Expression of a $G_{\alpha\gamma}/G_{\alpha i}$ Chimera That Constitutively Activates Cyclic AMP Synthesis*

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A chimeric $G_{\alpha}$ subunit cDNA, referred to as $G_{\alpha}(38)$, was constructed containing the complete 5'-untranslated region of $G_{\alpha}$, the first 356 codons of the rat $G_{\alpha}$ and the last 36 codons and 428 base pairs of the 3'-untranslated region of the rat $G_{\alpha}$ cDNA. Transient expression of the $G_{\alpha}(38)$ protein in COS cells allowed detection of a chimeric protein which was recognized by antibodies generated against an internal $G_{\alpha}$ sequence as well as antibodies recognizing the carboxy terminus of $G_{\alpha}$. Chinese hamster ovary cell clones stably expressing the chimeric G-protein $\alpha$ subunit transcript ($G_{\alpha}(38)$) demonstrated an increased activity ratio of cyclic AMP-dependent protein kinase, although expression of the chimeric polypeptide could not be demonstrated presumably because of low expression of the mutant $\alpha$. Expression of the rat $G_{\alpha}$ transcript yielded clones that were similar to wild-type Chinese hamster ovary cells in regard to cyclic AMP levels and protein kinase activity. In the presence of methyl isobutylxanthine, a cyclic AMP phosphodiesterase inhibitor, cyclic AMP levels in clones expressing the $G_{\alpha}(38)$ transcript were 10-15-fold higher than $G_{\alpha}$ expressing clones. Adenylyl cyclase activation by guanosine 5'-O-(thiotriphosphate) (GTPγS) in membranes from clones expressing the $G_{\alpha}(38)$ transcript demonstrated a diminished lag time for maximal activation, indicating an increased relative GDP dissociation rate for the chimeric $G_{\alpha}$ subunit and an increase in total adenylyl cyclase activity relative to wild-type $G_{\alpha}$ expressing clones. Cholera extracts from membranes of $G_{\alpha}(38)$ expressing clones, when mixed with cyc S49 membranes, reconstituted an increased GTPγS-stimulated adenylyl cyclase activity and a diminished lag time for maximal activation compared to cholera extracts prepared from $G_{\alpha}$-expressing clones. The $G_{\alpha}(38)$ construct confers a dominant constitutive activation of adenylyl cyclase when expressed in cells in the presence of a background of wild-type $G_{\alpha}$.

GTP-binding regulatory proteins (G-proteins) are composed of $\alpha$, $\beta$, and $\gamma$ subunits. These heterotrimeric couple receptor activation to control of membrane-associated enzymes and ion channels (1-4). Specific receptors, upon agonist binding, catalyze the exchange of GDP for GTP at the guanine nucleotide-binding site in the $\alpha$-subunit ($G_{\alpha}$) of an associated G-protein (5). The GTP-activated $\alpha$ subunit then activates the appropriate effector enzyme or channel.

To date, a large number of G-proteins have been described including, but not limited to, $G_{\alpha}$ three $G_{i}$, $G_{o}$, and transducin ($G_{T}$) (1). These proteins are distinguished most clearly by the differences in amino acid sequences among the $G_{\alpha}$ subunits. Although highly conserved, the G-proteins appear to interact with very different receptor and effector molecules. Two lines of evidence indicate that the $G_{\alpha}$ carboxy terminus composes a part of the receptor-binding site or is a domain which is essential for controlling receptor activation of GDP/GTP exchange. First, pertussis toxin ADP-ribosylates a cysteine residue four amino acids from the carboxyl terminus in $G_{o}$, $G_{o}$, and $G_{T}$ and uncouples the G-protein from the appropriate receptor (1, 6). Second, the unc S49 mouse lymphoma cell mutant, whose $G_{i}$ cannot be activated by $\beta$-adrenergic or prosta glandin E2 receptors, has an arginine to proline mutation six amino acids from the carboxyl terminus of $G_{i}$ (7, 8). Thus, both ADP-ribosylation by pertussis toxin and an amino acid substitution within the carboxyl-terminal domain of the $G_{\alpha}$ subunit uncouple the G-protein from receptor activation.

Based on these findings, we predicted that appropriate genetic manipulation of the $G_{\alpha}$ carboxy-terminal sequence might result in activation rather than inactivation of the G-protein. In other words, appropriate changes in amino acid sequence at the $G_{\alpha}$ carboxy terminus could possibly mimic, at least in part, the conformational changes in the G-protein that occur upon interaction with agonist-activated receptor. We have approached this problem by substituting the $G_{\alpha}$ carboxy terminus with sequences predicted to have significantly different secondary structure from $G_{\alpha}$ but which are known to be functional at the carboxyl terminus of other G-proteins. In this report, we describe a $G_{\alpha}$ chimeric construct in which the last 38-carboxyl-terminal amino acids are replaced by the last 36-carboxyl-terminal residues of a $G_{\alpha}$ protein. When this chimeric transcript, referred to as $G_{\alpha}(38)$, is expressed in cells, cyclic AMP levels are persist-

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The abbreviations used are: G-protein, GTP-binding regulatory protein; $G_{i}$, adenylyl cyclase stimulatory G-protein; $G_{o}$, adenylyl cyclase inhibitory G-protein; $G_{b}$, brain G-protein; $G_{T}$, retinal G-protein; $G_{\alpha}(38)$, chimeric G-protein between $G_{i}$ and $G_{o}$; CHO, Chinese hamster ovary cells; bp, base pairs; GTPγS, guanosine 5'-O-(thiotriphosphate); kb, kilobase pairs; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylendiamine(oxyethylenenitrite)]tetraacetic acid.
ently elevated, resulting from a dominant, constitutively active Gs-like activity.

MATERIALS AND METHODS

Construction of Plasmids—Expression vector pCW1-neo is a pUC13 derivative containing the SV40 enhancer, replication origin, and early promoter (EcoRI-HindIII fragment) and the SV40 splicing and polyadenylation sequences (HindIII-BamHI fragment) from pKO-neo (gift from D. Hanahan, Cold Spring Harbor Laboratory). In addition, the plasmid carries the TAg gene for neomycin resistance under the control of MoLTR from Homo 6 (9). Insertion into the unique HindIII cloning site allows the expression of cloned cDNA under the control of the SV40 early promoter. In the expression plasmid pGa, the 1.56 kb HindIII-HindIII fragment spanning the 5′-translated region, the entire coding region, and 192 bp of the 3′-untranslated region of the rat Gαi (10) was inserted into pCW1-neo. The orientation of the insert was determined by restriction analysis. In constructing pGa(38), the rat Gαi cDNA was truncated with AvaII and blunt-ended with the Klenow fragment of DNA polymerase. The corresponding 3′ region of the rat Gαi was obtained as a 640-bp BgIII-HindIII fragment, and the BglII end was filled to form a blunt end with the Klenow fragment. The chimeric cDNA was obtained by ligation of the 1.26-kb HindIII-AvaII Gαi fragment and the 640-bp BgIII-HindIII Gαi fragment into pUC12 linearized with HindIII. The identity of the chimeric cDNA was verified by restriction analysis and DNA sequencing (11). The 1.9-kb HindIII-HindIII chimeric DNA was then inserted into the HindIII cloning site of pCW1-neo to produce the expression plasmid pGa(38).

DNA-mediated Gene Transfer and Cell Culture—CHO-K1 cells were grown in F-12 medium supplemented with 10% fetal calf serum and 100 units/ml penicillin and streptomycin. The transformed cells were plated using protoplast fusion (12). Twenty-four hours after transfection, the cells were placed in growth medium plus 500 μg/ml genetin (G418). G418-resistant clones were isolated using glass cyliners, subcloned at least one time, and propagated in selective medium for further analysis. Clones Ga(38) and 3 were isolated from the same transfection while Ga(38) 9 was isolated from an independent fusion. The Ga(38) clones are representative of the phenotype characterized by expression of these constructs in 4 Gaαi and 12 Gaαi(38) independent clones as determined by Southern analysis (13).

COS cell transfections were performed using DEAE-dextran as described by Ausubel et al. (14). Clones were screened for protein expression 65–80 h after the transfection.

Northern Blot Analysis—Appropriate cell clones harvested by trypsinization and pelleted by centrifugation were resuspended in 1 ml of 500 mM sodium acetate, pH 5.5, 10 mM EDTA, 1 mg/ml heparin, and 1% SDS and briefly vortexed. An equal volume of buffered phenol + 0.3 mM sodium phosphate, 1.0 mg/ml heparin,

RESULTS AND DISCUSSION

Expression vector pCW1-neo and expression plasmids pGaαi and pGaαi(38) that were used to transfect COS and wild-type CHO cells are illustrated in Fig. 1. pGaαi contains the complete 5′-untranslated region, the entire coding region, and 192 bp of the 3′-untranslated region of the cDNA coding for the 52-kDa rat Gαi subunit (10). pGaαi(38) also contains the 5′-untranslated sequence but only the first 356 codons of the rat Gαi cDNA. The remaining 3′ end of pGaαi(38) is constructed from the last 36 codons and 428 bp of the 3′-untranslated region of the rat Gαi cDNA (10).

The aligned carboxyl-terminal amino acid sequences for Gααi, Gααi, and Gααi(38) are shown in Fig. 2. For Gααi and Gααi(38) the first 356 residues are identical. The construction of the Gααi(38) chimera resulted in a two amino acid (His-Tyr) deletion from Gααi at the switch site. This is followed by the 36 amino acids of Gααi. Of these 36 amino acids, there are 17 nonconserved residues between Gααi and Gααi. Within this same region, Gααi and Gααi have, respectively, 8 and 13 charged residues with a net charge of +2 for Gααi and −1 for Gααi. Charge distributions of the predicted amphipathic helices in this region of the Gα polypeptides are also significantly different (19). Finally, Gααi and Gααi(38) terminate with a cysteine followed by three uncharged residues which is the pertussis toxin-catalyzed ADP-ribosylation site in Gααi, Gααi, and Gα (1, 6).
Constitutively Active \( G_\alpha \) Chimera

**FIG. 1. Construction of \( pG_\alpha \) and \( pG_\alpha_{SL}(38) \).** The expression vector \( pCW1-neo \) is a \( pUC13 \) derivative containing the \( Tn5 \) gene (neomycin resistance), the SV40 enhancer, replication origin and early promoter (SV40EP), and the SV40 splice and polyadenylation sequences (SV40PA). Insertion of cDNA into the unique \( HindIII \)-cloning site permits expression under the control of the SV40 early promoter. \( pG_\alpha \) was obtained by inserting the 1.56-kb \( HindIII-HindIII \) fragment containing the rat \( G_\alpha \) cDNA into the \( HindIII \) site of \( pCW1-neo \). \( pG_\alpha_{SL}(38) \) was constructed by ligating the \( HindIII-HindIII \) fragment (1.9 kb) containing the \( G_\alpha/G_i \) chimeric cDNA into the \( HindIII \) site of \( pCW1-neo \) as described under "Materials and Methods.

**FIG. 2.** Amino acid sequence comparison between the carboxyl termini of \( G_\alpha \), \( G_\alpha_{SL}(38) \), and \( G_\alpha_i \). Panel A shows the alignment of the carboxyl-terminal 38-amino acid residues of \( G_\alpha \) with the carboxyl-terminal 36-amino acid residues of \( G_\alpha_i \) and \( G_\alpha_{SL}(38) \). The amino acids in the boxes represent conserved residues and conservative changes between \( G_\alpha \), \( G_\alpha_i \), and \( G_\alpha_{SL}(38) \). The deletion of 2 amino acid residues in \( G_\alpha_{SL}(38) \) and \( G_\alpha_i \) is represented by ---. Panel B shows the structure of the chimeric \( G_\alpha_{SL}(38) \), with nucleotide sequence and amino acid residues at the switch site. The chimera contains the first 356-amino acid residues of \( G_\alpha_i \) and the last 36-amino acid residues of \( G_\alpha \), as indicated.

In transient COS cell transfections, the expression of both \( G_\alpha \) and \( G_\alpha_{SL}(38) \) proteins could be detected by Western blotting. Fig. 3 shows that the expression of the 52-kDa polypeptide of \( G_\alpha \) encoded by the cDNAs is enhanced by transfection of COS cells with either \( pG_\alpha \) or \( pG_\alpha_{SL}(38) \). Densitometry of the 42- and 52-kDa bands indicated an increase in the 52-kDa band relative to the 42-kDa band of approximately 3-fold for \( G_\alpha \) and 2-fold for \( G_\alpha_{SL}(38) \)-transfected COS cells relative to control or \( G_\alpha_{SL}(38) \)-transfected cells. Panel B of Fig. 3 shows that an antiserum which recognizes the carboxyl terminus of \( G_\alpha_i \) (14) also recognized the 52-kDa polypeptide, in addition to the 41-kDa \( G_\alpha_i \) peptide, but not \( G_\alpha \). For reasons that are unclear, the antibody recognizing the \( G_\alpha \) carboxyl terminus does not react particularly well with the \( G_\alpha_{SL}(38) \) polypeptide. This may reflect differences in the conformation of the 52-kDa chimeric polypeptide relative to the 41-kDa \( G_\alpha \) polypeptide. Nevertheless,
The blots were probed with 1.56-kb nick-translated rat Ga cDNA and subjected to autoradiography at –80°C for 12 h. Panel A, lane 1, Gaα(38); lane 1, clone Gaα; lane 2, wild-type CHO-K1 cells. Panel B, lane 1, wild-type CHO-K1 cells; lane 2, pCW1-neo-transfected clone (no insert); lane 3, clone Gaα(38); lane 4, Gaα(38); lane 5, clone Gaα(38). In panel C, 1–2 μg (lanes 1 and 2) and 4 μg (lanes 3–6) of total RNA for each lane was analyzed as described above. The 640-bp BglII-HindIII fragment of the Gaα cDNA was nick-translated and used as a probe. Lane 1, pCW1-neo-transfected clone; lane 2, Gaα clone 14; lane 3, wild-type CHO-K1 cells; lane 4, clone Gaα(38); lane 5, clone Gaα(38); lane 6, clone Gaα(38). The size of the endogenous Gaα mRNA in CHO cells is approximately 1.9 kb (panel A, lane 2; panel B, lanes 1 and 2). The expressed rat Gaα (panel A, lane 2) and the chimeric Gaα(38) mRNAs (panel A, lane 1 and panel B, lanes 3–5) correspond to 2.5- and 3.0-kb bands, respectively. The endogenous Gaα mRNA in CHO cells is detected as two distinct messages of size 1.9 and 2.4 kb (panel C).

### TABLE I

**Cyclic AMP and cyclic AMP-dependent protein kinase activity in CHO clones expressing Gaα(38)**

Stable G418-resistant CHO clones expressing the pGaα or pGaα(38) cDNA constructs were assayed for basal cyclic AMP levels and cyclic AMP-dependent protein kinase activity as described under “Materials and Methods.” Phenotype of each clone has remained stable for greater than 50 cell doublings. A cyclic AMP-dependent protein kinase activity ratio greater than 0.3 does not linearly correlate with cyclic AMP levels. This results in the same apparent kinase activity for Gaα(38) clones 2, 3, and 9 even though the basal cyclic AMP levels are 1.5–3-fold higher in clone 9 relative to clones 2 and 3. Results represent two independent cyclic AMP measurements, and protein kinase activity is representative of two to three experiments for each clone.

| Clone  | Cyclic AMP | Cyclic AMP-dependent protein kinase |
|--------|------------|------------------------------------|
|        | pmol/mg    | pmol/mg/min                        |
|        | cyclic AMP | +cyclic AMP | –cyclic AMP | +cyclic AMP |
| Wild type | 83.8, 8.0 | 67         | 980        | 0.07       |
| Gaα    | 7.8, 7.1   | 88         | 1164       | 0.08       |
| Gaα(38) | 21.6, 25.8 | 422        | 1190       | 0.36       |
| Gaα(38) | 12.8, 17.8 | 456        | 1258       | 0.36       |
| Gaα(38) | 12.5, 17.5 | 398        | 1126       | 0.35       |

less, the immunoblots with the Gaα and Gaα antibodies confirm the chimeric nature of the Gaα(38) polypeptide.

To characterize the phenotype resulting from the expression of the chimeric Gaα(38) construct, stable transfectants were isolated in CHO cells. The expected size of the plasmid-expressed Gaα and Gaα(38) mRNAs are 2.5 and 3.0 kb, respectively, whereas the endogenous Gaα mRNA is 1.8 kb. Therefore, it was possible to screen G418-resistant clones by Northern analysis and detect the expression of the plasmid expressed Gaα transcripts. Fig. 4 shows the Northern analysis of CHO cell clones isolated after transfection with pGaα, pGaα(38), or a pCW1-neo control construct and probed using Gaα cDNA. While wild-type CHO cells transcribed with pCW1-neo express only endogenous Gaα mRNA, clones transfected with pGaα or pGaα(38) express the predicted plasmid encoded messages. Fig. 4 also shows the same RNA preparations probed with the BglII-HindIII fragment of a Gaα cDNA (10), encoding the last 108 bases of the coding region and the entire 3′-untranslated region. The Gaα(38) transcript was detected in addition to the endogenous Gaα transcripts, verifying the chimeric nature of the 3.0-kb mRNA. Each clone analyzed varied one from another, within a 3-fold range, in the level of expression of Gaα or Gaα(38) mRNA. Southern analysis of genomic DNA verified the independence of each Gaα and Gaα(38) 2, 3, and 9 clones (not shown).

In G418-resistant clones expressing the Gaα(38) transcript, basal intracellular cyclic AMP is elevated, and cyclic AMP-dependent protein kinase activity is significantly activated relative to other CHO clones (Table I). The Gaα(38) clones vary in basal cyclic AMP levels but are all 1.5–2.5-fold higher than wild-type CHO, Gaα clones, and pCW1-neo clones. Similarly, the CAMP-dependent protein kinase activity ratio is elevated 3–4-fold over Gaα, wild-type, and pCW1-neo controls. In the Gaα(38) clones, elevated cyclic AMP and kinase activity has been a stable phenotype for more than 150 cell doublings. No Gaα, Gaα, or pCW1-neo clone has demonstrated this phenotype, indicating that the constitutively elevated cyclic AMP levels in Gaα(38) clones is a consequence of expression of this transcript. In addition, we have now transfected other cell lines with the Gaα(38) construct and isolated stable G418-resistant clones with elevated cyclic AMP levels and protein kinase activity.

In the presence of the phosphodiesterase inhibitor methyl isobutylxanthine (MIX) on cyclic AMP levels in Gaα (C) and Gaα(38) (D) clones. Cells were grown to 75% confluence in 50-mm Petri dishes, the cells were washed one time, and incubated for 10 min in Dulbecco’s minimum essential medium and 10 mM NaHEPES, pH 7.5, in the presence or absence of 1 mM methyl isobutylxanthine, and cyclic AMP extracted and quantified as outlined under “Materials and Methods.” Cyclic AMP measurements in Gaα and Gaα(38) clones were determined in duplicate in three different experiments. Values represent the means of the three experiments where values varied less than 15% in all cases.

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diesterase is not dramatically altered in its properties in \(G_{\alpha(i)}(38)\) relative to wild type. Cellular export of cyclic AMP was similar in wild-type and \(G_{\alpha(i)}(38)\) expressing clones (not shown), indicating that changes in cyclic AMP extrusion was not responsible for the dramatic effect of methyl isobutyxanthine in the chimeras-expressing clones. The most likely explanation is a compensatory increase in cyclic AMP phosphodiesterase \(G_{\alpha(i)}(38)\) clones. Similar compensatory changes in phosphodiesterase levels have been described in other cell types (20). Nonetheless, even in the absence of phosphodiesterase inhibitors, the \(G_{\alpha(i)}(38)\) construct is capable of constitutively elevating cyclic AMP levels and activating cyclic AMP-dependent protein kinase in the presence of a background of wild-type \(G_{\alpha} \).

Activation of adenylyl cyclase by guanine nucleotides in membranes isolated from \(pG_{\alpha(i)}(38)\)-transfected clones differs in two significant ways relative to preparations from wild-type, \(pG_{\alpha} \), and \(pCW1-neo\) clones (Fig. 6). First, membranes from three independent \(G_{\alpha(i)}(38)\) clones demonstrate a significant decrease in the time required to achieve maximal adenylyl cyclase activation by GTP\(\gamma S\). This diminished lag time is somewhat variable but reproducible among \(G_{\alpha(i)}(38)\) clones 2, 3, and 9 relative to \(G_{\alpha} \), wild-type or \(pCW1-neo\) clones. In general, \(G_{\alpha(i)(38)}\) has the highest level of chimeric mRNA and shortest lag. In contrast, \(G_{\alpha(i)(38)}\) has the lowest chimeric mRNA level of the three clones, relative to the endogenous \(G_{\alpha}\) mRNA, and has less of a decreased lag than either clones \(G_{\alpha(i)(38)}\) or 2 compared with the two control clones, \(G_{\alpha}\) or \(pCW1-neo\).

The second change in guanine nucleotide-activated adenylyl cyclase observed with expression of the \(G_{\alpha(i)(38)}\) chimera was that GTP\(\gamma S\) and fluoride-activated adenylyl cyclase activities were reproducibly greater in membranes from the \(G_{\alpha(i)(38)}\) clones compared with \(G_{\alpha}\) or \(pCW1-neo\) clones. This observation has been found with several membrane preparations where caution was taken to prepare membranes from different clones on the same day and when cells were seeded and harvested at similar densities. This finding is consistent with the \(G_{\alpha(i)(38)}\) product increasing adenylyl cyclase activity and thus, elevating cyclic AMP levels relative to wild-type or \(G_{\alpha}\) clones. It is interesting to note that clone \(G_{\alpha(i)(38)}\), which has the shortest lag in GTP\(\gamma S\) activation of adenylyl cyclase and the highest basal cyclic AMP levels, also has the highest activity in the presence of GTP, consistent with a more active adenylyl cyclase system as a result of expressing the \(G_{\alpha(i)(38)}\) transcript.

In an attempt to normalize for differences in cloning expression of \(G_{\alpha(i)(38)}\), the ratio of GTP\(\gamma S\)/GTP-stimulated adenylyl cyclase activity was plotted as shown in panel F, Fig. 6. The GTP\(\gamma S\)/GTP ratio indicates the relative adenylyl cyclase activation intrinsic for the two guanine nucleotides. This comparison between clones emphasizes the more rapid activation of adenylyl cyclase in response to GTP\(\gamma S\) with \(G_{\alpha(i)(38)}\)-expressing CHO clones relative to \(G_{\alpha}\) or \(pCW1-neo\) clones. The simplest interpretation of the decreased lag is that, relative to wild-type \(G_{\alpha}\), the \(G_{\alpha(i)(38)}\) construct has a more rapid GDP dissociation rate allowing faster GTP\(\gamma S\) binding and activation. However, the greater apparent intrinsic adenylyl cyclase activity in clones expressing \(G_{\alpha(i)(38)}\) cannot be readily explained simply by enhanced GDP dissociation.

As a direct measure of G-protein activity, cholate extracts from membranes of \(G_{\alpha}\) and \(G_{\alpha(i)(38)}\)-expressing clones were mixed with cyc-549 membranes, which lack \(G_{\alpha}\) (21), and assayed for GTP and GTP\(\gamma S\)-stimulated adenylyl cyclase activity (22). Fig. 7 shows that cholate extracts from \(G_{\alpha(i)(38)}\) membranes reconstituted a GTP\(\gamma S\)-activated adenylyl cyclase activity with both a greater maximal activity and decreased lag time required to reach maximal adenylyl cyclase activation compared with a \(G_{\alpha}\)-expressing clone. Similar findings were observed with other \(G_{\alpha(i)(38)}\)-expressing clones when activities were compared in the reconstitution assay with cyc- membranes relative to either \(G_{\alpha}\)-expressing clones or wild-type CHO cells.

Fig. 8 shows that the enhanced adenylyl cyclase activity observed with extracts from \(G_{\alpha(i)(38)}\) is linear with increases
ing protein concentration, indicating a direct relationship between G-protein addition in the reconstitution assay and the enhanced activity observed in the GaLi(38)9 clone relative to clone GaL. Furthermore, Fig. 9 shows that at equal relative activities in the reconstitution assay the lag time to reach maximal GTPγS-activated adenylyl cyclase activity is about 3.2 min with cholate extracts from GaLi(38)9 and 6.5 min with the extract from the GaL clone. The inset of Fig. 9 also shows that the adenylyl cyclase activity measured with GTP in the reconstitution assay is greater with extracts from GaLi(38)9 relative to the GaL clone. Thus, similar kinetic properties of adenylyl cyclase activation are observed with either intact membranes from clones expressing plasmid-derived GaLi(38) or GaL transcripts and with cholate extracts from these membranes using the reconstitution assay with S49 cya membranes.

The reconstitution assay utilizing cya membranes is a direct measure of G-protein activity in the cholate extracts of donor membranes. The phenotype observed with cells expressing the GaLi(38)9 transcript is easily observed in the G-protein regulation of adenylyl cyclase. Plasmid expression of a normal GaL transcript had no effect on the phenotype of CHO cells. Thus, the utility of the GaLi(38)9 construct is that it is dominant among a background of wild-type GaL in constitutively elevating cyclic AMP synthesis in cells.

At present, the molecular basis for the GaLi(38)9 phenotype is unclear. We have no evidence that the difference in 3'-untranslated regions of pGaL and pGaLi(38)9 influence the phenotype of the cells. It also appears that the GaL(38) polypeptide is expressed at low levels in CHO cells and has eluded detection with currently available antibodies. The pGaL(38) construct, however, was shown to code for a chimeric protein in transient COS cell transfections. Several characteristics of the GaL(38) phenotype can be defined. First, increased activation of adenylyl cyclase is observed for both GTP and GTPγS. Second, expression of GaL(38) results in the activation of adenylyl cyclase and is not the result of constitutively active Gα, chimera.
phosphodiesterase inhibition or altered cellular cyclic AMP extrusion. Third, we have found that pertussis toxin treatment of $G_{\alpha_{38}}$ expressing clones did not influence the properties of adenyl cyclase activation in these clones relative to wild-type cells. Nor does pertussis toxin treatment of CHO cells mimic expression of the $G_{\alpha_{38}}$ transcript. The constitutive elevation of cyclic AMP with $G_{\alpha_{38}}$ expression does not, therefore, appear to be due to an inhibition of $G_{i}$, but appears to be due to a constitutively active $G_{\alpha}$-like activity. Because of the low level of expression in CHO cells it is not clear whether $G_{\alpha_{38}}$ directly interacts with catalytic adenyl cyclase or influences the regulation of the endogenous $G_{\alpha}$ polypeptide. We have been unable to express $G_{\alpha_{38}}$ in S49 cells in an attempt to answer this question, presumably because of the constitutively active nature of this construct, and the fact that S49 cells are killed by elevated cyclic AMP levels. Using other constructs such as pG$\alpha_{38}$, we have been able to successfully transfec S49 cells and express functional $G_{\alpha}$ proteins suggesting expression of $G_{\alpha_{38}}$ is lethal to cytoskeleton-infected cells. However, the same phenotype as described for $G_{\alpha_{38}}$/CHO cells has now been defined in other cell types such as CCL39 fibroblasts, indicating the constitutive activation of cyclic AMP synthesis is a characteristic of the $G_{\alpha_{38}}$ chimera.

Immunoblot analysis, using antisera raised against peptides corresponding to the carboxyl terminus of $G_{\alpha}$ and $G_{\alpha_{38}}$ and an antiserum raised against $\beta_{i}$ indicated the phenotype observed with expression of $G_{\alpha_{38}}$ cannot be explained by an alteration in the expression of endogenous $G_{\alpha}$, $G_{\beta}$, or $G_{\alpha_{38}}$ subunits. Fig. 10 shows that both $G_{\alpha}$ and $G_{\alpha_{38}}$ cause similar changes in the expression of G-protein subunits. Relative to wild type, expression of $\alpha_{i}$ and $G_{\alpha_{38}}$ cDNAs increased $\beta_{i}$ subunit levels 3- and 1.8-fold, respectively. Similarly, $\alpha_{i}$ and $\alpha_{s}$ subunits were increased 1.7- and 3-fold for $G_{\alpha}$ and 1.3- and 3-fold for $G_{\alpha_{38}}$-expressing clones. Because $G_{\alpha}$ expression has little measurable influence on cyclic AMP levels in CHO cells, and both $G_{\alpha}$ and $G_{\alpha_{38}}$ influence $\alpha_{i}$, $\alpha_{s}$, and $\beta_{i}$ subunit expression similarly, these changes do not appear to induce the phenotype of $G_{\alpha_{38}}$-activation of adenyl cyclase.

To our knowledge, this is the first demonstrated strategy for the constitutive activation of cyclic AMP synthesis by the stable expression of a chimeric mutant $G_{\alpha}$ cDNA. Bacterial expression and reconstitution of the chimeric protein with the $\beta_{i}$-subunit complex and adenyl cyclase will be required to understand the molecular mechanism for the $G_{\alpha_{38}}$ effect on cyclic AMP synthesis. Given the conservation in sequence among the $G_{\alpha}$ subunits of the various G-proteins similar constructs may give similar constitutively active $G_{i}$ or $G_{\alpha}$ phenotypes. This should provide an important new strategy for studying the regulation of cell function by G-proteins.

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FIG. 10. Immunoblot analysis of steady-state levels of endogenous G-proteins in $G_{\alpha}$ and $G_{\alpha_{38}}$ relative to wild-type CHO cells. Cholate extracts of membranes from wild-type (lanes 1, 4, and 7), $G_{\alpha}$ (lanes 2, 5, and 8) and $G_{\alpha_{38}}$ (lanes 3, 6, and 9) CHO cells were prepared and 100 µg of extract protein resolved by SDS-polyacrylamide gel electrophoresis on 12.5% acrylamide gels. Proteins were transferred to nitrocellulose and immunoblotted as described under "Materials and Methods" with antisera recognizing the $\beta_{i}$-subunit (lanes 1-3), the $G_{\alpha}$ carboxyl terminus (lanes 4-6), and the $G_{\alpha}$ carboxyl terminus (lanes 7-9). As shown in Fig. 3, the $G_{\alpha}$ carboxyl-terminal antibody appears to poorly recognize $G_{\alpha_{38}}$, and the chimera is not readily visualized in these blots. Filters were exposed for 8, 12, 24, and 48 h, and each lane was scanned by densitometry three times and averaged for the value of the appropriate $\beta$, $\alpha_{i}$, or $\alpha_{s}$ band. Densitometric values were averaged for the 12- and 24-h exposures. Assigning an arbitrary unit of 1.0 for $\beta$, $\alpha_{i}$, and $\alpha_{s}$ in wild-type CHO cells, $G_{\alpha}$ cells expressed 3.3, 1.7 and $G_{\alpha_{38}}$ cells expressed 1.5, 3, and 1.3 units of $\beta$, $\alpha_{i}$, and $\alpha_{s}$, respectively. The determinations are representative of three different experiments with different wild-type, $G_{\alpha}$, and $G_{\alpha_{38}}$ CHO clones.

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α subunits were increased 1.7- and 3-fold for Ga and 1.3- and 3-fold for Ga(38)-expressing clones. Because Ga expression has little measurable influence on cyclic AMP levels in CHO cells, and both Ga and Ga(38) influence αi, αs, and β subunit expression similarly, these changes do not appear to induce the phenotype of Ga(38) activation of adenyl cyclase.

To our knowledge, this is the first demonstrated strategy for the constitutive activation of cyclic AMP synthesis by the stable expression of a chimeric mutant Gaα cDNA. Bacterial expression and reconstitution of the chimeric protein with the βγ-subunit complex and adenyl cyclase will be required to understand the molecular mechanism for the Ga(38) effect on cyclic AMP synthesis. Given the conservation in sequence among the Gaα subunits of the various G-proteins (19) similar constructs may give similar constitutively active Gα or Go phenotypes. This should provide an important new strategy for studying the regulation of cell function by G-proteins.