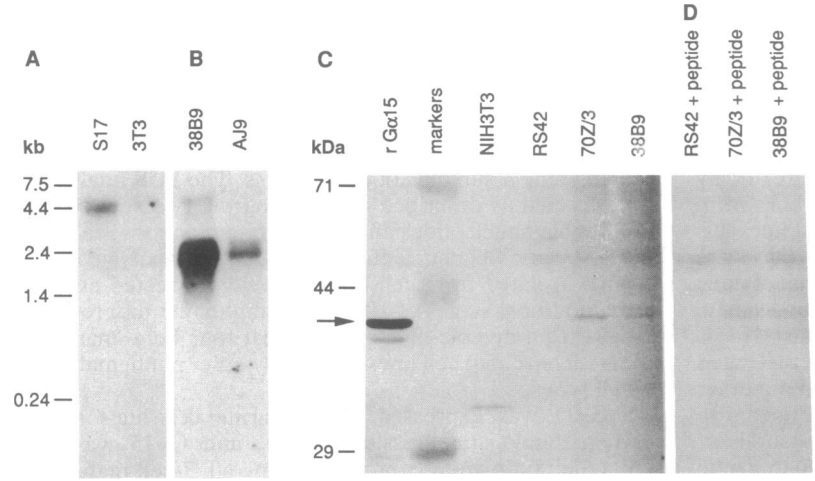

FIG. 3. Northern and Western analysis of Gα14 and Gα15. (*A* and *B*) Gα14 (*A*) and Gα15 (*B*) transcripts were detected on Northern blots containing 5 μg of poly(A)$^+$ RNA isolated from the indicated cell lines. (*C*) Gα15 protein was detected in the membrane protein fractions (50 μg) of two B-cell lines by Western blot analysis using affinity-purified polyclonal antiserum raised against a peptide sequence unique to the carboxyl terminus of Gα15 in mice. (*D*) Detection of Gα15 protein was blocked by coincubation of antisera with the Gα15 peptide.

most myeloid cell lines but not in most T-cell lines. Gα15 mRNA was expressed in all myeloid lineages that were analyzed and in B-cell lines that were arrested at various developmental stages from progenitor to mature cell types. Gα15 was not expressed in most T-cell lines or in any of the stromal cell lines; it was very weakly expressed in NIH 3T3 and P19 cells and undetectable in other nonhematopoietic cell lines. Expression of $G\alpha_q$ and Gα15 in a few of the murine T-cell lines may reflect the previously observed expression of characteristically B-cell-restricted genes in immature T-cell tumors (34, 35). The general pattern of expression among murine T-cell lines suggests that Gα11 is the predominant α subunit of the G_q class in T lymphocytes.

The expression pattern of Gα15 is similar to that of human Gα16. Both genes are expressed predominately in hematopoietic lineages, although Gα16 appears to be more prevalent in T-cell lines (18). Gα15 and Gα16 are probably the mouse and human homologues of a specific α subunit. Southern blots of mouse or human genomic DNA digested with several different restriction enzymes showed the same hybridization patterns when probed with either Gα15 or Gα16, and chromosomal mapping data demonstrated that Gα15 and Gα16 cosegregated with the same two flanking loci in mouse and humans (T.M.W., N. Jenkins, N. Copeland, and A. Olsen, unpublished observations). These results were unexpected because Gα15 and Gα16 exhibit only 85% amino acid identity whereas the mammalian homologues of other α subunits are >95% identical.

Nine distinct PLC proteins have been identified (36) and, recently, $G\alpha_q$ was found to mediate PTX-insensitive stimulation of PLC β1 but not PLC γ1 or δ1 (14). Although the precise effectors in the signal-transduction cascades mediated by Gα14 and Gα15 are unknown, their similarity to $G\alpha_q$ suggests that they may also activate the PLC β isotypes. Indeed, transient transfection assays suggest that all members of the G_q class can activate PLC β1 (C. H. Lee, D. Wu, and M.I.S., unpublished observations). It is not clear from this result why some cell types express all four members of the G_q class if the primary role of these α subunits is to activate PLC. Perhaps they differentially activate the PLC β isotypes *in vivo*. Other effector interactions are possible and not necessarily mutually exclusive, including regulation of various ion channels. The α subunits in the G_q class could also be functionally distinguished by their receptor interactions. With the exception of Gα11, expression of these α subunits within the hematopoietic compartment is restricted to certain lineages or developmental stages. An attractive model is that these α subunits are components of specific signaling pathways that mediate hematopoietic and stromal cell interactions, thus contributing to the regulation of hematopoiesis.

Note Added in Proof. The mouse Gα14 cDNA is homologous to the bovine $G_L1\alpha$ cDNA reported by Nakamura *et al.* (37).

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