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Myristoylation of the $G\alpha_{i2}$ polypeptide, a G protein α subunit, is required for its signaling and transformation functions

(*gip2*/GTPase inhibition/myristic acid/signal transduction)

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ABSTRACT **GTPase-inhibiting mutations of the α subunit (α_{i2}) of the G protein, G_{i2}, result in constitutive activation of α_{i2} signal transduction functions. GTPase-inhibited α_{i2} mutant polypeptides, referred to as gip2 oncoproteins, have glutamine-205 mutated to leucine (α_{i2}Q205L). Expression of the α_{i2}Q205L polypeptide inhibits adenylyl cyclase stimulation, constitutively activates p42 mitogen-activated protein kinase, and transforms Rat 1a fibroblasts. The α_{i2} polypeptides are N-terminal-myristoylated, but the function of myristoylation is unclear in α_{i2} signal transduction. We have tested the requirement for myristoylation on the ability of the α_{i2}Q205L mutant polypeptide to constitutively regulate signal pathways and cell transformation. When expressed in Rat 1a cells, the nonmyristoylated α_{i2}Q205L polypeptide is membrane associated but is unable to regulate adenylyl cyclase or p42 mitogen-activated protein kinase and does not induce cellular transformation. We conclude that myristoylation is absolutely necessary for α_{i2}Q205L signal transduction and regulation of effector enzymes in the cell.**

Protein N-myristoylation involves the covalent attachment of a 14-carbon myristoyl fatty acid to the N-terminal glycine of a limited number of cellular proteins (1, 2). Among this group of proteins, cotranslational N-myristoylation appears to be important in protein localization and biological activity (2). Significant attention has been recently focused on the role of protein N-myristoylation because of its requirement for the fibroblast transforming ability of cellular oncoproteins such as v-Src and v-Abl (3–5).

A subset of G protein α subunit family members, namely α_{i1}, α_{i2}, α_{i3}, α_o, and α_z, are N-terminal-myristoylated (6–8). It has been proposed that N-terminal amino acid residues and myristoylation of specific α subunits is involved in their membrane association (7–10). This conclusion is based on several observations. (*i*) Overexpression of nonmyristoylated mutant α_{i1} and α_o polypeptides in COS cells results in its cytoplasmic accumulation (7, 8). (*ii*) Replacement of myristate with the less-hydrophobic analog 10-propoxydecanoate (or 11-oxymyristate) markedly diminished membrane interactions of the α_{i1} polypeptide in transfected COS cells (8). (*iii*) Tryptic digestion removes a 2-kDa N-terminal fragment and releases guanosine 5′-[γ-thio]triphosphate (GTP[γS])-activated α_s and α_i subunit polypeptides from the membrane (9, 11). (*iv*) Deletion of codons 2–6 of α_{i1} results in a soluble GTP-binding polypeptide (12). However, it is highly unlikely that N-terminal myristoylation simply provides a hydrophobic anchor for membrane attachment for the α_i, α_o, and α_z polypeptides. In addition to membrane attachment, it is predicted that myristoylation fulfills a specific and possibly unique function for α_i, α_o, and α_z subunits. This prediction is based on the fact that other G protein α subunits (i.e., α_s and α_q) are bound to the plasma membrane, interact with $\beta\gamma$ subunit complexes, and regulate appropriate effector enzymes in the absence of N-terminal myristoylation (7, 13–15). Also, several cytoplasmic proteins are myristoylated (1, 2), indicating that N-terminal myristate is not sufficient for plasma membrane localization. A potential role of N-terminal myristoylation would be the involvement of myristic acid in the interaction of selected α subunits with membrane proteins required for G_i, G_o, or G_z signal transduction in the cell. This would be similar to the proposed role of N-terminal myristoylation of v-Src and its interaction with cellular proteins required for Src signal transduction and cellular transformation by v-Src (3–6, 16).

Until recently, it was difficult to approach the question of whether myristoylation was important in G protein α subunit-mediated signal transduction. It has now been shown that GTPase-inhibiting mutations constitutively activate G protein α subunit-mediated signal transduction functions (17, 18). One such mutation changes the glutamine-205 codon to a leucine codon in the α_{i2} gene (α_{i2}Q205L). The GTPase-inhibited α_{i2}Q205L mutant polypeptide has been found to constitutively inhibit adenylyl cyclase stimulation in several cell types (19, 20). In addition, α_{i2}Q205L is a tissue-selective oncogene referred to as *gip2* (21), capable of causing transformation of Rat 1a fibroblasts (22, 23). The activated signal transducing ability of the gip2 oncoprotein also results in the constitutive stimulation of the p42 mitogen-activated protein kinase [p42 MAP kinase (24)]. Thus, the activated nature of the *gip2* oncogene product provides the necessary dominant responses, in a background of the wild-type endogenous α_{i2} gene product, for determining the requirement of myristoylation in α_{i2} signal transduction.

METHODS

Cell Transfection. COS-1 cells were transfected as described (25) with 1 μg of plasmid DNA by using the DEAE-dextran procedure. Sixty-five hr after transfection, cells were harvested, suspended in lysis buffer [20 mM Tris·HCl, pH 8.0/2 mM $MgCl_2$/1 mM EDTA/1 mM 2-mercaptoethanol/aprotinin (0.02 unit/ml)], and lysed by passage five times through a 27-gauge needle. Nuclei were removed by centrifugation at 1000 × *g* for 5 min. Membranes were then separated from cytosol by centrifugation at 150,000 × *g* for 1 hr.

Virus-packaging GP+E-86 cells were transfected with 15 μg of pMV-7 DNA (26) containing the appropriate α_{i2} insert by electroporation with a Bio-Rad Gene Pulsar (500 V and 25 μF). Transfected GP+E-86 cells were selected by growth in G418 (800 μg/ml) for 2 weeks. GP+E-86 cell supernatants containing retrovirus carrying wild-type or mutant G protein α_{i2} cDNAs were collected and characterized as described (22).

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Abbreviations: MAP, mitogen-activated protein; GTP[γS], guanosine 5′-[γ-thio]triphosphate.

Rat 1a cells were infected with the appropriate virus by incubation overnight in the presence of Polybrene (8 μg/ml). On the following day, the culture medium was replaced, and 24 hr later the cells were placed in G418 (800 μg/ml). Focus formation was determined 2 weeks after infection in the absence of G418 (22).

The α_{i2} mutant containing a glycine-2 to alanine replacement (8) was kindly provided by Susan M. Mumby (University of Texas Southwestern Medical Center). This mutant α_{i2} polypeptide is not myristoylated when expressed in cells after transfection (ref. 8 and data not shown). Glutamine-205 was mutated to leucine in the nonmyristoylated Gly-2 → Ala mutant α_{i2} cDNA as described (19, 22).

Pertussis Toxin-Catalyzed ADP-Ribosylation and Tryptic Digestion of Mutant α_{i2} Polypeptides. Rat 1a cell membranes were used for pertussis toxin-catalyzed [^{32}P]ADP-ribosylation of α_{i2} subunits as described (19). Membranes (11 μg) expressing the various [^{32}P]ADP-ribosylated α_{i2} polypeptides were incubated for 10 min at 30°C in the absence or presence of 100 μM GTP or 1 μM GTP[γS]. Membranes were then digested with 0.5 μg of trypsin for 3, 10, or 30 min at 30°C. The reaction was stopped by the addition of SDS sample buffer and boiling. Proteins were resolved by SDS/PAGE on an 11.5% polyacrylamide gel. The [^{32}P]ADP-ribosylated proteins were detected by autoradiography.

Measurement of cAMP. The radioimmunoassay kit from Amersham was used as described (19) to measure cellular cAMP levels according to the manufacturers directions.

Measurement of MAP Kinase Activity. The indicated Rat 1a cell clones (4×10^6 cells) were seeded in 10-cm dishes in Dulbecco's modified Eagle's medium (DMEM) containing 5% (vol/vol) fetal calf serum and 5% (vol/vol) calf serum. Twenty-four hours later the cells were placed in DMEM with 0.1% bovine serum albumin for 16 hr to induce quiescence. Cells were rinsed and harvested by scraping in ice-cold phosphate-buffered saline. Cells were then lysed in 50 mM glycerol 2-phosphate, pH 7.2/100 μM sodium vanadate/2 mM $MgCl_2$/1 mM EGTA/0.5% Triton X-100/leupeptin (10 μg/ml)/aprotinin (0.02 unit/ml)/1 mM dithiothreitol. The cell lysates were centrifuged for 5 min in a Microfuge to remove insoluble cell components and 0.5 ml of the supernatants was loaded onto a Mono Q FPLC (Pharmacia) column equilibrated in 50 mM glycerol 2-phosphate, pH 7.2/100 μM sodium vanadate/1 μM EGTA/1 mM dithiothreitol. Proteins were eluted with a linear gradient of NaCl (0–0.4 M), and 1-ml fractions were collected. Aliquots (20 μl) of the fractions were assayed as described (27) for MAP kinase activity using the synthetic peptide containing residues 662–681 of the epidermal growth factor receptor [EGFR-(662–681)] (15 min at 30°C).

Immunoblots of α_{i2} and β Subunit Polypeptides. Anti-α_{i2} antiserum raised against a 12-amino acid peptide corresponding to the C terminus of the transducin α subunit was used for α_{i2} polypeptide immunoblot analysis (22). Anti-β subunit antiserum recognizing β_1 and weakly recognizing β_2 polypeptides was used for detection of β subunits (19).

RESULTS

Expression of Myristoylated and Nonmyristoylated α_{i2} Polypeptides. Membrane and cytosolic distributions of α_{i2}, α_{i2}Q205L, and their nonmyristoylated counterparts (Myr$^-$$\alpha_{i2}$ and Myr$^-$$\alpha_{i2}$Q205L) were analyzed on immunoblots. As described for transient COS cell assays with α_{i1} and α_o (7, 8), a fraction of the nonmyristoylated α_{i2} polypeptides was found in the cytosol (Fig. 1*A*). No myristoylated α_{i2} or α_{i2}Q205L polypeptide was detected in the cytosol. The Myr$^-$$\alpha_{i2}$ and Myr$^-$$\alpha_{i2}$Q205L polypeptides actually appeared to be expressed at higher levels than the myristoylated forms α_{i2} and α_{i2}Q205L, respectively. This was especially apparent when

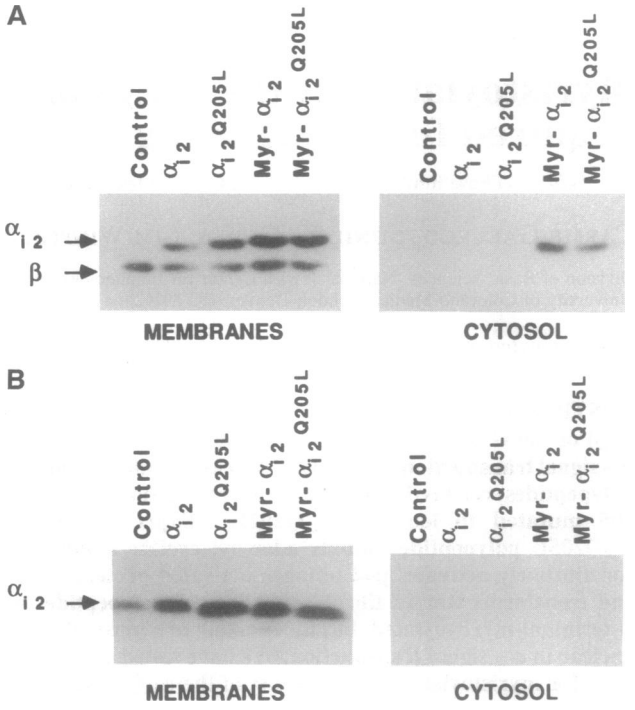

FIG. 1. Cellular distribution of wild-type and mutant α_{i2} polypeptides in COS and Rat 1a cells. Membrane and cytosolic distribution of α_{i2} polypeptides was analyzed on immunoblots after transient expression in COS-1 cells (*A*) or stable expression in Rat 1a fibroblasts (*B*). Control cells were COS cells transfected with expression vector lacking α_{i2} cDNA Rat 1a cells infected with an expression vector lacking α_{i2} cDNA. Cells were lysed and membranes and cytosol were separated. Proteins from membranes and cytosol (100 μg) were resolved by SDS/PAGE and transferred to nitrocellulose. Cell proteins were analyzed on an immunoblot with a rabbit antiserum recognizing the C terminus of α_{i2} (22). COS cell proteins were also analyzed on an immunoblot with a rabbit antisera recognizing the β subunit, showing that with overexpression of α_{i2} there was no change in β subunit polypeptide expression. ^{125}I-labeled protein A was used to detect α_{i2} and β polypeptides on the immunoblots. The COS cell blot (*A*) was exposed for 25 hr and the Rat 1a blot (*B*) was exposed for 72 hr.

the membrane and cytosolic fractions were summed. In these experiments, a substantial fraction of the Myr$^-$$\alpha_{i2}$ and Myr$^-$$\alpha_{i2}$Q205L polypeptides remained membrane-associated. Large dilutions in either high or low ionic strength buffers did not remove additional immunoreactive α_{i2} polypeptides from the COS cell membranes. The β subunit was only found in the membrane fraction and its expression was changed little, if any, even with the large increase in expression of the various α_{i2} polypeptides. Similar to the findings of others (7–10), we interpret these results to indicate that the N-myristoylation contributes to the membrane attachment of α_{i2} polypeptides but that additional considerations related to its primary amino acid sequence are also involved.

The total immunoreactive α_{i2} band was increased 2- to 3-fold in Rat 1a cells stably expressing the myristoylated and nonmyristoylated α_{i2} and α_{i2}Q205L polypeptides (Fig. 1*B*). The immunoreactive α_{i2} polypeptides were detected only in the membrane fraction and not in the cytosol. This finding indicates that, at the level of expression achieved in Rat 1a cells, all of the detectable immunoreactive α_{i2} polypeptides were membrane-associated. This is similar to the membrane association of the stably expressed nonmyristoylated form of the yeast GPa1 α subunit involved in the regulation of pheromone signal transduction (28).

The myristoylated and nonmyristoylated forms of α_{i2} and α_{i2}Q205L were solubilized from Rat 1a membranes using 0.1% Triton X-100 (Fig. 2*A*). This low concentration of

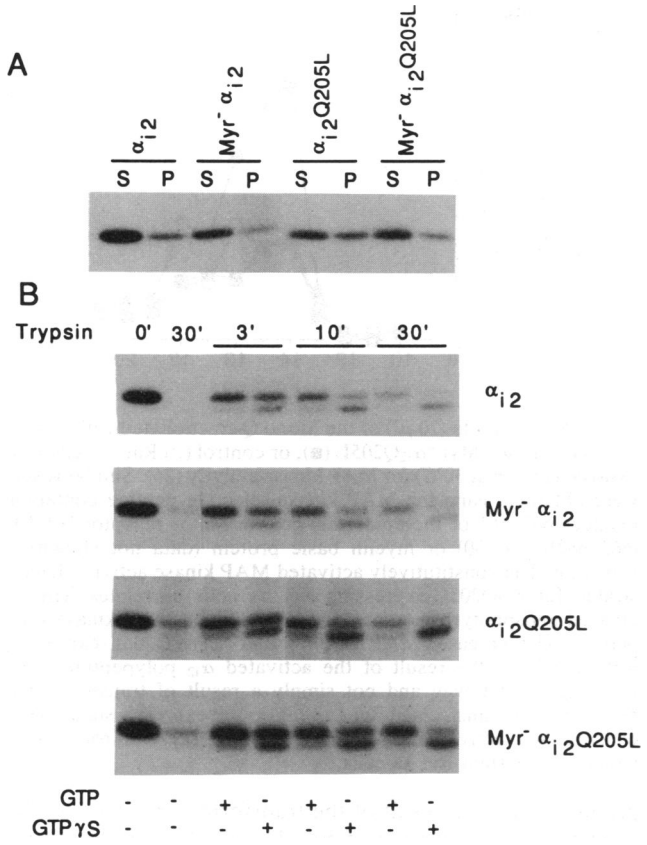

FIG. 2. (*A*) Membranes from Rat 1a cells expressing the indicated wild-type and mutant α_{i2} polypeptides were treated with 0.1% Triton X-100 for 30 min on ice. The preparation was then centrifuged at 100,000 × *g* for 30 min at 4°C. The supernatants (lanes S) and pellets (lanes P) were removed by SDS/PAGE and transferred to nitrocellulose. Proteins were analyzed on an immunoblot with the anti-α_{i2} peptide antiserum as described in Fig. 1. (*B*) Rat 1a cell membranes were [^{32}P]ADP-ribosylated and limited tryptic digestion of the [^{32}P]ADP-ribosylated α_{i2} polypeptides was performed. The 41-kDa α_{i2} band was degraded in a time-dependent manner upon addition of trypsin to the reaction mixture. The lower 39-kDa band present at 3, 5, and 10 min of trypsin treatment is most prominent in the presence of GTP[γS] but clearly is evident when the Myr⁻α_{12}Q205L- and α_{i2}Q205L-expressing membranes are incubated with GTP. The reactions were performed in parallel and gels were exposed for 36 hr. The results are representative of three experiments.

nonionic detergent solubilized 60–70% of the total immunoreactive polypeptides, indicating that the solubilization properties of the expressed myristoylated and nonmyristoylated α_{i2} polypeptides were indistinguishable.

In a GDP-bound state, G protein α subunits are highly sensitive to digestion by trypsin. When in an activated GTP[γS]-bound state, tryptic digestion removes an ≈2-kDa fragment from the N terminus (29). Fig. 2*B* shows that a trypsin-generated 39-kDa α_{i2} fragment was observed in the presence of GTP[γS] with membranes expressing each of the different recombinant α_{i2} polypeptides. In this experiment, the α_{i2} polypeptides were [^{32}P]ADP-ribosylated using pertussis toxin prior to trypsin treatment. We have shown (19) that the α_{i2}Q205L polypeptide is a substrate for pertussis toxin and that the ADP-ribosylation is similar to the level of expression of the recombinant α_{i2} polypeptide. In the absence of guanine nucleotide the [^{32}P]ADP-ribosylated polypeptides were degraded by trypsin. At each time point, in the presence of GTP[γS], a 39-kDa α_{i2}-tryptic fragment was observed. Reproducibly, it was found that the α_{i2}Q205L and Myr⁻α_{i2}Q205L polypeptides were somewhat less trypsin sensitive, suggesting a more stable activated conformation than the α_{i2} polypeptides. This is consistent with the previously characterized activated state of the α_{i2}Q205L polypeptide (17–20) and is manifested by a more prominent 39-kDa tryptic fragment in the presence of GTP[γS] than is observed with the α_{i2} polypeptides.

With membranes expressing the Myr⁻α_{i2}Q205L and α_{i2}Q205L polypeptides, a 39-kDa tryptic fragment was also generated at each time point in the presence of GTP. The 39-kDa fragment generated in the presence of GTP was significantly more prominent than that observed with the membranes expressing the Myr⁻α_{i2} or α_{i2} polypeptides. The ability to generate the α_{i2}Q205L and Myr⁻α_{i2}Q205L 39-kDa fragments in the presence of GTP was, however, less than that observed with GTP[γS]. This is likely due to the residual GTPase activity in the α_{i2}Q205L polypeptides (17, 18) and the probable differential α subunit conformations assumed with binding of GTP vs. GTP[γS]. Cumulatively, the data indicate that in Rat 1a cell membranes the Myr⁻α_{i2} and Myr⁻$_{i2}$Q205L polypeptides functionally behave similar to the myristoylated forms of α_{i2} and α_{i2}Q205L in their solubilization and ability to assume a GTP- and GTP[γS]-activated conformation.

Transformation Potential of α_{i2}Q205L and Myr⁻α_{i2}Q205L. The α_{i2}Q205L and Myr⁻α_{i2}Q205L Rat 1a cells expressed similar levels of recombinant α_{i2} polypeptide. However, the phenotypic consequence of the expression of each was

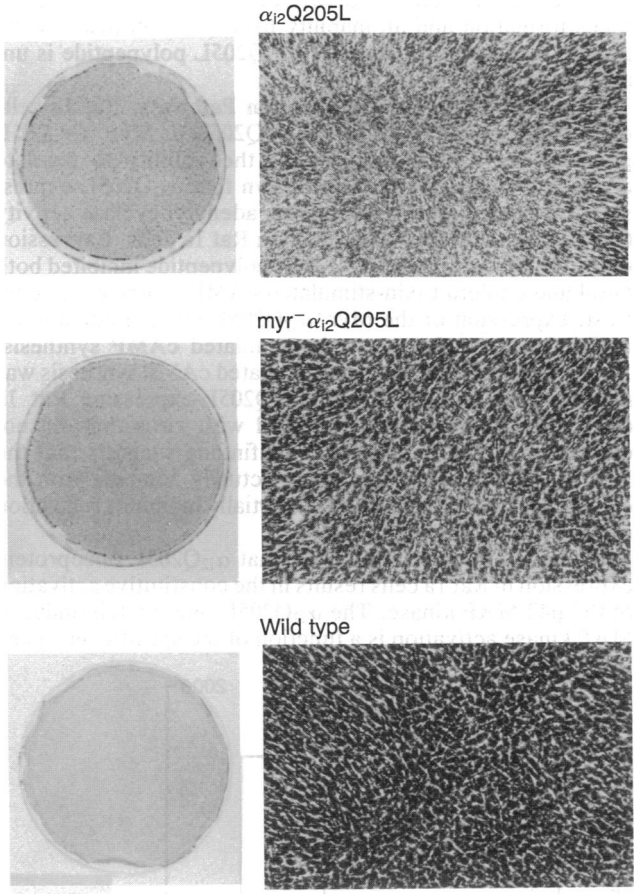

FIG. 3. Transformation potential of α_{i2}Q205L (*gip2*) and Myr⁻α_{i2}Q205L. Rat 1a cells were infected with equal titers of virus encoding the α_{i2}Q205L or Myr⁻Q205L constructs or with virus without a cDNA insert (wild-type neomycin resistant). Twelve days after infection representative fields were photographed (*Right*) and the cells were then fixed and stained with Giemsa (*Left*). Only the expression of the myristoylated α_{i2}Q205L polypeptide induced focus formation. Myr⁻α_{i2}Q205L expression appeared to increase cell density but did not induce focus formation. The results are representative of three experiments.

Table 1. Growth in soft agar of Rat 1a cells expressing α_{i2} and Myr$^-\alpha_{i2}$Q205L

Clone	Number of colonies
Neo	10*
α_{i2}Q205L	1662
Myr$^-\alpha_{i2}$Q205L	8*

Rat 1a cells (5000 cells per 60-mm dish) expressing α_{i2}Q205L or Myr$^-\alpha_{i2}$Q205L or a control clone infected with virus not encoding an α_{i2} cDNA (Neo) were grown in soft agar in DMEM containing 10% fetal calf serum. Fourteen days later colonies were counted in two dishes for each condition. The values represent the average number of colonies for each condition and vary by <10% between replicate dishes.

*Neo- and Myr$^-\alpha_{i2}$Q205L-expressing cells form colonies that were 5–10 times smaller in size than α_{i2}Q205L clones and are thus called minute colonies.

dramatically different. The *gip2* oncogene readily transforms Rat 1a cells (22, 23). Expression of the GTPase-inhibited α_{i2}Q205L polypeptide in Rat 1a cells induced a loss of contact inhibition and focus formation (Fig. 3). The Myr$^-\alpha_{i2}$Q205L polypeptide had some ability to increase cell growth but was unable to induce focus formation (Fig. 3). Myr$^-\alpha_{i2}$Q205L was also unable to induce soft agar colony growth of Rat 1a cells (Table 1). This was in contrast to expression of the *gip2* oncogene product, the α_{i2}Q205L polypeptide, that efficiently induced soft agar colony growth of Rat 1a cells. The lack of focus formation and an inability to induce soft agar colony growth indicates that the Myr$^-\alpha_{i2}$Q205L polypeptide is unable to transform Rat 1a cells.

Regulation of Signal Transduction Pathways. Rat 1a cells expressing similar levels of the α_{i2}Q205L or Myr$^-\alpha_{i2}$Q205L polypeptides were compared for their ability to regulate cAMP synthesis. It has been shown that α_{i2}Q205L expression in cells constitutively inhibits adenylyl cyclase activity (19, 20). Fig. 4 verifies this result in Rat 1a cells. Expression of the GTPase-inhibited α_{i2}Q205L polypeptide inhibited both basal and cholera toxin-stimulated cAMP synthesis. In contrast, expression of the Myr$^-\alpha_{i2}$Q205L polypeptide did not inhibit basal or cholera toxin-stimulated cAMP synthesis. Both basal and cholera toxin-stimulated cAMP synthesis was reproducibly greater in Myr$^-\alpha_{i2}$Q205L-expressing Rat 1a cells than in control cells infected with virus that did not contain an α_{i2} cDNA insert. This finding suggests that the Myr$^-\alpha_{i2}$Q205L polypeptide may actually compete with the endogenous α_{i2} polypeptide and partially inhibit its regulation of adenylyl cyclase.

We have also determined (24) that α_{i2}Q205L oncoprotein expression in Rat 1a cells results in the constitutive activation of the p42 MAP kinase. The α_{i2}Q205L oncoprotein-induced MAP kinase activation is a function of the specific oncogene

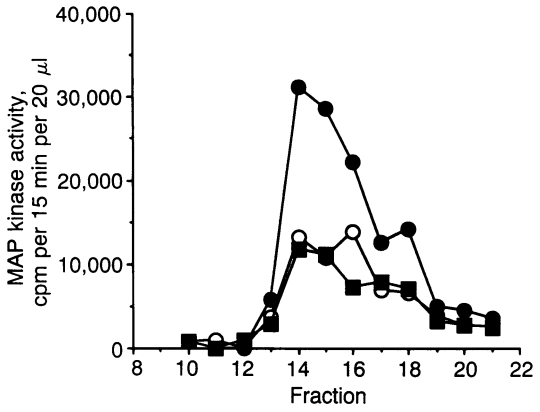

FIG. 5. Aliquots (20 μl) of the Mono Q-fractionated cell extracts of α_{i2}Q205L (●), Myr$^-\alpha_{i2}$Q205L (■), or control (○) Rat 1a cells were assayed (15 min at 30°C) for MAP kinase activity (24). Similar results were obtained using the MAP kinase-selective peptide containing residues 662–681 of the epidermal growth factor receptor [EGFR-(662–682)] (24, 30) or myelin basic protein (data not shown) as substrate. The constitutively activated MAP kinase activity demonstrated for α_{i2}Q205L-expressing Rat 1a cells correlates with increased phosphotyrosine content of the 42-kDa MAP kinase polypeptide. The increased MAP kinase activity in α_{i2}Q205L-expressing Rat 1a cells is the result of the activated α_{i2} polypeptide signal transduction pathway and not simply a result of transformation, because v-*ras*-transformed Rat 1a cells do not express a significantly constitutively activated MAP kinase activity (24). Control cells are neomycin-resistant Rat 1a cells.

product and not a result of the transformed phenotype. For example, transformation of Rat 1a cells by v-*ras* does not significantly activate MAP kinase. Fig. 5 shows Mono Q FPLC profiles of the MAP kinase activity from control and α_{i2}Q205L- and Myr$^-\alpha_{i2}$Q205L-expressing Rat 1a cells. The Myr$^-\alpha_{i2}$Q205L-expressing cells failed to activate MAP kinase activity in contrast to the α_{i2}Q205L-expressing cells that had a MAP kinase activity three times greater than the control cells.

Cumulatively, these results show that the Myr$^-\alpha_{i2}$Q205L polypeptide is unable to transduce α_{i2} signals in Rat 1a cells even though it is functionally capable of assuming a GTP-liganded conformation. This contrasts with the myristoylated α_{i2}Q205L polypeptide that constitutively activates α_{i2} signal transduction pathways.

DISCUSSION

Expression of the *gip2* oncogene that encodes the polypeptide α_{i2}Q205L, due to its inhibited GTPase activity, constitutively drives α_{i2} signal transduction pathways in the ab-

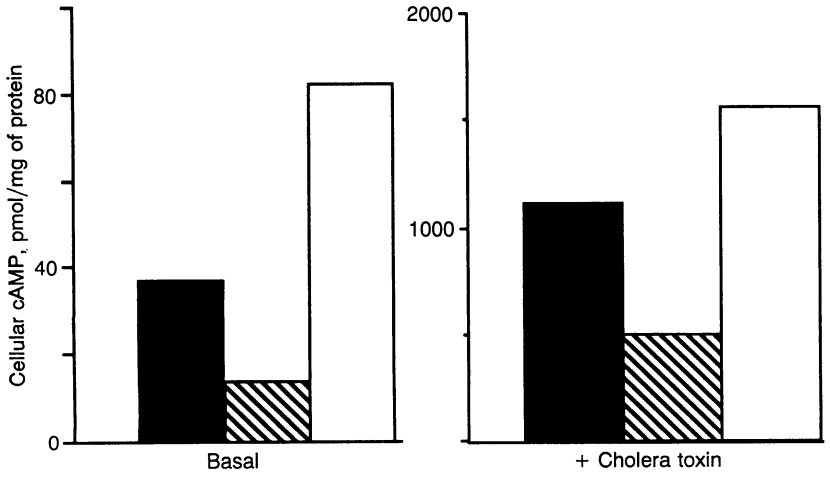

FIG. 4. Measurement of cAMP synthesis in α_{i2}Q205L- and Myr$^-\alpha_{i2}$Q205L-expressing clones. Rat 1a cells expressing the α_{i2}Q205L or Myr$^-\alpha_{i2}$Q205L polypeptides were analyzed for basal and cholera toxin-stimulated cAMP levels. Cells were incubated for 3 hr with or without 200 ng of cholera toxin and then washed three times with 20 mM Hepes-buffered Hank's balanced salt solution (pH 7.5). Cells were then incubated for 10 min in the presence of 500 μM isobutylmethylxanthine (for inhibition of cAMP phosphodiesterase). The medium was then aspirated, the cells were lysed in ice-cold 65% ethanol, and cAMP levels were measured by radioimmunoassay. The values represent the mean of triplicate dishes that varied by <10% in cAMP content for each condition. The results are representative of two experiments. Control cells are neomycin-resistant Rat 1a cells. Bars: solid, control cells; hatched, α_{i2}Q205L; open, Myr$^-\alpha_{i2}$Q205L.

sence of any hormone receptor activation (18–20, 22, 23). It was unexpected that Myr$^-\alpha_{i2}$Q205L was unable to activate α_{i2} signal pathways. The Myr$^-\alpha_{i2}$ and Myr$^-\alpha_{i2}$Q205L polypeptides were shown to be functionally capable of assuming a GTP or GTP[γS]-liganded conformation similar to their myristoylated counterparts in Rat 1a cell membranes. In addition, bacterially expressed α_{i1} and α_o that are not myristoylated were shown to regulate K^+ and Ca^{2+} channels in reconstitution experiments with patched membranes (14, 15). Recombinant nonmyristoylated α_{i2} also couples to receptors, exchanges GDP, and hydrolyzes GTP efficiently (14). Thus, the nonmyristoylated α_{i2} polypeptide is biochemically functional but is unable to transduce signals when expressed in mammalian cells.

We propose that myristoylation of α_{i2} polypeptides is required for its proper organization in the plasma membrane for interaction and regulation of effector enzymes. The α_{i2}Q205L polypeptide is activated in the absence of receptor stimulation (14, 19, 20, 22, 23), so the major role of myristoylation must be after the GDP/GTP exchange reaction. The dominant activated nature of the α_{i2}Q205L polypeptide, therefore, identifies the role of myristoylation in effector enzyme regulation. Thus, myristoylation may provide a myristate group that serves as a ligand covalently attached to the α_{i2} polypeptide that enhances or regulates interaction of α_{i2} with specific proteins in the cell. These proteins, as yet unidentified, must be components of the G_{i2} protein signal transduction network.

The concept of a "receptor" for myristoylated proteins has been proposed in relation to v-Src (4, 16). The evidence supports a role for myristate in combination with primary amino acid sequence that dictates specific interactions of myristoylated proteins with "receptors" (16). Only a subset of G protein α subunits are myristoylated (6, 8). The findings indicate that myristoylation is not required for intrinsic enzymatic activity but rather is required for proper regulation of signal transduction. The myristoylated α_i, α_o, and α_z polypeptides have an additional level of regulation in their control of signal transduction pathways relative to nonmyristoylated G protein α subunits (i.e., α_s and α_q). This may explain why recombinant nonmyristoylated α_{i2} fails to regulate purified adenylyl cyclase, whereas recombinant α_s readily stimulates cyclase activity (14). Additional proteins associated with the plasma membrane would be predicted to be involved in G_{i2} signal transduction and adenylyl cyclase regulation. One example of myristate-enhanced α subunit interaction with other proteins involves the G protein $\beta\gamma$ subunit complex. N-terminal myristoylation was shown to enhance the interaction of recombinant α_o polypeptides with $\beta\gamma$ subunit complexes bound to agarose (31). The constitutively activated character of the α_{i2}Q205L polypeptide, however, suggests that proteins other than $\beta\gamma$ subunits that preferentially interact with myristoylated α_{i2} polypeptides are involved in G_{i2} signal transduction. The requirement of α_{i2} N-terminal peptide sequence for membrane association is also similar to that for the Src polypeptide (3–5, 7, 8). A myristoylated peptide, consisting of amino acids 2–12 of the Src polypeptide, was shown to specifically bind to a 32-kDa membrane-associated protein (16). The 32-kDa Src-binding protein in the plasma membrane was proposed to be a component of the myristate-Src receptor required for Src signal transduction (16). The myristate-Src receptor does not appear to be a general myristate binding protein but is specific for the Src peptide. This observation suggests there may also be unique myristate-α_i binding proteins in the plasma membrane required for G_{i2} signal transduction. If myristate-α_i binding proteins exist, the mechanisms of signal transduction mediated by myristoylated G protein α subunits will involve distinct regulatory pathways that are not used by nonmyristoylated G protein α subunit polypeptides. Identification and purification of the proposed myristate-α_{i2} receptor will be necessary to define its role in the G_{i2} signal transduction network. This will be true not only for understanding G_{i2} regulation of adenylyl cyclase but also of other effectors such as MAP kinase regulatory proteins (30, 32) and the pathways contributing to cellular transformation induced by the gip2 oncoprotein.

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