The C-terminal Tail Preceding the CAAX Box of a Yeast G Protein γ Subunit Is Disposable for Receptor-mediated G Protein Activation in Vivo*

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The γ subunits of heterotrimeric G proteins are required for receptor-G protein coupling. The C-terminal domains of Gγ subunits can contact receptors and influence the efficiency of receptor-G protein coupling in vitro. However, it is unknown whether receptor interaction with the C terminus of Gγ is required for signaling in vivo. To address this question, we cloned Gγ homologs with diverged C-terminal sequences from five species of budding yeast. Each Gγ homolog functionally replaced the Gγ subunit of the yeast *Saccharomyces cerevisiae* (STE18 gene product). Mutagenesis of *S. cerevisiae* Ste18 likewise indicated that specific C-terminal sequence motifs are not required for signaling. Strikingly, an internal in-frame deletion removing sequences preceding the C-terminal CAAX box of Ste18 did not impair signaling by either of its cognate G protein-coupled pheromone receptors. Therefore, receptor interaction with the C-terminal domain of yeast Gγ is not required for receptor-mediated G protein activation in vivo. Because the mechanism of G protein activation by receptors is conserved from yeast to humans, mammalian receptors may not require interaction with the tail of Gγ for G protein activation in vivo. However, receptor-Gγ interaction may modulate the efficiency of receptor-G protein coupling or promote activation of Gβγ effectors that co-cluster with receptors.

Heterotrimeric GTP-binding proteins consisting of α, β, and γ subunits relay signals from receptors for many hormones, neurotransmitters, and sensory stimuli (1). Activated receptors promote GDP release and GTP binding to G proteins, resulting in dissociation of GTP-bound Gα subunits from Gβγ complexes, which trigger cellular responses. To activate G proteins, receptors are thought to act as a “lever” to move Gα relative to Gβγ by contacting a surface ~30 Å from the nucleotide binding site of the Gα subunit (2–5). In this model, Gβ catalyzes nucleotide exchange, because it interacts with the lip of the nucleotide binding pocket formed by Switch 1 and part of Switch 2 of the Gα subunit (6–8). Thus, movement of Gβ relative to Gα would allow Gβ to pull on the lip of the nucleotide binding pocket, inducing GDP release and subsequent GTP loading (4). Consistent with this mechanism, a mutant of Goα has been identified that requires association with Gβ to trigger a signal independently of a receptor (5). Gβ may also provide a receptor binding site that is important for G protein activation (9–12).

Gγ subunits function indirectly to promote G protein activation, because they do not contact Gα subunits (6–8). Gγ subunits can bind receptors, as indicated by studies of rhodopsin and transducin, which show that βγ binds activated rhodopsin and promotes activation of the α subunit (13, 14). Furthermore, rhodopsin preferentially activates transducin containing its cognate γ subunit (γ1; see Ref. 15); other receptors likewise couple preferentially with G proteins containing certain Gγ subunit isoforms *in vitro* (16–18). Selective coupling appears to be mediated by the diverged C termini of Gγ subunits, because peptides corresponding to the last 14 residues of γ1 stabilize the active conformation of rhodopsin (metalpha) and competitively inhibit rhodopsin-mediated activation of transducin (19).

These and other observations have suggested that receptors possess independent binding sites for the C-terminal domains of Gα and Gγ subunits (13, 14, 19). Thus, one variation of the “lever hypothesis” of G protein activation requires simultaneous binding of the C termini of Gα and Gγ to independent sites on a receptor (5), although other variations of this model do not (2, 3).

In certain signaling pathways, receptor interaction with the C terminus of Gγ may have a non-essential role in G protein activation. Studies have shown that γ7, which interacts poorly with M2 or M4 muscarinic acetylcholine receptors *in vitro*, supports 2-fold more efficient coupling with these receptors than does a Go heterotrimer containing γ5, whose tail binds these receptors *in vivo* (16). Furthermore, muscarinic receptor coupling to Go in *in vitro* is enhanced when the heterotrimer contains γ5 with a scrambled C-terminal sequence that does not bind muscarinic receptors (20). However, it remains to be established whether receptor binding to the C termini of Gγ subunits is an obligate or modulatory event for G protein activation *in vivo*.

We established previously that the C-terminal domain of Gγ promotes receptor-G protein coupling by analyzing mutations affecting the Gγ subunit (STE18 gene product) of the yeast *Saccharomyces cerevisiae*. In yeast, a single type of G protein heterotrimer transduces signals from mating pheromone receptors (21), providing a simple system to evaluate Gγ function genetically and biochemically. We found that Gγ truncated at position 94 (13 residues preceding the CAAX box) associates with Gβ but strongly impairs receptor-G protein coupling *in vitro* (agonist binding was low affinity and insensitive to...
GTPyS)$^1$ (22). This receptor coupling defect was not because of lack of C-terminal prenylation or palmitoylation, because Gα bearing a substitution (C107S) inactivating its CAAX box associated with Gα and Gβ and supported efficient receptor-G protein coupling in vitro (agonist binding was high affinity and sensitive to GTPyS). Therefore, peptide sequences between residue 94 and the CAAX box of yeast Gα, equivalent to the receptor binding domains of mammalian Gα subunits (14, 15, 18, 19, 23, 24), were proposed to promote receptor-G protein coupling.

Here we have investigated whether the C-terminal domain of yeast Gα is required for receptor-mediated signaling in vivo. By analyzing the function of a diverged family of Ste18 homologs from several species of budding yeast and amino acid substitutions affecting the C terminus of Ste18 we provide evidence indicating that the C-terminal domain preceding the CAAX box of Gα is dispensable for receptor-mediated signaling in vivo.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Media**—The *S. cerevisiae* strains used in these studies are listed in Table 1. *S. cerevisiae* cells were grown in standard synthetic medium containing adenine and amino acid supplements but lacking appropriate nutrients to select for cells containing plasmids.

**Plasmids, Oligonucleotide-directed Mutagenesis, and Sequence Analysis**—STE18 was expressed from the PGK promoter, and terminator sequences were cloned into the yeast high copy plasmid pRS425 as follows. A fragment containing the PGK promoter and terminator separated by a multicloning site containing restriction sites for EcoRI, HindIII, and BamHI was excised from the pPGK vector (25) by digesting with XhoI and SalI and inserted into pRS425 (26) cut with XhoI and SalI. The resultant plasmid was digested with SalI and NotI and circularized to produce pRS425PGK, which has unique EcoRI, HindIII, and BamHI sites for expression cloning. Wild-type and mutant fragments of the STE18 coding region were cloned as HindIII-BamHI fragments into pRS425PGK to express the native, untagged forms of these Ste18 subunits were expressed from their normal chromosomal loci and therefore were limiting.

**Cloning and Expression of STE18 Homologs from Five Divergent Saccharomyces Species**—The genome sequences of five species of yeast of varying relativeness to *S. cerevisiae* (Saccharomyces bayanus (strain NRRL Y-11845), Saccharomyces castellii (NRRL Y-12830), Saccharomyces kluyveri (NRRL Y-12851), Saccharomyces kudriavzevi (IFO 1802), and Saccharomyces mikatae (IFO 1815); provided by M. Johnston, Washington University Genome Sequencing Center) were searched by BLAST for STE18 homologs. As is the case in *S. cerevisiae*, the genome of each of these species of yeast encoded a single STE18 homolog (data not shown). Contig sequences encoding each STE18 homolog were provided by P. Clifton and M. Johnston (Washington University Genome Sequencing Center) and will be deposited in GenBank™ by those investigators. Primers used to amplify only the coding region of each STE18 homolog were designed such that the resultant amplification products could be cloned as HindIII-BamHI fragments into pRS425PGK as follows. Genomic DNA from each species of yeast (obtained from M. Johnston, Washington University School of Medicine) and the following primers were used: forward S. bayanus, 5’-GGGACAGCGTGCAGTCGAACGTTG-3’ and reverse S. bayanus, 5’-GGCCGATCTTACATAAGGCTACAAACTG-3’; for- ward S. kudriavzevi, 5’-GGGACAGCGTGCAGTCGAACGTTG-3’ and reverse S. kudriavzevi, 5’-GGCCGATCTTACATAAGGCTACAAACTG-3’; forward S. kluyveri, 5’-GGCCGATCTTACATAAGGCTACAAACTG-3’ and reverse S. kluyveri, 5’-GGCCGATCTTACATAAGGCTACAAACTG-3’. The amplification conditions using Taq polymerase were as follows: 96°C for 5 s, 30 cycles of 96°C for 20 s, 45°C for 2 min, and 68°C for 2 min; 72°C for 10 min. The resultant PCR frag- ments were sequemcntially digested with BamHI and HindIII and cloned into pRS425PGK to express the native, untagged forms of these Ste18 homologs in *S. cerevisiae*. The correct nucleotide sequence of each construct was confirmed by sequencing.

**Random Mutagenesis of S. cerevisiae STE18 and Identification of STE18 Dominant-negative Mutants**—A plasmid suitable for performing random mutagenesis of codons 79–110 in the STE18 gene was con- structed by introducing a silent KspI site into sequences encoding amino acids 77–79 and a KanI site immediately downstream of the STE18 stop codon in plasmid pVT-HA-STE18, resulting in pVT-HA-STE18-KK. Oligonucleotides containing 1% of the three incorrect bases at each position spanning the coding region for amino acids 79–110 of Ste18 were synthesized and inserted into pVT-HA-STE18 which was wild-type, and the remaining contained mutations affecting vari- ous codons throughout the targeted region, as expected. To select for dominant-negative STE18 mutations, we introduced the pool of 1500 plasmids into the wild-type *S. cerevisiae* strain RK511-6B. Portions of the transformation mixture were plated on selective media that lacked or contained a dose of α-factor (1 µM) sufficient to arrest growth of the parent strain expressing wild-type Ste18. Colonies resistant to α-factor were recovered at a frequency of 3%. Plasmid dependence of the α-factor-resistant phenotype was demonstrated by recovery of α-factor sen- sitivity following plasmid loss on media containing 5-fluoroorotic acid. Plasmids conferring α-factor resistance were isolated from yeast cells and sequenced. The same pool of mutagenized DNA was also subjected to a screen for STE18 dominant-negative mutations in which wild-type cells (RK511-6B) were transformed with the library, and random colonies were picked and screened for impaired response to α-factor in growth arrest (halo) assays.

**Pheromone Response Assays**—Long-term (48 h) pheromone-induced growth arrest (halo) assays were used to determine the ability of cells to respond to varying concentrations of agonist (α-factor) as described previously (27). Sterile paper disks containing various amounts of synthetic agonist (α-factor; 15 pmol, 50 pmol, 150 pmol, 500 pmol, 1.5 nmol) were applied to lawns of cells embedded in soft agar. After incubation at 30°C for 2 days, zones of growth inhibition were measured, and plates were scanned electronically to record images. Unless indicated other- wise, halo assays were performed with STE18 cells expressing various wild-type or mutant STE18 alleles from the ADH or PGK promoter on high copy plasmids. Although various STE18 homologs and *S. cerevisiae* STE18 alleles were expressed from strong promoters on high copy plasmids, this would not result in overexpression of Gαβγ complexes, because Gα and Gβ subunits were expressed from their normal chromosomal loci and therefore were limiting.

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$^1$ The abbreviations used are: GTPyS, guanosine 5’-3-O-(thio)triphosphate; HA, hemagglutinin.
Missense Mutations Affecting the C-terminal Domain of Ste18, these results suggested that the C-terminal domain of Ste18 may not be critical for receptor-G protein coupling and signaling.

Despite exhibiting varying degrees of sequence conservation in their putative receptor coupling domains, all of the Ste18 homologs could correct the signaling defect of S. cerevisiae cells carrying a disruption of the chromosomal STE18 gene. As indicated by quantitative assays of agonist (α-factor)-induced growth arrest (Fig. 2), expression of Ste18 homologs most similar to S. cerevisiae Ste18 (S. bayanus, S. mikatae, or S. kudriavzevii) fully restored agonist responsiveness to the S. cerevisiae ste18Δ mutant (zones of growth inhibition were equivalent to those of ste18Δ cells expressing the S. cerevisiae STE18 gene from a plasmid). More strikingly, expression of either of the more highly diverged Ste18 homologs (S. kluveri and S. castellii) nearly completely restored agonist responsiveness to the ste18Δ mutant (zones of growth inhibition were slightly smaller or turbid). Because the C-terminal domains of these latter two Ste18 homologs are quite diverged (57 and 50% identity, respectively).
tide sequences preceding the CAAX box of *S. cerevisiae* Ste18 are important for receptor-mediated signaling, we constructed and analyzed several types of missense mutations affecting this domain. We first used alanine scanning mutagenesis to target residues 99–105, just upstream of the CAAX box, which is prenylated and palmitoylated and required for activation of the downstream mitogen-activated protein kinase cascade (30–32). Pairs of residues were substituted with an Ala-Ala dipeptide to create six mutants (M99A,S100A; S100A,N101A; N101A,S102A; S102A,N103A; N103A,S104A; S104A,V105A) that were analyzed for their ability to function when expressed from a plasmid in a ste18Δ mutant. As indicated by quantitative assays of agonist-induced growth arrest, expression of each of these mutants in a ste18Δ mutant resulted in wild-type or nearly wild-type response to agonist (Fig. 3A), suggesting that the side chains of residues 99–105 are dispensable for receptor-mediated signaling.

As an alternative to alanine scanning mutagenesis, we altered the charge distribution of selected residues within the C-terminal domain of Ste18. In one mutant, lysine 95 was changed to aspartic acid, and in another mutant three uncharged residues (Met-99, Ser-100, Asn-101) were all changed to aspartic acid. Expression of either of these mutants from a plasmid in cells carrying a deletion of the chromosomal STE18 locus fully restored agonist-dependent signaling (Fig. 3B), further suggesting that specific amino acid sequences in the C-terminal domain of Ste18 are not essential for receptor coupling and signaling in vivo.

Although suggestive, the preceding results could not exclude the possibility that the C-terminal domain of Ste18 contains several sequences or motifs that mediate receptor coupling in a functionally redundant manner. To address this possibility, we introduced a string of four alanine residues at positions 94–97 and 102–105 or eight alanine residues at positions 98–105. Strikingly, expression of any of these STE18 alleles from a plasmid fully rescued the ability of a ste18Δ mutant to respond to agonist (Fig. 3C).

We also considered that Ste18 containing a string of eight alanine residues (positions 98–105) preceding the CAAX box could cause a quantitative defect in receptor-mediated signaling that was difficult to detect by *in vivo* signaling assays. Therefore, to increase the sensitivity of these assays we expressed plasmid-born STE18 alleles in a ste18Δ mutant that expressed α-factor receptors lacking their C-terminal domains. Receptors lacking their C-terminal tail were used, because they are partially impaired for G protein coupling *in vitro* (33), which is manifested *in vivo* by zones of agonist-induced growth inhibition that are slightly turbid and have less distinct margins (Fig. 3D). Despite this partial impairment in function, truncated α-factor receptors signaled with similar efficiency whether wild-type Ste18 or mutant Ste18 containing eight alanine residues was expressed from a plasmid in ste18Δ cells (Fig. 3D). Therefore, this mutant form of Ste18 appeared to be highly functional even when receptor-G protein coupling was partially impaired by other mechanisms.

**Mutations throughout Sequences Encoding the C-terminal Domain Preserve Ste18 Function**—To address whether receptor coupling information is present elsewhere within the C-terminal domain of Ste18, we performed random oligonucleotide mutagenesis of sequences encoding residues 79–110. Sequences further upstream were spared, because they probably are required for association with Gα, as suggested by structural studies of mammalian Gβγ complexes (34). A pool of plasmids created by random oligonucleotide mutagenesis of this region contained point and frameshift mutations as indicated by sequencing random clones (data not shown). This plasmid pool was subjected to two types of analyses to identify mutations that potentially impair receptor coupling. In the first, we selected for plasmids that encode dominant-negative Gy subunits. Dominant-negative Gy mutants were selected by their ability to allow wild-type cells to form colonies on medium containing a high dose of agonist (α-factor; 1 μM) sufficient to arrest the growth of control cells. This approach yielded only mutations that inactivated or removed the CAAX box of Ste18 (truncation, frameshift, or missense mutants; see Table II), which is required for membrane targeting (31).

Because the previous selection method might have been biased for very strong dominant-negative STE18 mutations that nearly completely block signaling, our second approach used a screen that is capable of identifying plasmids in the mutagenized library that encode weaker dominant-negative Gy subunits. The screen was performed by introducing the library of mutagenized plasmids into wild-type cells, picking colonies at random, and assaying for impaired agonist-induced signaling, as indicated by formation of smaller or turbid zones of agonist-induced growth inhibition. Despite the ability of this assay to detect partial loss of function mutants (35), the only mutations that caused a detectable dominant-negative phenotype affected the CAAX box (truncation, frameshift, missense, or point mutations; see Fig. 4 and Table II). Therefore, these
The preceding results suggested that sequences preceding the CC box were retained, because palmitoylation and prenylation of Ste18 is required for downstream signaling (30–32), although these modifications are not required for receptor-G protein coupling in vitro (22). Strikingly, expression of this STE18 internal deletion allele (Δ94–105) from a plasmid fully restored the ability of a ste18Δ mutant to respond to agonist (a-factor; see Fig. 5). Expression of an N-terminally HA-tagged version of this internal deletion mutant protein, as well as two other mutants analyzed in this study (98–105Ala; C107S, a CAAX mutant) was confirmed by immunoblotting (Fig. 6). Therefore, the C-terminal region of Ste18 immediately preceding the CAAX box is dispensable for a-factor receptor-mediated G protein activation and signaling.

**Table II**

| Dominant-negative STE18 alleles | Dominant-negative selection | Dominant-negative screen | Pool |
|---------------------------------|-----------------------------|--------------------------|------|
| Missense mutations              | None                        | None                     | %    |
| Nonsense mutations              | Cys-107<sup>a</sup>         | Cys-107<sup>a</sup>      | 50   |
|                                 | G82R, Ser-100<sup>a</sup>   | Val-80<sup>b</sup>       |      |
|                                 | Leu-90, Ser90<sup>b</sup>   | Phe-86<sup>a</sup>       |      |
|                                 | A93D, Lys-95<sup>a</sup>    | I87L, L90F Gln-98<sup>a</sup> |      |
|                                 | ES9G, Lys-95<sup>a</sup>    | G82V, F85L, M99T, Cys-107<sup>a</sup> |      |
|                                 | Lys-95<sup>a</sup>          | N84T, I87L, Gln-94<sup>a</sup> |      |
|                                 | Cys-106<sup>a</sup>         | H85L, F86L, Ile-87<sup>a</sup> |      |
|                                 | F86I, S97G, Ser-100<sup>a</sup> |                      |      |
| Frameshift mutations            | V80L, I87M, Leu-90<sup>b</sup> | Met-99<sup>a</sup>       | 28   |
|                                 | Asn-97<sup>b</sup>          | Q98H, Met-99<sup>b</sup> |      |
|                                 | Asn-92<sup>b</sup>          | Δ84–87 Gln-88<sup>a</sup> |      |
|                                 | Val-80<sup>b</sup>          | As-81<sup>b</sup>        |      |
|                                 | F86L, Asn-92<sup>b</sup>    | Glu-78<sup>b</sup>       |      |
|                                 | M99T, N103T, V105D, Cys-107<sup>b</sup> | As-84<sup>b</sup> |      |
|                                 | S83P, Asn-84<sup>b</sup>    | Pro-79<sup>b</sup>       |      |
|                                 | G82V, F85L, M99T, Cys-107<sup>a</sup> | His-85<sup>b</sup> |      |
| CAAX missense mutations         | G89D, A93D, C107M, T108S    | S83L, C107W              | 7    |
|                                 | V105G, C107R                |                           |      |
|                                 | C107S                       |                           |      |

<sup>a</sup> Codon changed to nonsense mutation.

<sup>b</sup> Codon affected by frameshift mutation.

**Fig. 4.** Inhibition of wild-type Ste18 function by overexpressed dominant-negative STE18 alleles. The indicated wild-type (WT) and dominant-negative STE18 alleles were overexpressed from the ADH promoter on a high copy plasmid (pVT-HA-STE18) in a strain (RK511–6B) carrying a wild-type STE18 gene on the chromosome. The results of agonist (a-factor; 1.5 nmol/disk)-induced growth arrest assays are shown. Codons affected by nonsense and frameshift mutations are indicated by * and **, respectively.

results suggested that a functionally critical receptor-coupling domain may not be present within residues 79–107 of Ste18.
type or mutant Ste18 (Table III), and the ability of these cells to mate required the presence of a STE18 plasmid (data not shown). Therefore, we conclude that signaling by neither the \( \alpha \)-factor receptor nor the \( \alpha \)-factor receptor in vivo requires the C-terminal domain preceding the CAAX box of Ste18.

### DISCUSSION

The primary conclusion of this study is that the C-terminal domain preceding the CAAX box of the \( \gamma \) subunit encoded by the STE18 gene of the yeast \( S. cerevisiae \) is dispensable for signaling by its two cognate G protein-coupled receptors in vivo. Furthermore, we obtained no evidence indicating that alteration of the C-terminal domain of yeast \( \gamma \) has a strong modulatory effect on the efficiency of receptor-mediated signaling in vivo. These findings are in contrast to the well established and evolutionarily conserved requirement for receptor contact with the C-terminal domain of the \( \alpha \) subunit in the process of G protein activation (reviewed in Refs. 1, 2, 4, 21, and 38).

The results presented here and our previous studies suggest that caution must be exercised when assessing the potential importance of certain receptor-G protein contacts solely by biochemical approaches. In the in vitro system used to detect receptor-G protein coupling in yeast plasma membrane fractions, agonist binding affinity of the \( \alpha \)-factor receptor is sensitive to GTP\( \gamma \)S (i.e. becomes low affinity) only under conditions of high salt concentration and moderately elevated pH (40). At physiological salt concentration and pH, in contrast, agonist binding to \( \alpha \)-factor receptors in plasma membrane fractions is GTP\( \gamma \)S-insensitive (i.e. remains high affinity; see Ref. 40). Therefore, only at high salt concentration and elevated pH is it possible to show that a-factor receptor-G protein coupling in vitro requires the \( \gamma \) tail (22). However, these reaction conditions may decrease the stability of receptor-G protein complexes such that a functional interaction between the receptor and the \( \gamma \) tail becomes evident. In contrast, the \( \alpha \)-factor receptor-G protein interface may be more stable in vivo and therefore tolerates loss of sequences preceding the CAAX box of \( \gamma \).

How do our results impact current models of receptor-mediated G protein activation in which receptors are hypothesized to act as a lever to open the nucleotide binding pocket of \( \alpha \) (3, 5)? One variation of this model suggests that simultaneous contact between the receptor and the C-terminal domains of \( \alpha \) and \( \gamma \) is required for receptors to open the nucleotide binding site of \( \alpha \) and catalyze nucleotide exchange; however, our results exclude this model, at least in yeast. Instead, they support mechanisms in which the receptor contacts \( \alpha \), \( \beta \), and/or other regions of \( \gamma \) to activate the G protein. They are also consistent with a model in which receptor interaction with \( \alpha \) is responsible for perturbing the \( \alpha \) helix (2, 3), which does not invoke an essential role for receptor-\( \gamma \) interaction.

Are the C-terminal domains of \( \gamma \) subunits dispensable for receptor-mediated G protein activation in mammalian cells? Although this question requires further investigation, several considerations suggest that contacts between the tails of \( \gamma \) subunits and mammalian G protein-coupled receptors are unlikely to be essential for G protein activation in vivo. First, the mechanism of receptor-mediated G protein activation is conserved from yeast to humans, because similar domains of yeast and mammalian receptors and G proteins are required for receptor-G protein coupling (22, 35, 36, 40–43). Second, sequences preceding the CAAX boxes of mammalian and yeast \( \gamma \) subunits are not conserved, yet several types of mammalian receptors expressed in yeast can activate a G protein consisting of a yeast/mammalian \( \alpha \) chimera, yeast \( \beta \) (Ste4), and yeast \( \gamma \) (Ste18) (44–46). Third, \( \gamma \) carrying a scrambled amino acid sequence preceding its CAAX box does not bind M2 muscarinic acetylcholine receptors but can support efficient coupling of \( \gamma \alpha \) and \( \gamma \beta \) with these receptors in vitro (20). Fourth, \( \gamma \)\( \gamma \), which does not appear to interact with M2 or M4 muscarinic acetylcholine receptors in vitro, supports more efficient coupling with these receptors than does a G protein containing \( \gamma \) alone, whose C-terminal tail can bind these receptors in vitro (16).

In apparent contrast, however, there is evidence suggesting that the \( \gamma \) tail is important for coupling between muscarinic receptors and G proteins in vivo (24). Cytoplasmic injection of a geranylgeranylated peptide corresponding to the wild-type C terminus of \( \gamma \) strongly impairs the ability of muscarinic receptors to inhibit N-type Ca\(^{2+}\) channels in primary sympathetic neurons, whereas a scrambled version of this peptide or wild-type peptides corresponding to the C termini of \( \gamma \) or \( \gamma \) have no effect (24). These observations are consistent with the hypothesis that muscarinic receptors have a functionally important binding site for the C terminus of \( \gamma \). However, they are equally likely to suggest that the \( \gamma \) peptide blocks the ability of G\( \beta \)\( \gamma \) subunits to inhibit N-type Ca\(^{2+}\) channels by interfering with G\( \beta \)\( \gamma \) binding to the channel.

Because the C-terminal domains of \( \gamma \) subunits clearly have the ability to bind receptors (14–20, 22, 24), this interaction...
may have novel functions in yeast or mammalian cells. For example, this interaction might allow Gβγ subunits to remain bound to receptors after Gα subunits are activated, which could promote downstream signaling via Gβγ effectors that cluster with N-type Ca2+ channels due to release of Gβγ subunits from receptors that potentially cluster with N-type channels, decreasing the local concentration of Gβγ required to maximally inhibit these channels. Tethering of Gβγ subunits to receptors also has the potential to promote resetting of the system to the inactive state by facilitating capture of Gα subunits from active state by facilitating capture of Gα subunits from receptors. Indeed, the blocking effect of the Ca2+ peptide on muscarinic receptor-mediated inhibition of N-type Ca2+ channels may have novel functions in yeast or mammalian cells. For example, this interaction might allow Gβγ subunits to remain bound to receptors after Gα subunits are activated, which could promote downstream signaling via Gβγ effectors that cluster with N-type Ca2+ channels due to release of Gβγ subunits from receptors that potentially cluster with N-type channels, decreasing the local concentration of Gβγ required to maximally inhibit these channels. Tethering of Gβγ subunits to receptors also has the potential to promote resetting of the system to the inactive state by facilitating capture of Gα subunits after GTP hydrolysis. Therefore, further studies of receptor-Gγ interaction may reveal new insights into mechanisms that control the efficiency, fidelity, and kinetic control of G protein signaling.

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