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Molecular Cloning of Human $G\alpha_q$ cDNA and Chromosomal Localization of the $G\alpha_q$ Gene (GNAQ) and a Processed Pseudogene

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$G\alpha_q$ is the α subunit of one of the heterotrimeric GTP-binding proteins that mediates stimulation of phospholipase Cβ. We report the isolation and characterization of cDNA clones from a frontal cortex cDNA library encoding human $G\alpha_q$. The encoded protein is 359 amino acids long and is identical in all but one amino acid residue to mouse $G\alpha_q$. Analysis of human genomic DNA reveals an intronless sequence with strong homology to human $G\alpha_q$ cDNA. In comparison to $G\alpha_q$ cDNA, this genomic DNA sequence includes several small deletions and insertions that alter the reading frame, multiple single base changes, and a premature termination codon in the open reading frame, hallmarks of a processed pseudogene. Probes derived from human $G\alpha_q$ cDNA sequence map to both chromosomes 2 and 9 in high-stringency genomic blot analyses of DNA from a panel of human–rodent hybrid cell lines. PCR primers that selectively amplify the pseudogene sequence generate a product only when DNA containing human chromosome 2 is used as the template, indicating that the authentic $G\alpha_q$ gene (GNAQ) is located on chromosome 9. Regional localization by FISH analysis places GNAQ at 9q21 and the pseudogene at 2q14.3–q21.

INTRODUCTION

Guanine nucleotide-binding proteins (G-proteins) are a family of heterotrimeric proteins that couple cell-surface, seven-transmembrane domain receptors to intracellular signaling pathways (Simon *et al.*, 1991; Hepler and Gilman, 1992; Spiegel *et al.*, 1992; Conklin and Bourne, 1993). Receptor activation catalyzes the exchange of GTP for GDP bound to the inactive G protein α subunit, resulting in a conformational change and dissociation of the complex. The G protein α and $\beta\gamma$ subunits are capable of regulating various cellular effectors. Activation is terminated by a GTPase intrinsic to the $G\alpha$ subunit.

Sixteen mammalian genes coding $G\alpha$ subunits have been cloned and grouped into four classes, α_s, α_i, α_q, and α_{12}, based on their amino acid sequence similarity (Simon *et al.*, 1991; Spiegel *et al.*, 1992). Members of the $G\alpha_q$ subfamily (α_q, α_{11}, α_{14} and $\alpha_{15/16}$) stimulate the activity of phospholipase Cβ, resulting in cleavage of phosphatidylinositol 4,5-bisphosphate and release of inositol (1,4,5)-triphosphate and 1,2-diacylglycerol (Smrcka *et al.*, 1991; Taylor *et al.*, 1991; Wu *et al.*, 1992). These second messengers mediate increases in intracellular Ca^{2+} and protein kinase C activity, respectively. $G\alpha_q$ is ubiquitously expressed (Strathmann and Simon, 1990), and constitutively activated mutants of mouse $G\alpha_q$ can transform NIH 3T3 mouse fibroblasts and induce tumor formation (De Vivo *et al.*, 1992; Kalinec *et al.*, 1992). Mutations of other α subunit genes have previously been shown to cause disease in humans. For example, heterozygous inactivating mutations of $G\alpha_s$, the subunit responsible for stimulation of intracellular cAMP levels, are found in patients with Albright hereditary osteodystrophy (Patten *et al.*, 1990; Weinstein *et al.*, 1990). Mutations of amino acid residues involved in GTP hydrolysis in $G\alpha_s$ (*gsp*) and $G\alpha_{i2}$ (*gip*) result in constitutive activation and are associated with autonomous hyperfunction, hyperplasia, or tumors of endocrine organs (Landis *et al.*, 1989; Lyons *et al.*, 1990; Weinstein *et al.*, 1991; Suarez *et al.*, 1991).

As a prerequisite to examining the role of $G\alpha_q$ in normal and abnormal cell signaling in humans, we cloned human $G\alpha_q$ cDNA and performed chromosomal localization studies. Analysis of human genomic DNA revealed the presence of an intronless, processed $G\alpha_q$

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pseudogene that maps to a chromosome different from that of the authentic $G\alpha_q$ gene.

MATERIALS AND METHODS

Screening of human brain cDNA library. A mouse $G\alpha_q$ cDNA probe encompassing the 359-amino-acid coding region was generated using established methods for reverse transcription (RT) followed by PCR (Davis *et al.*, 1994). The mRNA template for RT was mouse brain poly(A)$^+$ mRNA (Clontech). Primer sequences were based on the published mouse $G\alpha_q$ nucleotide sequence (Strathmann and Simon, 1990): the gene-specific primer used for RT was 5′GGAGGGAAGAACCAG3′, and the primers for PCR were 5′CAGCGAGGCACTTCGGAAGA3′ (sense primer) and 5′TTCTGGGAGGCACGGTTAGA3′ (antisense primer). The identity of the single 1.1-kb PCR fragment obtained was confirmed by nucleotide sequence analysis of a plasmid subclone containing the fragment. The PCR fragment was labeled by random hexamer priming (BRL kit) and used for screening 1×10^6 members of a human temporal cortex cDNA library formed in the Lambda ZAP vector (Stratagene). Positive clones were plaque purified, the pBluescript plasmid sequences autoexcised as described by the manufacturer (Stratagene), and plasmids containing a 3-kb insert were sequenced.

Rapid amplification of cDNA end (RACE). A 5′ RACE procedure was performed to obtain the missing 5′ sequences of human $G\alpha_q$ cDNA using established protocols (Davis *et al.*, 1994). The gene-specific primer used for reverse transcription was 5′CCGTGAAGATGTTCTGATAC3′, and the primer used for PCR was 5′CTGCTTGGTGAAGCCCCTTTTA3′.

PCR amplification of genomic DNA with cDNA sequence primers. Human genomic DNA was prepared from blood or frozen pituitaries (kindly provided by the National Hormone and Pituitary Program) from normal subjects. PCR amplification of genomic DNA with primers derived from $G\alpha_q$ cDNA sequence (sense primer 560–579, antisense primer 760–779; Fig. 2) that were predicted to flank one or more introns (Kozasa *et al.*, 1988; Weinstein *et al.*, 1988) unexpectedly produced a PCR product that was identical in size (220 bp) to that observed when first-strand cDNA was used as a template. PCR was performed with 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 1 min of elongation at 72°C, for 40 cycles. The sequence of the PCR product differed slightly from that of $G\alpha_q$. To obtain additional genomic sequences flanking the novel fragment on the 5′ side, an additional PCR amplification was performed (1 min of denaturation at 94°C, 45 sec of annealing at 62°C, and 1 min of elongation at 72°C, 35 cycles) using an antisense primer specific for the genomic sequence at its 3′-terminal base (5′CTGACCTTTGGCCCCCTACATCGAA3′; 652–676, Fig. 2) and a degenerate sense primer [5′GG(ACT)GAGAG(CT)GG(GC)AA(AG)AG(CT)AC(GC)TT(CT)A3′; 184–208] based on computer alignment of sequences of G protein α subunits in GenBank. A 492-bp fragment of human genomic DNA was amplified and sequenced.

Screening of human genomic library for pseudogene. The labeled 492-bp PCR product was then used to screen 1×10^6 members of a human leukocyte genomic library. Hybridizing clones were plaque purified, and analytical preparations of λ bacteriophage DNA were made as described (Davis *et al.*, 1994). Positive inserts were subcloned into pGEM 4, and recombinant colonies were identified by colony hybridization. Plasmids containing 0.3- and 8-kb inserts were fully and partially sequenced, respectively.

Sequencing analysis. The nucleotide sequence of genomic and cDNA fragments was determined with bacteriophage promoter primers and gene-specific internal primers by cycle sequencing (fmol kit, Promega). PCR products were purified with the GlassMax kit (BRL) and used directly as templates for cycle sequencing.

Chromosomal mapping with somatic cell hybrids. Chromosomal localization was carried out by Southern analysis or PCR amplification of DNA from a human × rodent somatic cell hybrid panel (BIOS Laboratories, New Haven, CT). The human chromosomal content of cells used in this study is as reported previously (Miyasaka *et al.*, 1993), except that cell line 1006 lacks chromosome 8 and 1079 lacks chromosome 16. In addition, cell lines 010 (human chromosome 10), 016 (chromosome 16), and 852 (chromosome 2) were used. Cell line 016 is a human × mouse hybrid, and all others are hamster × human. For Southern analysis, ^{32}P-labeled probes derived from human $G\alpha_q$ coding or 3′-untranslated regions were hybridized to membranes containing somatic cell hybrid DNA digested with *Pst*I, *Taq*I, or *Hin*dIII. Hybridization was carried out overnight at 65°C, and final washes were at 65°C in 0.2× SSC, 0.2% SDS. For PCR analysis, genomic DNA from hybrid cell lines was screened for $G\alpha_q$ pseudogene sequence with the PCR primers (184–208 and 652–676) and under the cycling conditions described above.

Fluorescence in situ hybridization (FISH). Subchromosomal localization was performed by FISH. Briefly, two purified plasmids, one containing a 3-kb insert of $G\alpha_q$ cDNA and the other an 8-kb insert of the $G\alpha_q$ pseudogene and its 3′-flanking region, were labeled individually with biotin-14–dUTP (Boehringer Mannheim) using nick-translation and hybridized to standard normal male metaphase spreads, essentially as described (Baldini and Lindsay, 1994). Biotin-labeled DNA was detected using fluorescein–avidin DCS (Vector). Chromosome identification was performed with simultaneous DAPI (4,6-diamidino-2-phenylindole) staining, which produces a Q-banding pattern. Digital images were obtained using a cooled CCD camera (Photometrics) mounted on a standard fluorescent microscope (Leica). Fluorescein and DAPI fluorescence were recorded separately as gray-scale images and then merged using the software package NIH 1.57 (Ijdo *et al.*, 1992). Band assignments for the fluorescein signals were then determined.

RESULTS

Isolation and Sequence of Human $G\alpha_q$ cDNA Clones

Using a mouse $G\alpha_q$ cDNA probe encompassing the entire open reading frame of the protein (obtained as described under Materials and Methods), we isolated a clone from a human frontal cortex cDNA library that contained an insert about 3 kb in length. Nucleotide sequence analysis revealed a single, long open reading frame in the human cDNA that closely resembled $G\alpha_q$, but the clone was missing sequence encoding the first 46 amino acids found in mouse. To obtain the missing 5′ sequence, a 5′ RACE procedure was performed, using human brain mRNA as template, which generated a single 276-bp fragment. This fragment was subcloned, and sequences encoding the amino terminal end of the human $G\alpha_q$ protein were obtained. cDNA sequences encoding human and mouse $G\alpha_q$ are 95% identical (Fig. 1). Both proteins are predicted to be 359 amino acids long and differ in only one amino acid: residue 171 is serine in mouse $G\alpha_q$ and alanine in human $G\alpha_q$ (Fig. 1). When the human $G\alpha_q$ coding sequence is aligned with that of human $G\alpha_{11}$ (Jiang *et al.*, 1991), its closest relative in the G protein family, the amino acid identity is 89%, and the nucleotide sequence identity is 78% (data not shown). The close homology of mouse and human $G\alpha_q$ sequences indicates that the cDNA isolated from the brain library corresponds to the human $G\alpha_q$ gene and not to another member of the $G\alpha_q$ subfamily. Subsequently, Northern analysis with a probe containing the 3-kb $G\alpha_q$ cDNA sequence was shown to detect a poly(A)$^+$ RNA of greater than 5 kb in human brain, kidney, and mouse Swiss 3T3 cells (data not shown).

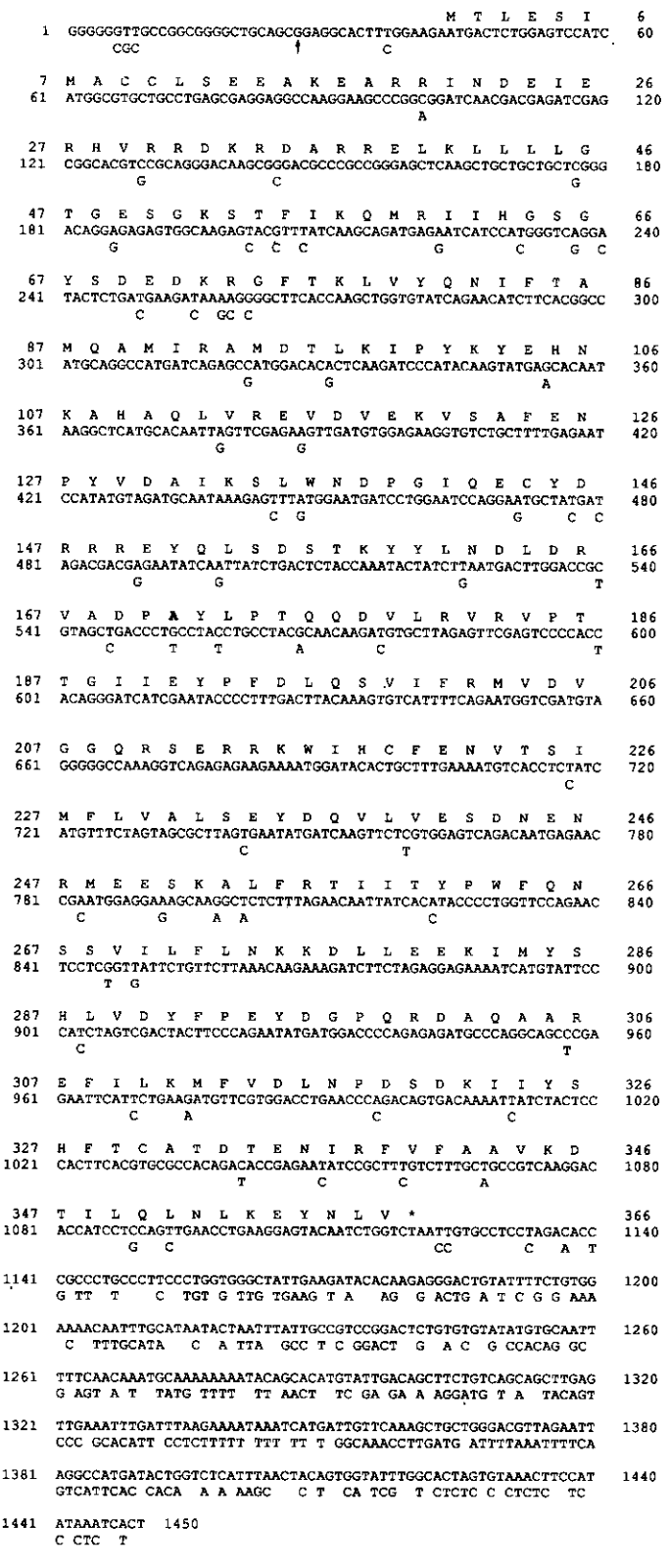

FIG. 1. Comparison of human and mouse $G\alpha_q$ sequence. The first row is predicted human amino acid sequence, the second row is human nucleotide sequence, and the third row shows mouse nucleotide sequence (only nucleotides that differ from human sequence are shown). The position of the only predicted amino acid difference is shown in bold (residue 171 is alanine in human $G\alpha_q$, serine in mouse). The arrow indicates the position of a single nucleotide that is absent in the mouse 5′-untranslated sequence.

Isolation and Partial Nucleotide Sequence Analysis of Genomic Clones Encoding a Human $G\alpha_q$ Processed Pseudogene

As a prelude to RT-PCR screening for activating $G\alpha_q$ mutations in human tumors, primers were designed (sense primer 560–579, antisense primer 760–779; Fig. 2) to amplify the region known to encode amino acids critically involved in GTP hydrolysis (De Vivo *et al.*, 1992; Kalinec *et al.*, 1992; Conklin *et al.*, 1992). Based on analogy to the known genomic structure of several other $G\alpha$ subunits (Kozasa *et al.*, 1988; Weinstein *et al.*, 1988), we strongly suspected that these primers would flank at least one intron in the genomic DNA. Curiously, amplification of normal genomic DNA with these primers produced a PCR product that was identical in size (220 bp) to that observed when first-strand cDNA was used as a template (data not shown), indicating an absence of introns in the amplified genomic DNA template. Direct nucleotide sequence analysis of PCR products from three normal subjects confirmed the absence of introns and revealed four single nucleotide differences between the genomic PCR product and the $G\alpha_q$ cDNA sequence: homozygous substitutions at positions 610 and 652 and heterozygous substitutions at positions 613 and 625. The lack of introns, as well as the fact that two of the observed nucleotide substitutions encoded nonconservative amino acid changes, raised the possibility that we had amplified a segment of a $G\alpha_q$ processed pseudogene, rather than the $G\alpha_q$ gene itself. However, no termination codon was observed in the open reading frame of the genomic PCR product, leaving open the remote possibility that this genomic PCR fragment might reflect a polymorphic $G\alpha_q$ allele or another closely related member of the $G\alpha_q$ gene family with no introns in this region. To obtain additional genomic sequences flanking the genomic PCR fragment on the 5′ side, an additional PCR amplification was performed using an antisense primer (652–676) specific for the genomic PCR product at its 3′-terminal base and a degenerate sense primer (184–208) located 5′ to sequences isolated in the first genomic PCR fragment (Fig. 2). The sequence of the degenerate primer was based on amino acid sequence that is highly conserved in most known $G\alpha$ subunits. Using a human genomic DNA template, a single 492-bp product was isolated. Direct nucleotide sequence analysis of the PCR product confirmed that this sequence also contained no introns and had two deletions and three insertions that shift the $G\alpha_q$ open reading frame, as well as multiple single base changes. One of the insertions is associated with a premature termination codon (Fig. 2). Taken together, these data indicate that the two overlapping PCR products represent segments of a $G\alpha_q$ processed pseudogene that cannot encode a functional $G\alpha_q$ subunit.

The 492-bp PCR fragment from the putative processed pseudogene was used to screen a human genomic library. Several bacteriophage clones were isolated, and hybridizing fragments were subcloned into plasmid vectors. Se-

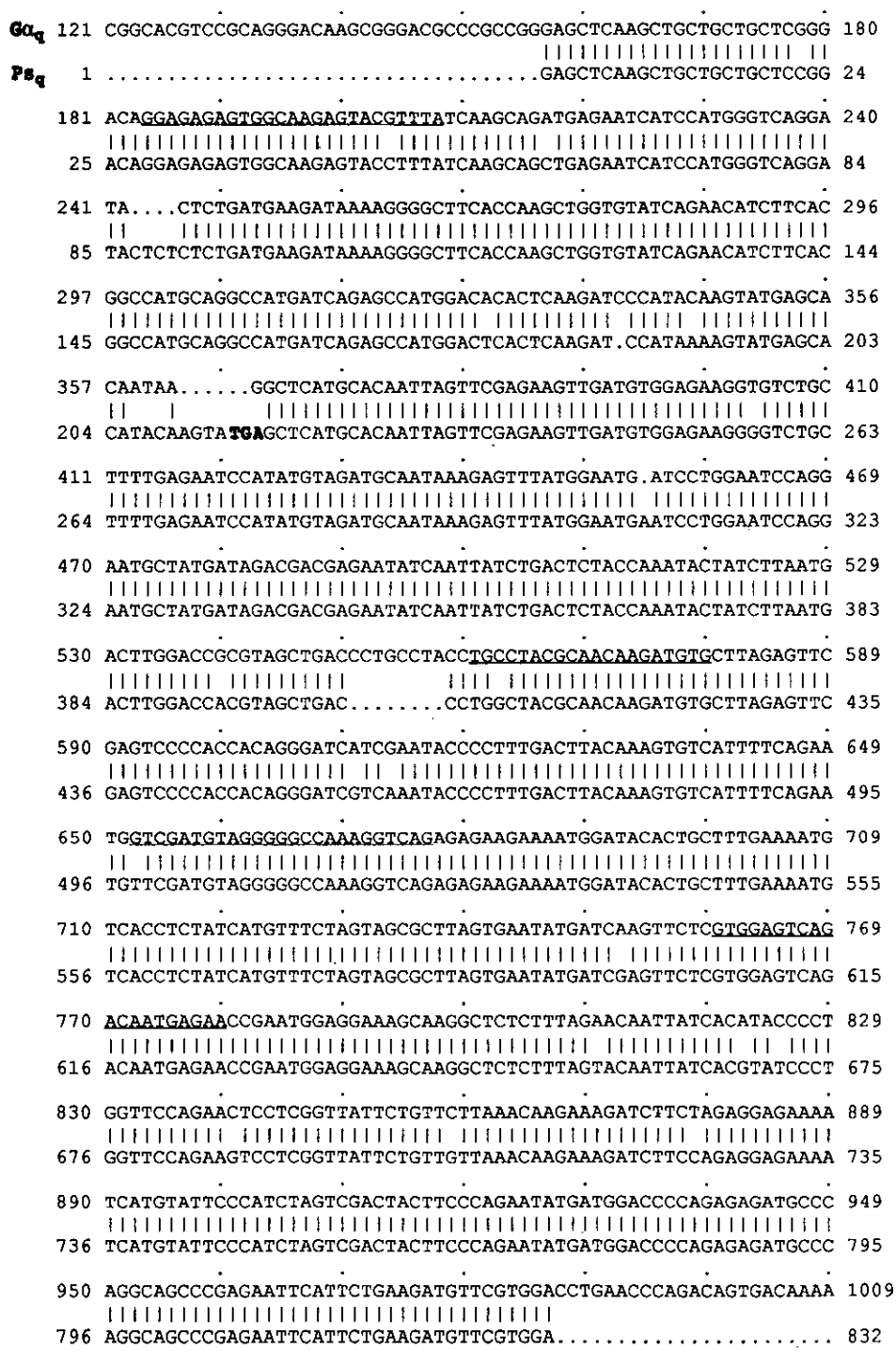

FIG. 2. Partial nucleotide sequence comparison between human $G\alpha_q$ and its pseudogene (Ps_q). There are two deletions, three insertions, and 20 single nucleotide substitutions in the available pseudogene sequence. A premature stop codon is in bold. The locations of PCR primer pairs 184–208, 652–676, 560–579, and 760–779 are underlined; numbering is based on $G\alpha_q$ cDNA sequence but the actual primer sequences are given under Materials and Methods. The Ps_q sequence that is shown was derived from two genomic clones. Heterozygous polymorphisms at positions corresponding to $G\alpha_q$ 613 (A/G) and 625 (G/C) were found in PCR products amplified from three different genomic DNA samples.

quence corresponding to that of the pseudogene PCR fragments was obtained, as well as 5′- and 3′-flanking sequences (Fig. 2). No introns were found. Compared to $G\alpha_q$, the sequence of the processed pseudogene contains a total of two deletions, three insertions, and 20 single nucleotide substitutions (Fig. 2).

Mapping the Human $G\alpha_q$ Gene to Chromosome 9q21 and Its Pseudogene to Chromosome 2q14.3–2q21

Restriction fragment probes (0.6–0.7 kb) derived either from the coding region or from 3′-untranslated sequences of the human $G\alpha_q$ cDNA were hybridized at

high stringency to DNA from a panel of somatic cell hybrids. Hybridization only occurred to genomic DNA derived from hybrid cell lines 734 (which contains human chromosomes 5, 9, and 18) and 852 (human chromosome 2 only). Cell line 734 is the only member of the panel that contains chromosome 9, and 852 is the only one that contains chromosome 2. Because the $G\alpha_q$ probe did not hybridize to other members of the panel that contain chromosomes 5 and 18 (Miyasaka *et al.*, 1993) we concluded that $G\alpha_q$-like sequence was located on chromosomes 2 and 9.

To determine the subchromosomal localization of the $G\alpha_q$ gene and its pseudogene, chromosome spreads were analyzed by FISH using two purified plasmids as the probes, one containing a 3-kb insert of $G\alpha_q$ cDNA and the other an 8-kb insert of the $G\alpha_q$ pseudogene and its 3′-flanking region. For each probe, 15 metaphase spreads were analyzed, and a specific hybridization signal was detected in 85% of metaphases on both chromatids of the two chromosomes 9 at band 9q21 and on both chromatids of the two chromosomes 2 at 2q14.3–q21. No other hybridization sites could be detected.

The pseudogene-selective PCR primer 652–676 was then used with primer 184–208 to amplify DNA derived from the panel of somatic cell hybrids. Rodent DNA was not amplified by these primers. Only human control DNA and DNA derived from hybrid cell line 852 was amplified, indicating that the pseudogene is located on chromosome 2 and that the authentic $G\alpha_q$ gene resides on human chromosome 9.

DISCUSSION

In this study, we have determined the nucleotide and predicted amino acid sequences of human $G\alpha_q$, identified the presence of a highly homologous processed pseudogene in the human genome, and mapped the human $G\alpha_q$ gene to chromosome 9q21 and the pseudogene to chromosome 2q14.3–q21. The human $G\alpha_q$ nucleotide sequence will be useful for studying the role of $G\alpha_q$ in normal and abnormal cell signaling in humans. For example, it is possible that activating missense mutations in human $G\alpha_q$ might be found in some human tumors, given the fact that a constitutively activated mutant of mouse $G\alpha_q$ can transform NIH 3T3 cells, generating clones that can form tumors in nude mice (De Vivo *et al.*, 1992; Kalinec *et al.*, 1992).

Reverse transcription of a tissue or tumor-derived mRNA followed by PCR amplification of a relevant coding region is a widely used protocol in gene expression analysis and in screening for mutations. Given the extraordinarily high nucleotide sequence identity observed between the pseudogene and the authentic human $G\alpha_q$ cDNA, it will clearly be critical to remove any contaminating genomic DNA from tissue mRNA samples before reverse transcription to avoid inadvertent amplification of pseudogene sequences. Alternatively, selective primers based on sequence mismatches can be designed. Preliminary results from PCR amplification of genomic DNA with $G\alpha_q$-selective primers suggests that, like most other $G\alpha$ subunit genes (Kozasa *et al.*, 1988; Weinstein *et al.*, 1988), the authentic $G\alpha_q$ gene contains introns.

Assignment of $G\alpha_q$ to human chromosome 9q21 adds to the number of G protein α subunit genes whose chromosomal localization is known (Blatt *et al.*, 1988; Wilkie *et al.*, 1992). The assignment of the $G\alpha_q$ gene to human chromosome 9q21 is consistent with the fact that $G\alpha_q$ has been mapped to mouse chromosome 19 (Wilkie *et al.*, 1992), since mouse chromosome 19 and human chromosome 9q have known linkage homologies (Guenet and Poirier, 1993). Many G protein α subunit genes segregate as tandem pairs in both mouse and human, including $G\alpha_{i2}$ and $G\alpha_{t1}$, $G\alpha_{i3}$ and $G\alpha_{t2}$, and $G\alpha_{11}$ and $G\alpha_{15/16}$ (Wilkie *et al.*, 1992). In the mouse, $G\alpha_q$ and $G\alpha_{14}$ are paired on chromosome 19. Based on these data, it seems reasonable to speculate that human $G\alpha_{14}$ may also map to human chromosome 9q.

It has been hypothesized that a processed pseudogene may arise by reverse transcription of a mature mRNA species with the introns removed, followed by reintegration of the reverse transcript at a new location (Leder *et al.*, 1981; Hollis *et al.*, 1982; Battey *et al.*, 1982). Since the location of the insertion site appears to be random, the chromosomal localization of the processed pseudogene is usually different from that of the authentic gene. This is indeed the case for the $G\alpha_q$ processed pseudogene and the previously reported $G\alpha_{i2}$ pseudogene (Magovcevic *et al.*, 1992). The close resemblance between $G\alpha_q$ cDNA and an apparently nonfunctional pseudogene indicates that the formation of the processed pseudogene is probably a relatively recent event. The extent of accumulated differences (five deletions or insertions and 20 point mutations over 832 bp of nucleotide sequence; Fig. 2) corresponds to 12 to 30 million years of evolution (Saitou and Ueda, 1994), suggesting that humans and possibly some primates may be the only species that possess the $G\alpha_q$ pseudogene.

REFERENCES

Baldini, A., and Lindsay, E. A. (1994). Mapping human YAC clones by fluorescence in situ hybridization using Alu-PCR from single yeast colonies. *In* "Methods in Molecular Biology. In Situ Hybridization Protocols" (K. H. A. Choo, Ed.), Vol. 33, pp. 75–85, Humana Press, New Jersey.

Battey, J., Max, E. E., McBride, W. O., Swan, D., and Leder, P. (1982). A processed human immunoglobulin epsilon gene has moved to chromosome 9. *Proc. Natl. Acad. Sci. USA* **79:** 5956–5960.

Blatt, C., Eversole-Cire, P., Cohn, V. H., Zollman, S., Fournier, R. E., Mohandas, L. T., Nesbitt, M., Lugo, T., Jones, D. T., and Reed, R. R. (1988). Chromosomal localization of genes encoding guanine nucleotide-binding protein subunits in mouse and human. *Proc. Natl. Acad. Sci. USA* **85:** 7642–7646.

Conklin, B. R., and Bourne, H. R. (1993). Structural elements of G alpha subunits that interact with G beta gamma, receptors, and effectors. *Cell* **73:** 631–641.

Conklin, B. R., Chabre, O., Wong, Y. H., Federman, A. D., and Bourne, H. R. (1992). Recombinant Gq alpha. Mutational activa-

tion and coupling to receptors and phospholipase C. *J. Biol. Chem.* **267:** 31–34.

Davis, L., Kuehl, M., and Battey, J. (1994). "Basic Methods in Molecular Biology," Appleton & Lange, Norwalk, CT.

De Vivo, M., Chen, J., Codina, J., and Iyengar, R. (1992). Enhanced phospholipase C stimulation and transformation in NIH-3T3 cells expressing Q209LGq-alpha-subunits. *J. Biol. Chem.* **267:** 18263–18266.

Guenet, J. L., and Poirier, C. (1993). Mouse chromosome 19. *Mamm. Genome* **4:** S261–S268.

Hepler, J. R., and Gilman, A. G. (1992). G proteins. *Trends Biochem. Sci.* **17:** 383–387.

Hollis, G. F., Hieter, P. A., McBride, O. W., Swan, D., and Leder, P. (1982). Processed genes: A dispersed human immunoglobulin gene bearing evidence of RNA-type processing. *Nature* **296:** 321–325.

Ijdo, J. W., Lindsay, E. A., Wells, R. A., and Baldini, A. (1992). Multiple variants in subtelomeric regions of normal karyotypes. *Genomics* **14:** 1019–1025.

Jiang, M., Pandey, S., Tran, V. T., and Fong, H. K. (1991). Guanine nucleotide-binding regulatory proteins in retinal pigment epithelial cells. *Proc. Natl. Acad. Sci. USA* **88:** 3907–3911.

Kalinec, G., Nazarali, A. J., Hermouet, S., Xu, N., and Gutkind, J. S. (1992). Mutated alpha subunit of the Gq protein induces malignant transformation in NIH 3T3 cells. *Mol. Cell. Biol.* **12:** 4687–4693.

Kozasa, T., Itoh, H., Tsukamoto, T., and Kaziro, Y. (1988). Isolation and characterization of the human Gs alpha gene. *Proc. Natl. Acad. Sci. USA* **85:** 2081–2085.

Landis, C. A., Masters, S. B., Spada, A., Pace, A. M., Bourne, H. R., and Vallar, L. (1989). GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. *Nature* **340:** 692–696.

Leder, A., Swan, D., Ruddle, F., D'Eustachio, P., and Leder, P. (1981). Dispersion of alpha-like globin genes of the mouse to three different chromosomes. *Nature* **293:** 196–200.

Lyons, J., Landis, C. A., Harsh, G., Vallar, L., Grünewald, K., Feichtinger, H., Duh, Q.-Y., Clark, O. H., Kawasaki, E., Bourne, H. R., and McCormick, F. (1990). Two G protein oncogenes in human endocrine tumors. *Science* **249:** 655–659.

Magovcevic, I., Ang, S. L., Seidman, J. G., Tolman, C. J., Neer, E. J., and Morton, C. C. (1992). Regional localization of the human G protein alpha i2 (GNAI2) gene: Assignment to 3p21 and a related sequence (GNAI2L) to 12p12–p13. *Genomics* **12:** 125–129.

Miyasaka, H., Choudhury, B. K., Hou, E. W., and Li, S. S. (1993). Molecular cloning and expression of mouse and human cDNA encoding AES and ESG proteins with strong similarity to Drosophila enhancer of split groucho protein. *Eur. J. Biochem.* **216:** 343–352.

Patten, J. L., Johns, D. R., Valle, D., Eil, C., Gruppuso, P. O., Steele, G., Smallwood, P. M., and Levine, M. A. (1990). Mutation in the gene encoding the stimulatory G protein of adenylate cyclase in Albright's hereditary osteodystrophy. *N. Engl. J. Med.* **322:** 1412–1419.

Saitou, N., and Ueda, S. (1994). Evolutionary rates of insertion and deletion in noncoding nucleotide sequences of primates. *Mol. Biol. Evol.* **11:** 504–512.

Simon, M. I., Strathmann, M. P., and Gautum, N. (1991). Diversity of G proteins in signal transduction. *Science* **252:** 802–808.

Smrcka, A. V., Hepler, J. R., Brown, K. O., and Sternweis, P. C. (1991). Regulation of polyphosphoinositide-specific phospholipase C activity by purified Gq. *Science* **251:** 804–807.

Spiegel, A. M., Shenker, A., and Weinstein, L. S. (1992). Receptor-effector coupling by G proteins: Implications for normal and abnormal signal transduction. *Endocr. Rev.* **13:** 536–565.

Strathmann, M., and Simon, M. I. (1990). G protein diversity: A distinct class of α subunits is present in vertebrates and invertebrates. *Proc. Natl. Acad. Sci. USA* **87:** 9113–9117.

Suarez, H. G., du Villard, J. A., Caillou, B., Schlumberger, M., Parmentier, C., and Monier, R. (1991). gsp mutations in human thyroid tumours. *Oncogene* **6:** 677–679.

Taylor, S. J., Chae, H. Z., Rhee, S. G., and Exton, J. H. (1991). Activation of the β1 isozyme of phospholipase C by a subunits of Gq class of G proteins. *Nature* **350:** 516–518.

Weinstein, L. S., Spiegel, A. M., and Carter, A. D. (1988). Cloning and characterization of the human gene for the alpha-subunit of Gi2, a GTP-binding signal transduction protein. *FEBS Lett.* **232:** 333–340.

Weinstein, L. S., Shenker, A., Gejman, P. V., Merino, M. J., Friedman, E., and Spiegel, A. M. (1991). Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N. Engl. J. Med.* **325:** 1688–1695.

Weinstein, L. S., Gejman, P. V., Friedman, E., Kadowaki, T., Collins, R. M., Gershon, E. S., and Spiegel, A. M. (1990). Mutations of the Gs-alpha subunit gene in Albright hereditary osteodystrophy detected by denaturing gradient gel electrophoresis. *Proc. Natl. Acad. Sci. USA* **87:** 8287–8290.

Wilkie, T. M., Gilbert, D. J., Olsen, A. S., Chen, X. N., Amatruda, T. T., Korenberg, J. R., Trask, B. J., de Jong, P., Reed, R. R., and Simon, M. I. (1992). Evolution of the mammalian G protein alpha subunit multigene family. *Nature Genet.* **1:** 85–91.

Wu, D. Q., Lee, C. H., Rhee, S. G., and Simon, M. I. (1992). Activation of phospholipase C by the alpha subunits of the Gq and G11 proteins in transfected Cos-7 cells. *J. Biol. Chem.* **267:** 1811–1817.