The N termini of two G protein \(\alpha\) subunits, \(\alpha_q\) and \(\alpha_{11}\), differ from those of other \(\alpha\) subunits in that they display a unique, highly conserved six-amino acid extension (MTLESI(M)). We recently showed that an \(\alpha_q\) deletion mutant lacking these six amino acids (in contrast to wild type \(\alpha_q\)) was able to couple to several different \(G_\alpha\) and \(G_{11}\)-coupled receptors, apparently due to promiscuous receptor/G protein coupling (Kostenis, E., Deytaryev, M. Y., Conklin, B. R., and Wess, J. (1997) J. Biol. Chem. 272, 19107–19110). To study which specific amino acids within the N-terminal segment of \(\alpha_{q/11}\) are critical for constraining the receptor coupling selectivity of these subunits, this region of \(\alpha_q\) was subjected to systematic deletion and alanine scanning mutagenesis. All mutant \(\alpha_q\) constructs (or wild type \(\alpha_q\) as a control) were coexpressed (in COS-7 cells) with the m2 muscarinic or the D2 dopamine receptors, two prototypical \(G_{16}\)-coupled receptors, and ligand-induced increases in inositol phosphate production were determined as a measure of G protein activation. Surprisingly, all 14 mutant G proteins studied (but not wild type \(\alpha_q\)) gained the ability to productively interact with the two \(G_{16}\)-linked receptors. Similar results were obtained when we examined the ability of selected mutant \(\alpha_q\) subunits to couple to the \(G_q\)-coupled \(\beta_2\)-adrenergic receptor. Additional experiments indicated that the functional promiscuity displayed by all investigated mutant \(\alpha_q\) constructs was not due to overexpression (as compared with wild type \(\alpha_q\)), lack of palmitoylation, or initiation of translation at a downstream ATG codon (codon seven). These data are consistent with the notion that the six-amino acid extension characteristic for \(\alpha_{q/11}\) subunits forms a tightly folded protein subdomain that is critical for regulating the receptor coupling selectivity of these subunits.

G protein-coupled receptors (GPCRs)\(^1\) regulate the activity of a large variety of effector systems via interaction with specific classes of heterotrimeric G proteins (consisting of \(\alpha\), \(\beta\), and \(\gamma\) subunits) that are attached to the cytoplasmic side of the plasma membrane (1–6). In most cases, an individual GPCR can only activate a distinct subset of the many structurally similar G proteins present in each cell (7, 8). How this selectivity is achieved at a molecular level is currently being explored by a great number of laboratories.

A large body of evidence indicates that multiple regions on the G protein \(\alpha\) subunits play key roles in receptor binding and dictating the selectivity of receptor/G protein interactions (2, 5, 6, 8, 9). Specifically, recent studies have shown that residues at the extreme C terminus of the G protein \(\alpha\) subunits are of fundamental importance for regulating receptor/G protein coupling selectivity (10–13), probably by directly contacting the receptor protein (14, 15). However, several lines of evidence indicate that other regions of \(G_\alpha\) also contribute to receptor binding and the selectivity of receptor recognition (2, 5, 6, 9). Biochemical studies suggest, for example, that the N-terminal portion of \(G_\alpha\) may also be in contact with the receptor protein (14, 16, 17). An early study demonstrated that a synthetic peptide corresponding approximately to the N-terminal \(\alpha\)N-helix of \(\alpha\)-transducin (Fig. 1) was able to prevent rhodopsin/ transducin interactions, presumably by directly binding to rhodopsin (14). Moreover, Higashijima and Ross (16) showed that a cysteine-substituted mastoparan, a receptor-mimetic peptide toxin, can be cross-linked to the extreme N terminus of \(G_\alpha\) subunits. Similar findings were obtained with a photoaffinity derivative of a receptor-mimetic peptide corresponding to the C-terminal portion of the third cytoplasmic loop of the \(\alpha_{2A}\)-adrenergic receptor (17).

In addition, functional analysis of an N-terminally truncated \(\alpha_q\) subunit suggests that the N terminus of \(G_\alpha\) subunits is also involved in modulating the fidelity of receptor/G protein recognition (18). \(G_\alpha_q\) and \(G_{11}\) contain a unique six-amino acid extension (MTLESI(M)) that is not found in other \(G_\alpha\) subunits (Fig. 1). This short sequence is highly conserved among all vertebrate species from which these subunits have been cloned so far (19–23). When this sequence was removed by deletion mutagenesis (18), the resulting mutant \(\alpha_q\) subunit (corresponding to \(G_{16}\) in Fig. 1) gained the ability to be activated by various \(G_{16}\)- and \(G_\alpha\)-coupled receptors which normally do not couple efficiently to wild type (full-length) \(\alpha_q\) (\(\alpha_{WT}\)). Based on these results, we speculated that the N-terminal extension characteristic for \(\alpha_{q/11}\) subunits may act as a “filter” by selectively preventing \(G_{16}\)- and \(G_\alpha\)-coupled receptors from contacting functionally critical \(\alpha_{q/11}\) residues. Alternatively, it is conceivable that the N-terminal extension exerts indirect conformational effects on adjacent \(\alpha_{q/11}\) domains, such as the functionally critical C terminus, thereby constraining the selectivity of receptor/\(\alpha_{q/11}\) interactions.

To examine which specific amino acids within the N-terminal segment of \(\alpha_{q/11}\) are critical for maintaining the selectivity of receptor recognition, we generated a large number of mutant \(\alpha_q\) subunits in which the N-terminal residues were progressively deleted or systematically replaced, either individually or in combination, with alanine. The ability of these mutant subunits to be activated by different \(G_{16}\)- and \(G_\alpha\)-coupled receptors was examined in cotransfected COS-7 cells. Surprisingly, all 14 mutant \(\alpha_q\) subunits studied, but not \(\alpha_{WT}\), displayed pro-

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1. The abbreviations used are: GPCR, G protein-coupled receptor; DMEM, Dulbecco’s modified Eagle’s medium; IP<sub>3</sub>, inositol monophosphate; PLC, phospholipase C; qWT, wild type \(G_{16}\); PAGE, polyacrylamide gel electrophoresis; HA, hemagglutinin.
nounced receptor coupling promiscuity. These results indicate that each single amino acid within the N-terminal extension of αq(2-7) subunits is critical for constraining the receptor coupling selectivity of these subunits. The most straightforward explanation for this finding is that the N-terminal portion of αq(2-7) forms a well defined protein subdomain that regulates the fidelity of receptor/αq(2-7) interactions.

EXPERIMENTAL PROCEDURES

Creation of Mutant G Protein Constructs—A mouse α1α2 cDNA (19) cloned into the pcDNA expression vector (24) was used as a template for polymerase chain reaction mutation. All wild type and mutant Gaq subunits contained an internal hemagglutinin (HA) epitope tag (DVPDYA; Ref. 24). The presence of the epitope tag, which replaced αq resid 125–130, did not affect the receptor and effector coupling properties of qWT (10, 11). The construction of the q(2-7) deletion mutant (formerly referred to as -6q) has been described previously (18). A silent BssHII site was introduced into q(2-7) at amino acid codons 18–19 to facilitate the generation of additional mutant Gα proteins. All mutant αq subunits were constructed by replacing a 57-base pair BamHI/BssHII fragment of q(2-7) with the corresponding DNA fragments (generated via polymerase chain reaction) containing the desired deletions or substitutions (Fig. 1). In the wild type and all mutant αq plasmids, the BamHI-site of the pcDNA polylinker was immediately followed by the initiating ATG codon. The correctness of all polymerase chain reaction-derived DNA sequences was verified by dideoxy sequencing of the mutant plasmids (25).

Coexpression of Receptor and G Protein Constructs—COS-7 cells were grown in Dulbecco’s modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum at 37 °C in a humidified 5% CO2 atmosphere. The cells were preincubated for 20 min at room temperature with 2 ml of antibody for the indicated G protein (1 μg of DNA/dish) and receptor constructs (4 μg of DNA/dish) by using a DEAE-dextran procedure (26). The following receptor expression plasmids were used: human m2 muscarinic receptor in pC3 (27), human D2 dopamine receptor in pCDNAI (28), and rat β2-adrenergic receptor in pSVL (29).

Phosphatidylinositol Hydrolysis Assays—About 18–24 h after transfections, cells were split into six-well dishes (approximately 0.4 × 106 cells/well) and labeled with 3 μCi/ml [3H]palmitate (50.0 Ci/mM, American Radiolabeled Chemicals Inc.) in 3 ml of DMEM containing 5% fetal calf serum at 37 °C, 5% CO2. Following removal of the medium, cells were metabolically labeled for 16 h with 200 μCi/ml [9,10–3H]palmitate (50.0 Ci/mM; American Radiolabeled Chemicals Inc.). The remaining chemicals were obtained through Sigma. (Amersham Pharmacia Biotech) and visualized using an enhanced chemiluminescence system (Amersham Pharmacia Biotech) and visualized using an enhanced chemiluminescence system (Amersham Pharmacia Biotech). The remaining chemicals were obtained through Sigma.

RESULTS

Construction and Expression of Mutant αq Subunits—Initially we prepared two series of N-terminally modified mutant αq subunits (Fig. 1). In one series, amino acids 2–7 (TLESIM) of αq single point mutants) or in combination (resulting in q(TLE → AAA) and q(SI → AA) (Fig. 1). All wild type and mutant αq subunits contained an internal hemagglutinin (HA) epitope tag replacing the HA epitope tag present in all G protein constructs. Samples containing 20 μg of membrane protein prepared from transfected COS-7 cells were resolved by SDSPAGE (13%), electroblotted onto nitrocellulose, and immunostained with the appropriate agonists for 1 h at 4 °C and then centrifuged for 1 min at 2,000 rpm in a refrigerated Eppendorf 5417R microcentrifuge. Supernatants were mixed with an equal volume of buffer A and 20 μl of Sepharose 4B beads (Sigma) co-purified with the 12CA5 monoclonal antibody (Boehringer Mannheim) (the antibody was coupled to CNBr-activated Sepharose 4B according to the manufacturer’s (Sigma) instructions). Samples were tumbled for 2 h at 4 °C and then centrifuged for 1 min at 2,000 rpm in an Eppendorf microcentrifuge to gently pellet the Sepharose beads. Pellets were washed twice with 500 μl of a 1.5 dilution of extraction buffer and resuspended in 20 μl of Laemmli sample buffer (Bio-Rad) without reducing agents. After heating the samples for 10 min at 70 °C, the beads were pelleted gently (same conditions as above), and supernatants were used for SDS-PAGE (13%). Gels loaded with 10% of the total sample volumes were subjected to Western blotting (see below). Gels run with the remaining 90% of the samples were soaked in 20–30 ml of Fluoro-Hance (Research Products International Corp.) for 30 min, dried, and subjected to fluorography at −80 °C for 4–6 weeks.

Western Blotting—All wild type and mutant Gaq subunits were detected with the 12CA5 monoclonal antibody directed against the HA epitope tag present in all G protein constructs. Samples containing 20 μg of membrane protein prepared from transfected COS-7 cells were resolved by SDS-PAGE (13%), electroblotted onto nitrocellulose, and probed with the 12CA5 antibody as described (11, 31). Immunoreactive proteins were detected by incubation with horseradish peroxidase-conjugated sheep anti-mouse IgG antibody (Amersham Pharmacia Biotech) and visualized using an enhanced chemiluminescence system (Amersham Pharmacia Biotech).

Drugs—All ligands used in this study were purchased from Research Biochemicals Inc. The remaining chemicals were obtained through Sigma.

Functional Role of N Terminus of Gαq

FIG. 1. Structure of αq deletion and point mutants. Amino acids marked with an arrow were replaced, either individually or in combination, with alanine residues. For comparison, the N-terminal portions of several other G protein α subunits are also shown. Sequences (human) were taken from Refs. 23 and 60 (note that the human αq sequence (23) shown here is identical to the corresponding mouse sequence (19)). Gaps were introduced to allow for maximum sequence identity. The position of the N-terminal segment of the α-helix, as revealed by x-ray crystallography (43, 44), is indicated.
expression of the m2 receptor with either vector DNA (pcDNA1) or qWT, followed by ligand stimulation, resulted only in a relatively small increase in PLCβ activity (approximately 3-fold; Fig. 3), a response known to be largely due to activation of PLCβ isozymes by G protein βγ subunits released upon m2 receptor-mediated activation of endogenous G_{μγ} proteins (36, 37). Remarkably, the m2 muscarinic receptor gained the ability to productively couple to all 14 mutant α subunits, resulting in a 6–11-fold stimulation of PLC activity (Fig. 3).

Essentially similar findings were obtained when we studied the ability of the D2 dopamine receptor, another G_{μγ}-coupled receptor, to interact with the various mutant G proteins. When coexpressed with qWT, the D2 receptor, upon stimulation with the selective D2 agonist, (-)-quinpirol (10 μM), was unable to induce an appreciable degree of PLC stimulation (Fig. 4). However, in the presence of either of the 14 mutant α subunits studied, a significant increase in inositol phosphate production was observed (ranging from 2- to 6-fold above basal; Fig. 4).

Interaction of Mutant α Subunits with a G_s-coupled Receptor—We next examined the ability of the G_s-coupled β2-adrenergic receptor to interact functionally with selected mutant α subunits (q(Δ2-7), q(T2A), q(L3A), q(E4A), q(SSA), q(I6A), and q(M7A)). Consistent with its known coupling preference (1), this receptor subtype, when stimulated with (-)-isoproterenol (200 μM), was unable to activate efficiently qWT (Fig. 5), mediating only an approximately 1.5-fold increase in IP production. However, the β2-adrenergic receptor gained the ability to interact productively with all examined mutant α subunits, leading to a 3–5-fold stimulation of PLC activity (Fig. 5).

Translation Start Site in Mutant α Subunits—As described above, all investigated mutant α subunits showed similar functional properties, displaying coupling promiscuity upon coexpression with different G_{μγ} and G_s-coupled receptors. As shown in Figs. 1 and 6A, the N-terminal six-amino acid extension (MTLESI) characteristic for α subunits is immediately followed by a second in-frame methionine codon (codon seven). If this second rather than the first methionine codon is used as a translation start site, all α constructs would direct the synthesis of the same N-terminally truncated protein (corresponding to q(Δ2-4)). To exclude such a mechanism as a potential cause of the observed functional promiscuity displayed by the different mutant α subunits, an additional experiment was designed. Frameshift mutations were introduced between the two N-terminal ATG codons (codons one and seven), using two mutant α constructs, q(L3A) and q(E4A), as model systems (Fig. 6A). In q(L3A)-FS, the deletion of a single nucleotide (C) within codon three in q(L3A) led to a shift of the amino acid reading frame resulting in a premature stop codon after 10 amino acids of nonsense sequence. In q(E4A)-FS, the insertion of a single nucleotide (C) after codon four in q(E4A) led to a changed amino acid reading frame and a premature stop codon after 59 amino acids of nonsense sequence (Fig. 6A).

If codon seven is in fact used as a translation start site, q(L3A)-FS and q(E4A)-FS would be expected to exhibit functional properties similar to those of q(L3A) and q(E4A), respectively. However, coexpression studies showed that the two frameshifted mutant α constructs, in contrast to q(L3A) and q(E4A), were unable to interact with the m2 muscarinic receptor (Fig. 6B). Following ligand stimulation (carbachol, 0.5 mM) of the m2 muscarinic receptor, q(L3A) and q(E4A) were capable of mediating a pronounced increase in inositol phosphate production (7–8-fold above basal), whereas the responses observed with q(L3A)-FS and q(E4A)-FS were not significantly different from those found with vector-transfected control cells (approximately 3-fold above basal; Fig. 6B).

In addition to the functional studies, G protein expression was monitored by Western analysis using the 12CA5 monoclonal antibody (Fig. 7). Whereas q(L3A) and q(E4A) were found to be properly expressed in a fashion similar to qWT (detectable at 42–45 kDa), no α protein was detected after transfection with the q(L3A)-FS and q(E4A)-FS mutant constructs (Fig. 7, lanes 3 and 5). In this set of experiments, a weak nonspecific band of >50 kDa was observed in all lanes (including vector control), indicating that it represents a cross-reacting COS-7 cell protein. Taken together, these observations demonstrate that the second methionine codon (codon seven) is not used (at least not to a detectable degree) as a translation start site in the studied mutant α constructs.

Palmitoylation Pattern of Wild Type and Mutant α Subunits—Previous studies (24, 38–42) have shown that α and α_{11}, like most other Gα subunits, are palmitoylated at cysteine residues located near the N termini (corresponding to Cys-9 and Cys-10 in Fig. 1). We therefore wanted to examine whether the promiscuous mutant α subunits differed from qWT in their palmitoylation patterns. Toward this goal, COS-7 cells transfected with qWT or selected mutant α subunits (q(Δ2-4), q(Δ2-7), q(TLE→AAA), q(SI→AA), q(T2A), and q(M7A)) were metabolically labeled with [3H]palmitic acid, followed by immunoprecipitation of α subunits by the 12CA5 monoclonal antibody, SDS-PAGE, and fluorography. Western analysis showed that the amounts of immunoprecipitated wild type and mutant α proteins were similar (Fig. 8A). The α subunits were the only immunoprecipitated proteins, since no labeled proteins were detected when cells were transfected with “empty” vector DNA. As shown in Fig. 8B, qWT and all mutant α subunits incorporated significant amounts of [3H]palmitate. However, the strength of the palmitoylation signal was generally weaker in the case of the mutant α subunits (40–75% compared with qWT, as determined by scanning densitometry).

DISCUSSION

The fidelity of receptor/G protein interactions critically depends on the ability of different GPCRs to discriminate between unique structural features of the Gα subunits (2, 5–9). Characteristically, most Gα subunits (when bound to βγ in a trimeric complex) can be efficiently activated by only certain functional classes of GPCRs (7, 8). In this study, we have identified a series of 14 mutant α subunits that display promiscuous receptor coupling. In contrast to qWT, all 14 mutant subunits investigated here could be activated by both G_{μγ} and G_s-coupled receptors. In these mutant G proteins, residues within the six-amino acid N-terminal extension that is characteristic for α_{11} subunits were systematically substituted with alanine (either individually or in combination) or progressively deleted.
The most straightforward explanation for the observed functional promiscuity displayed by all mutant $\alpha_q$ subunits is that the unique N terminus of $\alpha_{q11}$ subunits adopts a well defined three-dimensional structure that is critical for maintaining the coupling selectivity of these G proteins. Based on this concept, mutations within this structural motif are thought to interfere with its proper folding and its ability to regulate receptor/G protein coupling selectivity.

Although all mutant G proteins showed a qualitatively similar functional profile, quantitative differences in their ability to be activated by different $G_{i/o}$ and $G_s$-coupled receptors (m2 muscarinic, D2 dopamine, and $\beta_2$-adrenergic) were noted. Moreover, the observed patterns of G protein activities also varied among the three receptors used in this study. It is likely that the specific receptor residues involved in G protein binding differ between the three receptors, thus providing an explanation for the observed ability of these receptors to discriminate between different mutant $G_{\alpha_q}$ subunits which, in turn, are also likely to display subtle structural differences.

Recently, the atomic structures of two different G protein heterotrimers, $G_{i1}B_{2}G_{\gamma_2}$ (Ref. 43) and $G_{\alpha_{q5}}B_{2}G_{\gamma_1}$ (Ref. 44), have been resolved by x-ray crystallography. In these structures (but not in the free $\alpha$ subunits), the N-terminal segment of the $\alpha$ subunit (corresponding to residues 6–26 in $\alpha_{q}$) is $\alpha$-helically arranged and protrudes away from the “bulk” of Go. This
resulting increases in intracellular IP$_1$ levels were determined as de-

so-called α-N-helix (Fig. 1) is engaged in interactions with the βγ complex via docking along the first blade of the β-propeller, thus determining the location of the acylated N terminus of Ga (note, however, that the G protein structures were resolved using proteins devoid of lipid modifications). Unfortunately, the extreme N termini of the α subunits (corresponding to amino acids 1–10 in α$_q$; Fig. 1) were not observed in the x-ray structures, probably due to their conformational flexibility. The available structural information therefore provides little insight into the potential arrangement of the N-terminal 10 amino acids of α$_q$. Mutants.

Accumulating evidence indicates that several C-terminal regions of Ga (including the C-terminal tail, portions of the α5-helix, and the α4/β6 loop) are directly involved in receptor binding and play a key role in dictating the selectivity of receptor/G protein interactions (10–15, 45–49). X-ray crystallographic studies suggest that the N- and C-terminal segments of Ga subunits are conformationally linked (43, 50). One possibility therefore is that the N-terminal extension characteristic for α$_q$ subunits constrains their receptor coupling selectivity by preventing access of G$_a$- and G$_b$-coupled receptors (either due to steric hindrance or to allosteric effects) to functionally critical C-terminal Ga regions.

As outlined in the Introduction, biochemical studies suggest that residues within the N-terminal 30-amino acid segment of Ga may be involved in receptor binding (14). Since this Ga region is also known to be critical for binding of Ga to βγ complexes (43, 44), binding of the receptor to an N-terminal Ga site may contribute to triggering dissociation of the G protein heterotrimer into free α and βγ subunits. An alternative pos-

FIG. 6. Lack of functional coupling of the m2 muscarinic receptor to frameshifted mutant α$_q$ subunits. A, a structure of frameshifted mutant α$_q$ subunits. For comparison, the amino acid and nucleotide sequences of the N-terminal segment of wild type α$_q$ (qWT) are shown on top. The two N-terminal in-frame ATG codons (codons one and seven) are underlined. In q(L3A)-FS, the deletion of a single nucleotide (C) within codon three in q(L3A) leads to a shift of the amino acid reading frame resulting in a premature stop codon after 10 amino acids of nonsense sequence. Analogously, in q(E4A)-FS, the insertion of a single nucleotide (C) after codon four in q(E4A) leads to a changed amino acid reading frame and a premature stop codon after 59 amino acids of nonsense sequence. B, COS-7 cells were cotransfected with expression plasmids coding for the wild type m2 muscarinic receptor and vector DNA (pcDNAI), qWT, or the indicated frameshifted α$_q$ mutant constructs. For comparison, the q(Δ2-7), (L3A), and q(E4A) mutants were also included in this set of experiments. Transfected cells were incubated for 1 h (at 37 °C) with 0.5 mM carbachol, and the IP$_1$ levels were determined as described under “Experimental Procedures.”

FIG. 7. Immunoblot analysis of frameshifted mutant α$_q$ subunits expressed in COS-7 cells. COS-7 cells were transfected with vector DNA (pcDNAI), qWT, and various mutant α$_q$ constructs (for structures of mutant G proteins, see Figs. 1 and 6A). Equal amounts of membrane protein (20 μg) prepared from transfected COS-7 cells were analyzed by SDS-PAGE (13%) and Western blotting, using the 12CA5 monoclonal antibody as described under “Experimental Procedures.” The following mutant constructs were studied: q(Δ2-7) (lane 1), q(L3A) (lane 2), q(L3A)-FS (lane 3), q(E4A) (lane 4), and q(E4A)-FS (lane 5). Protein molecular mass standards (in kDa) are indicated. Please note that COS-7 cells transfected with q(L3A)-FS (lane 3) and q(E4A)-FS (lane 5) did not express detectable amounts of α$_q$ protein (observable at approximately 45 kDa in all other lanes, except vector). In this set of experiments, a nonspecific band of ~50 kDa was observed in all lanes (including vector control), indicative of a cross-reacting COS-7 cell protein. Three additional experiments gave similar results.

FIG. 8. Palmitoylation of wild type and mutant α$_q$ subunits expressed in COS-7 cells. COS-7 cells were transfected with vector DNA, qWT, and selected α$_q$ deletion and point mutants. Following labeling with [3H]palmitic acid (16 h; 200 μCi/ml), cells were lysed, and extracts were subjected to immunoprecipitation with the 12CA5 antibody as described under “Experimental Procedures.” A, aliquots of the immunoprecipitated proteins (10% of total samples) were analyzed by SDS-PAGE (13%) and Western blotting, using the 12CA5 monoclonal antibody. B, the remaining 90% of the samples were resolved by SDS-PAGE (13%) and analyzed by fluorography, as described under “Experimental Procedures.” The following mutant constructs were studied: q(Δ2-7) (lane 1), q(TLE → AAA) (lane 2), q(SI → AA) (lane 3), q(Δ2-4) (lane 4), q(M7A) (lane 5), and q(T2A) (lane 6). Protein molecular weight standards (in kDa) are indicated. Two additional experiments gave similar results.
The ability of a receptor to mediate G protein-dependent increases in PLC activity remains to be elucidated. Whether the coupling promiscuity allows us to distinguish between these different possibilities. Thus, another possibility is that the N terminus of the receptor plays key roles in triggering G protein activation (3, 8). Recent studies have shown that several positively charged residues located near the N terminal of a subset of GPCRs resembles those of two other G protein subunits, α1q and α12q, which appear to be the murine and human versions of the same gene (Ref. 51). Recent studies have shown that α1q and α12q, the murine and human versions of the same gene (Ref. 51), are not the murine and human versions of the same gene (Ref. 51). A sequence comparison indicates that the N-terminal eight amino acids of α15/16 (MARS/LTWG(R)) differ considerably from the corresponding α1/11 sequence (MTLESI(M)MA) (19, 51, 54). It therefore remains to be elucidated whether the coupling promiscuity displayed by α15/16 and all mutant αq constructs examined here is dependent on a similar molecular mechanism.

Like most other Gα subunits, αq and α11 are known to be reversibly palmitoylated at cysteine residues located near their N termini (corresponding to Cys-9 and Cys-10 in Fig. 1; Refs. 24 and 38–42). Studies with mutant αq constructs in which both N-terminal cysteine residues were replaced with serine or alanine suggest that palmitoylation facilitates membrane attachment of the α subunit (24, 38, 39). However, palmitoylation does not appear to be an absolute requirement for membrane targeting of αq (39). In addition, palmitoylation-defective mutant αq subunits were found to be severely impaired in their ability to mediate receptor-dependent increases in PLCγ activity (24, 38). However, in a reconstituted system, enzymatic removal of palmitate from purified αq had little effect on the ability of this subunit to interact with receptors and downstream effector enzymes (39), indicating that the N-terminal cysteine residues themselves rather than the palmitoyl moieties attached to them are of primary functional importance.

In the present study, all examined mutant αq subunits, similar to the approach used for the other Gα subunits, were found to incorporate significant amounts of [3H]palmitate (Fig. 8B). We noted, however, that the intensity of the palmitoylation signal was reduced in the case of the murine and human versions of the same gene (Ref. 51). The murine and human versions of the same gene (Ref. 51). The murine and human versions of the same gene (Ref. 51). A sequence comparison indicates that the N-terminal eight amino acids of α15/16 (MARS/LTWG(R)) differ considerably from the corresponding α1/11 sequence (MTLESI(M)MA) (19, 51, 54). It therefore remains to be elucidated whether the coupling promiscuity displayed by α15/16 and all mutant αq constructs examined here is dependent on a similar molecular mechanism.

Like most other Gα subunits, αq and α11 are known to be reversibly palmitoylated at cysteine residues located near their N termini (corresponding to Cys-9 and Cys-10 in Fig. 1; Refs. 24 and 38–42). Studies with mutant αq constructs in which both N-terminal cysteine residues were replaced with serine or alanine suggest that palmitoylation facilitates membrane attachment of the α subunit (24, 38, 39). However, palmitoylation does not appear to be an absolute requirement for membrane targeting of αq (39). In addition, palmitoylation-defective mutant αq subunits were found to be severely impaired in their ability to mediate receptor-dependent increases in PLCγ activity (24, 38). However, in a reconstituted system, enzymatic removal of palmitate from purified αq had little effect on the ability of this subunit to interact with receptors and downstream effector enzymes (39), indicating that the N-terminal cysteine residues themselves rather than the palmitoyl moieties attached to them are of primary functional importance.

In the present study, all examined mutant αq subunits, similar to qWT, were found to incorporate significant amounts of [3H]palmitate (Fig. 8B). We noted, however, that the intensity of the palmitoylation signal was reduced in the case of the murine and human versions of the same gene (Ref. 51). On the other hand, Western analysis of immunoprecipitated, [3H]palmitate-labeled G proteins indicated that most mutant αq subunits were expressed at levels similar to qWT (Fig. 8A). It is therefore conceivable that palmitoylation plays an inhibitory role in constraining the receptor coupling selectivity of αq, perhaps by specifying the spatial orientation and fold of the N terminus of αq. Consistent with this notion, biochemical studies have shown that the myristoyl moiety attached to the N terminus of αq is essential for the proper folding of the N-terminal αq segment (55). In addition, a previous study analyzing the coupling properties of a palmitoylation-defective mutant αq subunit suggests that palmitoylation inhibits αq signaling by an as yet unknown mechanism (56). Interestingly, Tu et al. (57) recently showed that palmitoylation of αq and α1 strongly inhibits the activity of several RGS proteins (regulators of G protein signaling) which are known to stimulate the GTPase activity of these α subunits. This finding indicates that palmitoylation of α subunits may represent a general mechanism for prolonging the lifetime of activated α subunits. However, such a mechanism cannot explain the functional properties of the mutant αq subunits investigated in this study, which appear to be palmitoylated less efficiently (as compared with qWT) and would therefore be expected to show reduced rather than increased functional activity.

In eucaryotic organisms, translation initiation is usually restricted to the first in-frame ATG (AUG in the case of mRNA) codon. However, several examples are known where translation is initiated from a downstream ATG codon (a process frequently referred to as “leaky scanning”), particularly when the first in-frame ATG codon is located in a “weak” context that considerably deviates from the consensus sequence (5′-CCUA/G/CCATGG-3′) for strong initiation codons (58, 59). The residues that exert the strongest effects on the efficiency of translation initiation are a −3 purine (A or G) and a +4 G (the A of the ATG codon is numbered +1, and nucleotides to the left have negative numbers). Inspection of the nucleotide sequences of the αq subunits shows that the second methionine codon (q(Δ2-7)) of the ATG codon is located in a clearly weaker context than the first one (codon one) (Fig. 6A). It was therefore important to demonstrate that the mutant αq constructs analyzed in this study do not allow translation initiation from codon seven, thus yielding the same, N-terminally truncated protein (q(Δ2-7)) that is known to be functionally promiscuous (18). Several observations clearly argue against such a mechanism. First, since the first ATG in qWT is located in the same weak context as all mutant αq plasmids (all constructs contain identical 5′-flanking sequences), leaky scanning should also lead to the synthesis of the promiscuous q(Δ2-7) subunit in the case of qWT. However, no such promiscuous coupling was observed when qWT was coexpressed with different Gq- and Gα-i coupled receptors. Second, a mutant αq construct in which the second ATG codon (codon seven) was replaced with an alanine codon (thus allowing only translation from codon one) showed functional properties similar to all other mutant αq constructs examined. Third, we constructed two mutant αq subunits, q(L3A)-FS and q(E4A)-FS, that differed from q(L3A) and q(E4A) only by the deletion and insertion of a single nucleotide, respectively (Fig. 6A). To avoid changing the nucleotide sequences immediately adjacent to the two ATG codons, these mutations were introduced 7–11 base pairs upstream of the second ATG codon. Functional studies showed that the two frameshifted mutant αq constructs did not allow the synthesis of (mutant) αq subunits capable of interacting with the m2 muscarinic receptor (Fig. 6B). Consistent with this finding, no q(Δ2-7) protein could be detected via Western blotting (Fig. 7). Taken together, these observations clearly exclude the possibility that the functional promiscuity of the N-terminal αq mutants studied here is due to leaky scanning and the use of the second methionine codon (codon seven) as a translation start site.

In conclusion, our data suggest a novel mechanism by which receptor/G protein coupling selectivity can be regulated. Elucidating the precise molecular mechanisms involved in this process should eventually lead to new insight into the complex processes governing the selectivity of receptor/G protein interactions.

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