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Mutated α Subunit of the G_q Protein Induces Malignant Transformation in NIH 3T3 Cells

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The discovery of mutated, GTPase-deficient α subunits of G_s or G_{i2} in certain human endocrine tumors has suggested that heterotrimeric G proteins play a role in the oncogenic process. Expression of these altered forms of $G\alpha_s$ or $G\alpha_{i2}$ proteins in rodent fibroblasts activates or inhibits endogenous adenylyl cyclase, respectively, and causes certain alterations in cell growth. However, it is not clear whether growth abnormalities result from altered cyclic AMP synthesis. In the present study, we asked whether a recently discovered family of G proteins, G_q, which does not affect adenylyl cyclase activity, but instead mediates the activation of phosphatidylinositol-specific phospholipase C harbors transforming potential. We mutated the cDNA for the α subunit of murine G_q in codons corresponding to a region involved in binding and hydrolysis of GTP. Similar mutations unmask the transforming potential of $p21^{ras}$ or activate the α subunits of G_s or G_{i2}. Our results show that when expressed in NIH 3T3 cells, activating mutations convert $G\alpha_q$ into a dominant acting oncogene.

Certain alterations of proteins involved in mitogenic signaling are known to cause profound aberrations in cell growth, including malignant transformation (1). One such example is the mutational activation of *ras* genes, whose altered forms are implicated in a wide variety of naturally occurring tumors (4). The *ras* proteins belong to a large family of signal-transducing molecules that bind guanine nucleotides and exchange GDP for GTP as a result of their interaction with specific activated receptors. The GTP-bound form of these proteins in turn regulates the activity of specific downstream effectors, until signal transduction is terminated by virtue of an intrinsic GTPase activity (6). Mutationally activated *ras* genes encode proteins with reduced ability to hydrolyze GTP, thus remaining largely in the active, GTP-bound form (4). Similar GTPase-inhibiting mutations have been found in genes for α subunits of G_s and G_{i2} (20, 21, 33), heterotrimeric G proteins that mediate the activation and inhibition, respectively, of adenylyl cyclase. These altered forms of G_s and G_{i2} were identified in a subset of human endocrine tumors (21, 33), raising the possibility that activated heterotrimeric G proteins also contribute to the oncogenic process. Expression of these mutant $G\alpha_s$ and $G\alpha_{i2}$ proteins in rodent fibroblasts activates and inhibits, respectively, endogenous adenylyl cyclase and causes alterations in cell growth (13, 25, 38, 40). Recently, a new family of heterotrimeric G proteins, G_q, has been identified (31). Its α subunits, designated α_q, α_{11}, α_{14}, α_{15}, and α_{16} (3, 31, 37), are highly related to one another. The G_q family of G proteins appears to be widely expressed, although some of its members are expressed in a tissue- or developmentally specific manner (3, 37). Available evidence suggests that G_q does not affect adenylyl cyclase activity, but instead mediates phosphatidylinositol-specific phospholipase C (PI-PLC) activation (11, 29, 32, 39). Since this biochemical coupling has been demonstrated for G_q, other members of the family are expected to behave similarly. Because the PI-PLC pathway is implicated in mitogenic signaling for a wide variety of growth factors (26, 35) and transforming receptors (2, 10, 16), we asked whether the G_q class of G proteins harbors oncogenic potential. Our results show that, in contrast with $G\alpha_{i2}$, NIH 3T3 cells transfected with an activated mutant of $G\alpha_q$ are fully transformed and that these cells are highly tumorigenic in athymic nude mice.

MATERIALS AND METHODS

Materials. *myo*-[2-^{3}H]inositol (15 Ci/mmol) was from Dupont, NEN Research Products. Tissue culture products used were Dulbecco's modified Eagle's medium (DMEM) (GIBCO), the neomycin analog G418 (GIBCO), and calf serum (Advanced Biotechnologies, Inc.). Oligonucleotides were synthesized by Lofstrand Laboratories, and the polymerase chain reaction kit was from Perkin-Elmer Cetus. AG1-X8 anion-exchange resin (100/200 mesh, formate form) was from Bio-Rad. All other chemicals were purchased from Sigma.

Expression vectors. The complete open reading frame of the murine $G\alpha_q$ cDNA was obtained by the polymerase chain reaction technique from mouse brain cDNA and cloned between the *Bam*HI site and the *Hin*dIII site of the pBluescript bacterial plasmid (Stratagene). The DNA sequence of the polymerase chain reaction product was identical to that of the published cDNA (31). Mutated α_q-G207T and α_q-Q209L were obtained by site-directed mutagenesis of double-stranded DNA by overlap extension, using polymerase chain reaction methodology (15). The mutagenic oligonucleotides had the following sequence: α_q-G207T, 5′-CC TTTGGCCCGTTACATCGAC-3′ and 5′-GTCGATGTAAC GGGCCAAAGG-3′; α_q-Q209L, 5′-CTCTGACCTTAGGCC CCCTAC-3′ and 5′-GTAGGGGGCCTAAGGTCAGAG-3′. Modified nucleotides are underlined. In both cases, a final amplified DNA fragment of 512 bp was obtained with the 5′ and 3′ oligonucleotides 5′-GAGAGGTTGATGTGGAGA

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AG-3' and 5'-GATTTTCTCCTCTAGAAG-3', respectively. Mutated DNA fragments were recombined with flanking wild-type sequences by using the internal *Nde*I and *Bgl*II restriction sites, and mutations were confirmed by dideoxynucleotide sequencing. The *Hin*dIII site in pBluescript was modified to a *Not*I site by using appropriate linkers, and the complete coding sequences were excised by *Not*I digestion and transferred into the *Not*I site of a modified mammalian expression vector, pZipNeoSV(X) (pZN) (7). Construction of wild-type and mutated $G\alpha_{i2}$ expression plasmids has been described previously (13).

Transfection of NIH 3T3 cells. Plasmid DNA transfection of NIH 3T3 (16) cells was performed by the calcium phosphate precipitation technique, as modified by Wigler et al. (36). Transformed foci were scored after 2 to 3 weeks as previously described (10). Mass populations expressing transfected genes were selected for ability to grow in the presence of geneticin (G418) (GIBCO). Individual transformed foci were isolated with the aid of cloning cylinders and maintained in DMEM–10% calf serum.

Colony formation in semisolid medium and tumorigenesis in nude mice. For assays of anchorage-independent growth, suspensions of 5×10^3 cells in 0.3% agar in DMEM–10% calf serum were laid onto medium containing 0.55% agar as described previously (13). Colonies over 0.10 mm in diameter were scored after 15 days of culture. For tumorigenicity, actively growing cells were dislodged from tissue culture plates with EDTA and resuspended in DMEM at a concentration of 2×10^5 viable cells per ml. Cells (10^5, 0.5 ml) were injected subcutaneously into 4- to 8-week-old female athymic NIH mice (*nu/nu*), and animals were examined twice per week for tumor formation.

Immunoblotting. Lysates from confluent cells were analyzed by immunoblotting as described previously (13). Briefly, 40 μg of cellular proteins was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose. Filters were incubated with anti-$G\alpha_q$ serum (29), and bands were visualized after treatment with ^{125}I-protein A by autoradiography as previously described (13).

PI hydrolysis. For determination of inositol phosphates (IPs), subconfluent cultures of NIH 3T3 cell transfectants were labeled to equilibrium with *myo*-[^{3}H]inositol (10 μCi/33-mm plate). The labeled medium was removed, and cells were rinsed twice with phosphate-buffered saline (PBS) and incubated for 4 h in serum-free DMEM or DMEM plus 10% calf serum. Cultures were aspirated, rinsed once with cold PBS, and treated with 1 ml of cold 6% trichloroacetic acid. Treated cells were kept on ice for 30 min to extract the water-soluble IPs. The IPs were separated by anion-exchange chromatography as previously described (10). Inositol lipids were extracted from insoluble cell residue twice with chloroform-methanol-concentrated HCl (200:100:1 by volume). Phase split was induced by adding 0.5 ml of chloroform and 0.5 ml of 0.1 N HCl to each 1.5-ml sample. After mixing and a brief centrifugation, an aliquot of the lower phase was dried under N_2 and counted. For each cell line, total counts for IP_1, IP_2, and IP_3 were normalized by the radioactive counts in phospholipids. For *myo*-[^{3}H]inositol incorporation, subconfluent cultures of transfected cells were rinsed twice with DMEM and then incubated for 4 h in serum-free DMEM or DMEM plus 10% dialyzed calf serum. Media were supplemented with *myo*-[^{3}H]inositol (2 μCi/ml/33-mm plate), and at different intervals, phospholipids were extracted as described above. Radioactivity in the phosphoinositide pools was normalized to the protein concentration in the cell lysates.

TABLE 1. Focus-forming activity of wild-type and mutated α subunits of G proteins[a]

DNA construct	Focus-forming activity (no. of foci)/μg of DNA	Colony-forming activity (no. of colonies)/μg of DNA
pZN	<1	>500
pZNα_q-wt	<1	236 ± 18
pZNα_q-G207T	<1	235 ± 28
pZNα_q-Q209L	27 ± 8	4 ± 1
pZNα_{i2}-wt	<1	346 ± 24
pZNα_{i2}-Q205L	<1	320 ± 44
pT24	2,160 ± 172	ND[b]

[a] Wild-type or mutated $G\alpha_q$ or $G\alpha_{i2}$ cDNAs were inserted into an expression vector, pZipNeoSV(X) (pZN) (7), and 0.05 to 2 μg of plasmid DNA was transfected into NIH 3T3 murine fibroblasts, using the human *ras* oncogene (pT24) (27) as a control. Cultures were scored both for colony-forming activity in the presence of G418 (GIBCO) and for focus formation 2 to 3 weeks after transfection. Data shown represent mean values ± SE of triplicate plates from three independent experiments.

[b] ND, not determined.

RESULTS

Transfection of mutated α_q induces focus formation in NIH 3T3 cells. We mutated mouse α_q at codons corresponding to the highly conserved G-3 region of G proteins (6). The G-3 region is involved in binding and hydrolysis of GTP and has the Gα consensus sequence DVGGQ. A similar motif is also present in most low-molecular-weight GTP-binding proteins of the *ras* family (4). We generated cDNAs that encoded threonine instead of glycine at position 207 (α_q-G207T) or leucine instead of glutamine at position 209 (α_q-Q209L). Mutations analogous to α_q-Q209L constitutively activate the α subunits of G_s or G_{i2} (13, 25, 38, 40). Furthermore, mutation of the corresponding sequence in the *ras* gene (codon 61) increases steady-state levels of GTP-bound $p21^{ras}$ and unmasks its oncogenic potential (4). Thus, mutation of glutamine 209 in $G\alpha_q$ would be expected to produce a constitutively activated G protein. Whereas mutations similar to α_q-G207T activate *ras* (codon 59 mutations) (4), the analogous mutation is inhibitory for α_{i2} (24).

Plasmids carrying wild-type (wt) or mutated murine α_q cDNAs were constructed such that their expression was driven by the Moloney murine leukemia virus long terminal repeat, a potent transcriptional promoter in NIH 3T3 cells. Each construct also contained a dominant selectable marker, *neo* (7), that conferred resistance to geneticin (G418). Genes for wild-type α_{i2} (α_{i2}-wt) or mutants analogous to α_q-Q209L (α_{i2}-Q205L) cloned in the same expression vector or the human *ras* oncogene (pT24) (27) were used as controls. The number of G418-resistant colonies was similar for each transfected plasmid, except for the α_q-Q209L construct, which induced very few resistant colonies (Table 1). Only α_q-Q209L or the human *ras* oncogene induced focus formation (Table 1 and Fig. 1). *ras* displayed a higher focus-forming activity, and these foci were larger and more diffuse than α_q-Q209L-induced foci, which were compact and punctate (Fig. 1). Cells from individual α_q-Q209L foci displayed a typical transformed morphology (see below) that was retained during repeated subculturing. Cells also grew as foci on a lawn of untransfected NIH 3T3 cells (data not shown). Thus, we conclude that α_q-Q209L acts as a focus-forming oncogene.

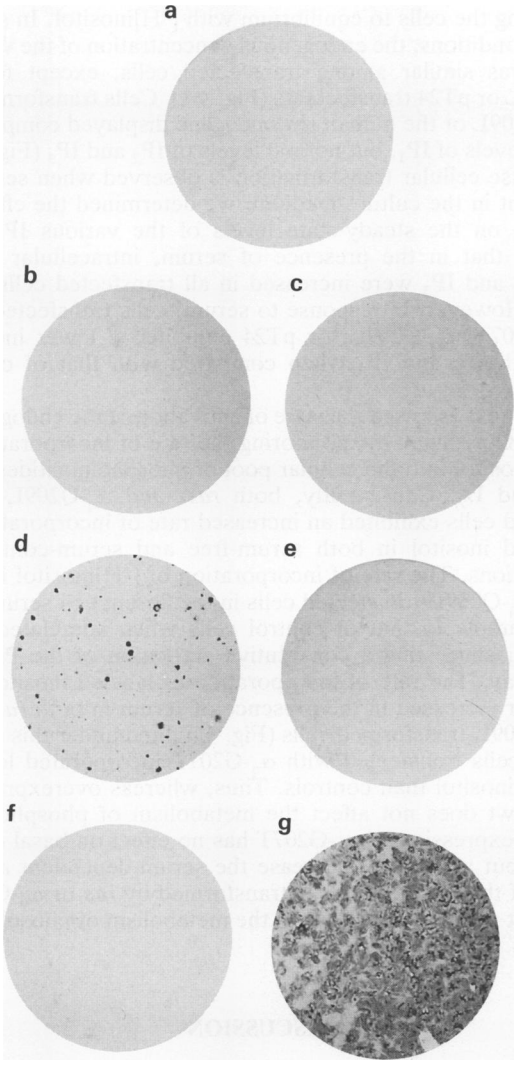


FIG. 1. Focus-forming activity of wild-type and mutated Gα cDNAs. NIH 3T3 cells were transfected with 2 μg of pZN (a), pZNα_q-wt (b), pZNα_q-G207T (c), pZNα_q-Q209L (d), pZNα_{i2}-wt (e), or pZNα_{i2}-Q205L (f) or 0.2 μg of pT24 (g) plasmid DNA. Cultures were maintained in DMEM plus 10% calf serum, and cells were stained 3 weeks after transfection.

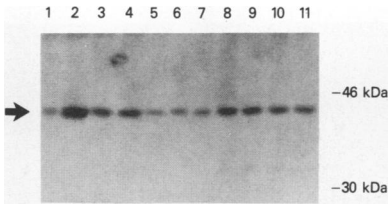


FIG. 2. Expression of Gα_q protein in transfected NIH 3T3 cells. Lysates containing 40 μg of cellular protein from G418-selected NIH 3T3 cells transfected with plasmid pZN (lane 1), pZNα_q-wt (lane 2), pZNα_q-G207T (lane 3), pZNα_q-Q209L (lane 4), pZNα_{i2}-wt (lane 5), pZNα_{i2}-Q205L (lane 6), or pT24 (lane 7) or from four individual pZNα_q-Q209L-induced foci (lanes 8 to 11) were fractionated by SDS-PAGE and transferred to nitrocellulose as described previously (13). Filters were incubated with anti-Gα_q serum (29), and after treatment with ^{125}I-protein A, bands were visualized by autoradiography as previously described (13). Products of Gα_q genes are indicated by an arrow. The electrophoretic mobility of molecular size standards is shown.

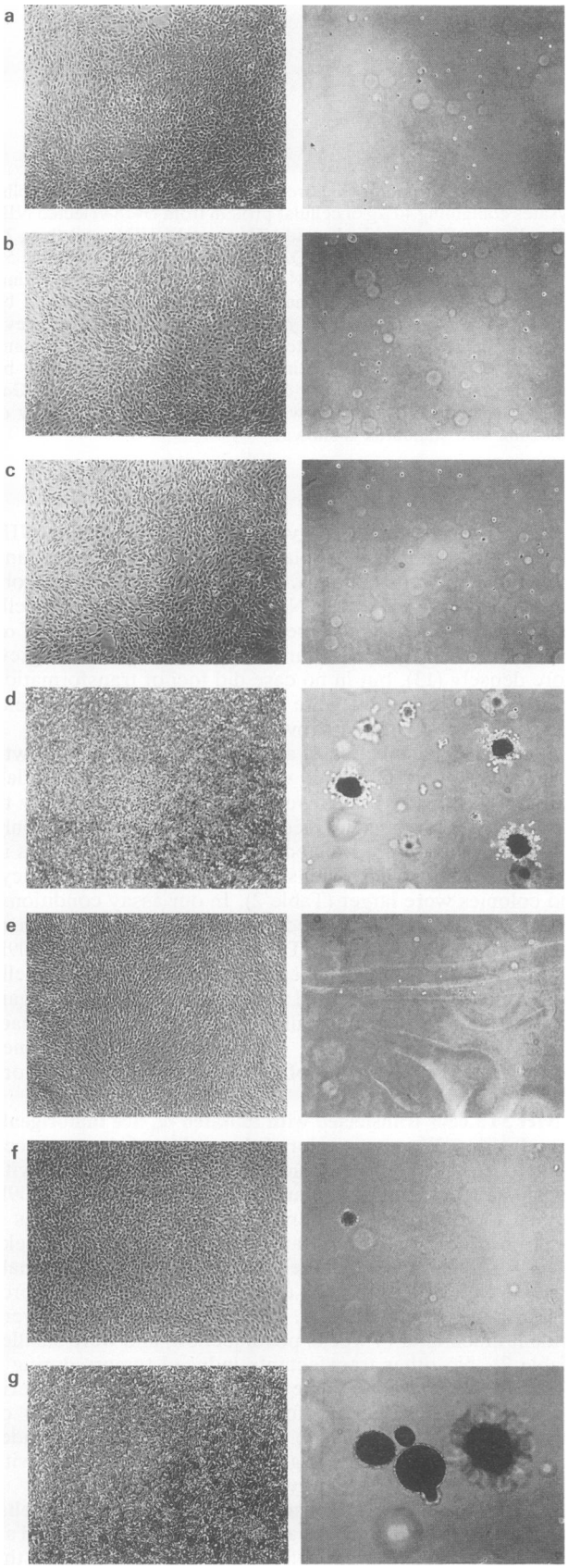
Expression of α_q in transfected cells. We examined the expression of α_q in lysates of transfected, G418-selected cells. As shown in Fig. 2, immunoreactive Gα_q was readily detectable in vector-transfected NIH 3T3 cells, and it was remarkably overexpressed in α_q-wt transfectants (Fig. 2). Expression of α_q-G207T or α_q-Q209L proteins was more limited, approximately twofold greater than that of the endogenous G protein. Similar results were obtained with lysates from cloned α_q-Q209L foci (Fig. 2). Our data demonstrate that a limited expression of α_q-Q209L is sufficient to induce focus formation. In contrast, even when overexpressed, α_{i2}-wt, α_{i2}-Q205L (13; data not shown), α_q-wt, and α_q-G207T were not able to induce focus formation.

Effect of α_q on NIH 3T3 cell proliferation. We next studied growth properties of these transfected NIH 3T3 cells. Cultures transfected with α_q-Q209L were readily distinguishable from parental or control transfected cells (Fig. 3). While control cells grew in a monolayer and showed the characteristic fusiform morphology, α_q-Q209L-transfected NIH 3T3 cells were rounded, displayed refractile cell bodies, and grew in a crisscross pattern. Similar morphology was observed in pT24-transfected NIH 3T3 cells, but not in cells transfected with the wild type or other mutated forms of α_q or α_{i2}. Particularly, cells transfected with α_{i2}-Q205L grew more densely (13), but in no case did foci of transformation arise even when cells were cultured for up to 3 weeks postconfluence (data not shown).

Because the acquisition of anchorage-independent growth of fibroblasts correlates well with their malignant potential, transfected NIH 3T3 cells were assayed for their ability to form colonies in semisolid medium. As shown in Fig. 3, only α_q-Q209L- and *ras*-transfected cells formed large colonies in soft agar. *ras*-transfected cells plated with greater efficiency, and colonies were larger (Table 2). In our assay conditions, *ras*-transformed cells formed compact colonies, whereas colonies formed by NIH 3T3 transfected with α_q-Q209L displayed a more diffuse appearance (Fig. 3). NIH 3T3 cells expressing α_{i2}-Q205L formed very few colonies in soft agar, and the colonies were smaller and exhibited a compact morphology (Fig. 2; Table 2), although selected clones expressing very high levels of α_{i2}-Q205L protein grew more efficiently in soft agar (13).

NIH 3T3 cells transfected with mutated α_q are tumorigenic in nude mice. We next explored the ability of the same cell lines to form tumors in nude mice. Mice injected with transfectants bearing the human *ras* oncogene or α_q-Q209L formed large tumors. Tumors were evident as early as 1 week postinjection for *ras*-transfected cells or after 2 weeks for α_q-Q209L-transfected cells (Table 2). Some animals injected with NIH 3T3 cells expressing the α_{i2}-Q205L protein also developed tumors, although these tumors were evident much later (4 weeks postinjection) and were smaller (Table 2). No other transfectants induced tumors during 7 weeks of observation (Table 2). Thus, by several criteria including focus induction, focus formation on a lawn of contact-inhibited fibroblasts, morphology, anchorage-independent growth, and tumorigenicity, cells transfected with the α_q-Q209L cDNA are fully malignant.

Effect of α_q on PI metabolism in transfected NIH 3T3 cells. To determine whether cells transformed by Gα_q displayed an altered metabolism of phosphoinositides, we examined the steady-state level of IPs in transfected NIH 3T3 cells after



labeling the cells to equilibrium with [^{3}H]inositol. In serum-free conditions, the endogenous concentration of the various IPs was similar among transfected cells, except for α_q-Q209L or pT24 transfectants (Fig. 4A). Cells transformed by α_q-Q209L or the human *ras* oncogene displayed comparable high levels of IP_1, but normal levels of IP_2 and IP_3 (Fig. 4A). Because cellular transformation is observed when serum is present in the culture medium, we determined the effect of serum on the steady-state levels of the various IPs. We found that in the presence of serum, intracellular levels of IP_1 and IP_3 were increased in all transfected cells (Fig. 4B). However, in response to serum, cells transfected with α_q-G207T, α_q-Q209L, or pT24 exhibited a lower increase in both IP_1 and IP_3 when compared with that of control cells.

We next assessed the state of activation of the endogenous PI-PLC pathway by monitoring the rate of incorporation of [^{3}H]inositol into the cellular pool of phosphoinositides (Fig. 4C and D). Consistently, both *ras*- and α_q-Q209L-transformed cells exhibited an increased rate of incorporation of labeled inositol in both serum-free and serum-containing conditions. The rate of incorporation of [^{3}H]inositol in *ras*- and α_q-Q209L-transfected cells in the absence of serum was comparable to that of control cells when stimulated with serum, suggesting a constitutive activation of the PI-PLC pathway. The rate of incorporation of labeled inositol was further increased in the presence of serum in both *ras*- and α_q-Q209L-transformed cells (Fig. 4D), and under this condition, cells transfected with α_q-G207T incorporated less labeled inositol than controls. Thus, whereas overexpression of α_q-wt does not affect the metabolism of phosphoinositides, expression of α_q-G207T has no effect on basal conditions but appears to decrease the serum-dependent activation of this pathway. Cells transformed by *ras* or α_q-Q209L exhibit marked alterations in the metabolism of phosphoinositides.

DISCUSSION

Somatic mutations in α_s or α_{i2} genes have recently been found in human tumors (20, 21, 33). Such tumors were restricted to those tissues in which mutated α_s or α_{i2} could mimic mitogenic effects of trophic hormones that normally stimulate or inhibit adenylyl cyclase (21, 33), respectively. However, we have shown that activation of receptors effectively coupled through G_i to inhibition of adenylyl cyclase does not transform NIH 3T3 cells (10) and fails to induce reinitiation of DNA synthesis in quiescent cells (9a). Consistently, we and others have demonstrated that constitutively activated $G\alpha_{i2}$ induces alterations in cell growth, but fails to induce a fully transformed phenotype when expressed in NIH 3T3 cells (13, 25; present study). Thus, alterations in G proteins or receptors coupled to G proteins might contribute to oncogenesis only in cells capable of

FIG. 3. Morphology and soft agar growth of G418-resistant NIH 3T3 cell transfectants. Cultures transfected with pZN (a), pZNα_q-wt (b), pZNα_q-G207T (c), pZNα_q-Q209L (d), pZNα_{i2}-wt (e), pZNα_{i2}-Q205L (f), or pT24 (g) were G418 selected and photographed when nearly confluent (left panel) (×10). Human *ras* transfectants were not treated with G418. Cells were trypsinized, and aliquots containing 1×10^3 to 5×10^3 transfected cells were plated in 0.3% agar containing DMEM plus 10% calf serum as described previously (13). Photographs were taken after 15 days of growth (right panel) (×10).

TABLE 2. Soft agar growth and tumorigenicity of NIH 3T3 cells transfected with wild-type and mutated α subunits of G proteins[a]

Gene	Growth in agar		Tumorigenicity in nude mice		
	Colony efficiency (%)	Colony size (mm)	No. of tumors/ no. of injections[b]	Latency (days)	Tumors >1 cm by 40 days
neo	<0.5	<0.1	0/5	>40	0/5
α_q-wt	<0.5	<0.1	0/5	>40	0/5
α_q-G207T	<0.5	<0.1	0/5	>40	0/5
α_q-Q209L	19	0.15–0.35	5/5	14–17	5/5
α_{i2}-wt	<0.5	<0.1	0/5	>40	0/5
α_{i2}-Q205L	3	0.10–0.30	1/5	28–>40	0/5
T24	>80	0.15–0.35	5/5	6–12	5/5

[a] Colony formation in semisolid medium was determined after 2 weeks of culture in 0.3% agar in DMEM plus 10% calf serum as described previously (13). Colony-forming efficiency = (number of colonies observed/number of cells seeded) × 100. For tumorigenicity, 10^5 cells (0.5 ml) were injected subcutaneously into 4- to 8-week-old female athymic NIH mice (*nu/nu*). Mice were examined twice per week for tumor formation. Latency is defined as the time interval required for a palpable tumor to arise. All animals with tumors exceeding 2.0 cm during the observation period were immediately sacrificed.

[b] Tumors were scored as positive when greater than 5 mm in diameter by 28 days postinjection.

utilizing their linked pathways to induce mitogenesis. Alternatively, increased mitogenesis might play a key role in the promotion or progression of neoplastic transformation, but might not be sufficient itself to induce the malignant phenotype. For example, the GTPase-deficient, constitutively activated α subunit of G_s fails to transform Swiss 3T3 cells (40), despite elevated cytoplasmic levels of cyclic AMP, which is a proliferation mediator for this particular cell line (26, 40).

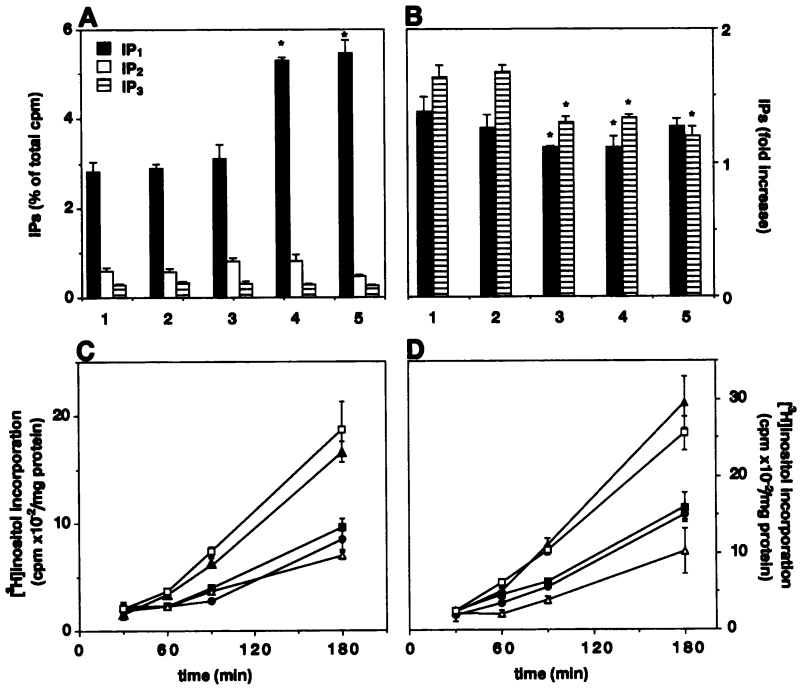


FIG. 4. Steady-state levels of IPs (A and B) and time course of incorporation of *myo*-[^{3}H]inositol into the phosphoinositide pool (C and D) of transfected NIH 3T3 cells. For determination of IPs, subconfluent cultures of NIH 3T3 cells transfected with pZN (column 1), pZNα_q-wt (column 2), pZNα_q-G207T (column 3), pZNα_q-Q209L (column 4), or pT24 (column 5) were labeled to equilibrium with *myo*-[^{3}H]inositol (10 μCi/33-mm plate). The labeled medium was removed, and cells were rinsed twice with PBS and incubated for 4 h in serum-free DMEM (A) or DMEM plus 10% calf serum (B). Cultures were aspirated, and cells were treated as described in Materials and Methods. Water-soluble IPs were extracted and separated by anion-exchange chromatography as previously described (10). Inositol lipids were extracted from insoluble cell residue as described in Materials and Methods, and the radioactivity was counted. For each cell line, total counts for IP_1 (■), IP_2 (□), and IP_3 (▤) were normalized by the radioactive counts in phospholipids. In panel B, data are expressed as the fold increase in intracellular levels of IPs when cells were cultured in the presence of 10% serum with respect to cells in serum-free medium. Intracellular levels of IP_2 were not affected by the treatment (data not shown). For *myo*-[^{3}H]inositol incorporation, subconfluent cultures of cells transfected with pZN (■), pZNα_q-wt (●), pZNα_q-G207T (△), pZNα_q-Q209L (▲) or pT24 (□) were rinsed twice with DMEM and then incubated for 4 h in serum-free DMEM (C) or DMEM plus 10% dialyzed calf serum (D). Media were supplemented with *myo*-[^{3}H]inositol (2 μCi/ml/33-mm plate), and at the indicated intervals, phospholipids were extracted as described above. Data represent the radioactivity in the phosphoinositide pools, normalized to the protein concentration in cell lysates. Mean values ± standard error of triplicate samples were determined from three independent experiments. *, $P < 0.05$ when compared with pZN-transfected cells. Data obtained from pZNα_{i2}-wt or pZNα_{i2}-Q205L transfectants did not differ significantly from those of control cells and are not shown.

Therefore, the relationship between stimulation of cell proliferation and transformation by G proteins remains to be precisely defined.

Alterations in the metabolism of phosphoinositides have frequently been implicated in cellular transformation (8, 12). For example, the inhibition of this biochemical pathway with immunological reagents has unveiled its critical role in mitogenesis and/or transformation induced by both activated tyrosine kinases and $p21^{ras}$ (23, 30). Furthermore, receptors that couple through G proteins to the activation of PI-PLC can effectively transform NIH 3T3 cells (2, 10, 17). Thus, continuous activation of the PI-PLC pathway might result in cellular transformation. By several different approaches, it has been recently shown that G_q or its highly homologous G protein, G_{11}, can at least mediate receptor-activated PI-PLC (11, 29, 32, 39). As expected, transient overexpression in COS-7 cells of α_q-Q209L but not of wild-type α_q induces a remarkable elevation in intracellular levels of all IPs (39, 39a). In NIH 3T3 cells, however, our biochemical data are consistent with an increased basal turnover of PI in cells transformed by α_q-Q209L, but in these stable transfectants, the steady-state levels of IP_2 and IP_3, products of this pathway, are not demonstrably affected. In addition, the presence of serum further increases the activation of the PI-PLC pathway, but poorly elevates intracellular levels of IP_3 in α_q-Q209L. This inconsistency can be the result of adaptive changes in established cells transformed by α_q-Q209L, or of cells selected by their ability to rapidly metabolize IP_3 (see below).

In addition to cellular transformation, the low colony-forming activity of the α_q-Q209L expression plasmid might represent a growth inhibitory effect. A similar situation, namely, growth inhibition and transformation, has been previously shown for the potent v-*abl* oncogene (34, 41) and more recently for the c-*fgr* proto-oncogene (28). In an effort to further characterize the apparent growth inhibition by mutated α_q-Q209L, we used an approach similar to that previously described by Goff et al. (9). Each DNA construct used in this study was cotransfected together with an expression plasmid carrying a *gpt* selectable marker. Upon selection for *gpt* expression, cells cotransfected with pZNα_q-Q209L consistently induced 70 to 80% fewer colonies than those cotransfected with other plasmids (data not shown). These data strongly suggest that expression of α_q-Q209L is toxic for cells. Recently, Wu et al. (39) arrived at a similar conclusion after cotransfecting expression plasmids carrying a similar mutant of α_q and a different reporter gene. Thus, it is likely that whereas limited expression of the α_q-Q209L protein is transforming, higher expression is cytotoxic.

The mechanism for inhibition of cell growth or toxicity by α_q-Q209L is unknown. It is possible that decreased cell proliferation or viability is a consequence of high levels of intracellular Ca^{2+} induced by increased IP_3, thus selecting for cells that can metabolize IP_3 more rapidly. This hypothesis is consistent with the lack of elevated IP_3 in cells transformed by α_q-Q209L despite an increased activity of the endogenous PI-PLC pathway. Nevertheless, this issue is not resolved and warrants further investigation. We expect that further studies with suitable inducible promoters directing the expression of activated α_q might provide a means for more precisely identifying those G_q-linked pathways directly involved in cellular transformation and/or growth inhibition or cytotoxicity.

The focus-forming activity of receptors coupled through G proteins to PI-PLC is several orders of magnitude higher than that of activated G_q (2, 10). This discrepancy could be explained by the dual transforming and toxic effects of activated G_q. Alternatively, other biochemical pathways might be activated by transforming G protein-coupled receptors, which, in addition to PI hydrolysis, would result in higher transforming efficiency. In this regard, recent studies have suggested that activation of the PI-PLC pathway by tyrosine kinase growth factor receptors is not sufficient to induce DNA synthesis (14, 22). Thus, it would be interesting to know whether this is also the case for G protein-coupled receptors.

Our present results demonstrate that a mutated α_q gene can act as a fully transforming oncogene. This finding is consistent with previous observations that receptors coupled through G proteins to PI hydrolysis but not to inhibition of adenylyl cyclase are capable of transforming rodent fibroblasts (10). Furthermore, our findings strongly suggest that genes for α_q and its related G-protein α subunits (α_{11}, α_{14}, α_{15}, and α_{16}) can be considered proto-oncogenes. The recent observation that genes for proteins that are thought to interact with and probably inhibit this class of G proteins are frequently mutated in certain common human tumors (5, 18, 19) supports the idea that alterations in signaling through G_q or related G proteins might be relevant to naturally occurring tumorigenesis. We expect that further studies on the oncogenic pathways activated by transforming mutants of $G\alpha_q$ or their coupled receptors will yield valuable information about malignant transformation and help identify new targets for therapeutic intervention in neoplastic disease.

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