Dual Lipid Modification of the Yeast G\textsubscript{Y} Subunit Ste18p Determines Membrane Localization of G\textbeta\textgamma

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The pheromone response in the yeast 	extit{Saccharomyces cerevisiae} is mediated by a heterotrimeric G protein. The G\textbeta\textgamma subunit (a complex of Ste4p and Ste18p) is associated with both internal and plasma membranes, and a portion is not stably associated with either membrane fraction. Like Ras, Ste18p contains a farnesyl-directing CaaX box motif (C-terminal residues 107 to 110) and a cysteine residue (Cys 106) that is a potential site for palmitoylation. Mutant Ste18p containing serine at position 106 (mutation ste18-C106S) migrated more rapidly than wild-type Ste18p during sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The electrophoretic mobility of wild-type Ste18p (but not the mutant Ste18p) was sensitive to hydroxylamine treatment, consistent with palmitoyl modification at Cys 106. Furthermore, immunoprecipitation of the G\textbeta\textgamma complex from cells cultured in the presence of \textsuperscript{3}Hpalmitic acid resulted in two radioactive species on nonreducing SDS-PAGE gels, with molecular weights corresponding to G\textbeta\textgamma. Substitution of serine for either Cys 107 or Cys 106 resulted in the failure of G\textbeta\textgamma to associate with the membrane. The Cys 107 substitution also resulted in reduced steady-state accumulation of Ste18p, suggesting that the stability of Ste18p requires modification at Cys 107. All of the mutant forms of Ste18p formed complexes with Ste4p, as assessed by coimmunoprecipitation. We conclude that tight membrane attachment of the wild-type G\textbeta\textgamma depends on palmitoylation at Cys 106 and prenylation at Cys 107 of Ste18p.

Heterotrimeric G proteins (containing G\alpha, G\beta, and G\textgamma subunits) are peripheral-membrane proteins that are coupled to cell surface receptors with seven membrane-spanning domains. They mediate response to various extracellular stimuli such as light, odorants, and hormones. Activated receptors stimulate the G\beta\gamma subunits (a complex of Ste4p and Ste18p) is associated with both internal and plasma membranes, and a portion is not stably associated with either membrane fraction. Like Ras, Ste18p contains a farnesyl-directing CaaX box motif (C-terminal residues 107 to 110) and a cysteine residue (Cys 106) that is a potential site for palmitoylation. Mutant Ste18p containing serine at position 106 (mutation ste18-C106S) migrated more rapidly than wild-type Ste18p during sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The electrophoretic mobility of wild-type Ste18p (but not the mutant Ste18p) was sensitive to hydroxylamine treatment, consistent with palmitoyl modification at Cys 106. Furthermore, immunoprecipitation of the G\textbeta\textgamma complex from cells cultured in the presence of \textsuperscript{3}Hpalmitic acid resulted in two radioactive species on nonreducing SDS-PAGE gels, with molecular weights corresponding to G\textbeta\textgamma. Substitution of serine for either Cys 107 or Cys 106 resulted in the failure of G\textbeta\textgamma to associate with the membrane. The Cys 107 substitution also resulted in reduced steady-state accumulation of Ste18p, suggesting that the stability of Ste18p requires modification at Cys 107. All of the mutant forms of Ste18p formed complexes with Ste4p, as assessed by coimmunoprecipitation. We conclude that tight membrane attachment of the wild-type G\textbeta\textgamma depends on palmitoylation at Cys 106 and prenylation at Cys 107 of Ste18p.

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We provide evidence for palmitoylation of Ste18p and define the roles for farnesyl and palmitoyl modifications in subcellular localization. Our results are consistent with unpublished results of Manahan and Linder (29), who have found that Ste18p is palmitoylated when expressed in S\textsuperscript{2}9 insect cells.

**MATERIALS AND METHODS**

**Yeast strains and plasmids.** Strains used in this study are isogenic with strain W303-1A and are described in Table 1. Strains W303-1A, HCl106S, HCl107S, and BR11426 were transformed with the SacI-XbaI fragment of plasmid pBR808 (39) containing raml::HIS3 to generate strains S09-A, S10-A, S11-A, and S14-A, respectively. PCR was used to confirm the genetic structure of the resulting recombinants. Strains S161-1 and S171-1 were generated by selecting for 5-fluoroorotic acid-resistant derivatives of strains BR11426 and S14-A, respectively. Plasmids M70p2, M70p2C106S, and M70p2C107S (48) are high-copy-number YEp plasmids that contain the ste18-C106S, ste18-C107S, and ste18-C107S alleles, respectively, under transcriptional control of the ADH1 promoter. Plasmid pH1121 contains the STE18 coding sequence under transcriptional control of the ADH1 promoter. It was constructed by digesting high-copy-number plasmid M91p1 (provided by M. Whiteway) with BglII and replacing the **URA3-containing**

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ing BiP fragment with a BiP fragment containing the LEU2 gene from plasmid YEp13 (20). Plasmid pEL37 (provided by E. Leberer) is a single-copy plasmid that contains the HIS3 gene, as well as the STE4 and GPA1 coding sequences under transcriptional control of the GAL1,10 promoter. Plasmid pRS313 is a single-copy vector plasmid containing the HIS3 gene.

Results and Discussion. YM-1 is a rich liquid medium (17). Minimal glucose medium (18) and minimal galactose medium (20) were supplemented with auxotropic requirements as described previously. – URA + CAA is supplemented minimal glucose medium containing 0.1% casamino acids (Difco) and lacking uracil (20). Dodecyl-β-maltoside, cholesterol hemisuccinate, protein A-Sepharose beads, and glyceraldehyde were from Sigma Chemical Co. Primary and secondary antibody preparations were described previously (20).

Preparation of cleared lysates. Unless indicated otherwise, cleared lysates were prepared from cells growing exponentially in 150 ml of YM-1 medium. Cultures were poured over ice, and the cells were collected by centrifugation. After two washes with ice-cold membrane buffer (10 mM Tris acetate [pH 7.6], 1 mM magnesium acetate, 0.1 mM EDTA, 8% glycerol, 0.1 mM diithiothreitol) containing 100 µg of phenylmethylsulfonyl fluoride (PMSF)/ml and 10 µg of pepstatin A/ml, the cells were resuspended in 0.5 ml of the same buffer and lysed by mechanical disruption with glass beads. Unbroken cells were removed by centrifugation for 5 min at 330 × g. Cells containing plasmids were cultured in – URA + CAA instead of YM-1. Protein concentrations were determined by using the bicinchoninic acid reagent (Pierce).

Immunoblotting methods and quantitation. Protein samples were diluted 1:3 with sample buffer containing 50% (wt/wt) urea (20), except for the immunoprecipitation procedure. Samples were heated for 10 min at 37°C and resolved on SDS–D–dodecylmaltoside/ml, 0.4 mg of cholesterol hemisuccinate/ml, and 0.25 M NaCl. After the course of this study.

Membranes were fractionated on Renografin gradients as described previously (40), except that the gradient contained protease inhibitors (100 µg of PMSE and 10 µg of pepstatin A/ml) and additional protease inhibitors were added to the fractions at the same concentrations. The volume of each fraction loaded on the SDS-polyacrylamide gel was proportional to the fraction of the volume of culture was incubated for 2 h in the presence of [3H]palmitic acid (50 Ci/mmol) in the presence of the fatty acid synthesis inhibitor cerulenin (2 µg/ml). The levels of incorporation of radioactivity into the cells were 54, 51, and 60%, respectively, for the three cultures. As described above for the immunoprecipitation procedure, the cells were disrupted with glass beads, cleared lysates were prepared, membrane proteins were solubilized with detergent, and complexes containing Ste4p were precipitated with anti-Ste4p antiserum. Half of the preparation was resolved on each of two nonreducing SDS–18% polyacrylamide gels. One gel was fixed and treated with 1 M Tris-HCl, pH 7, and the other was treated with 1 M NH4OH, pH 7, as described elsewhere (43). Gels were processed for autoradiography by using En350 universal autoradiographic enhancer (NEN Life Sciences) and exposed for 3 months at –80°C. Diphosphoryl W303 containing plasmids pH21 and pEL37 and the control strain containing plasmids pH21 and pRS313 were processed identically except that minimal galactose medium was used for both strains and levels of incorporation of radioactivity were 34 and 33%, respectively.

Hydroxylamine treatment of cleared lysates. Cleared lysates were prepared as described above except that the cells were disrupted in a buffer containing 10 mM Tris acetate (pH 7.6), 2 mM EDTA, 0.1 mM diithiothreitol, 100 µg of PMSE/ml, and 10 µg of pepstatin A/ml. Samples were diluted to 5 mg of protein per ml, treated with an equal volume of 0.5 M hydroxylamine, incubated for 45 min at 30°C, and then processed for SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting.

RESULTS

Electrophoretic mobilities and relative abundance of the ste18 mutant proteins. Proteins containing the C-terminal CaaX box motif are subject to a series of posttranslational processing steps consisting of prenylation of the cysteine followed by proteolysis of the aaX residues and carboxymethylation of the prenylated cysteine. When the CaaX motif contains methionine, serine, or glutamine in the X position, it specifies farnesylation of the cysteine residue, whereas sequences containing leucine in the X position specify geranylgeranylation (reviewed in reference 49). As for mammalian Ras, the yeast Ras1p and Ras2p proteins each contain a farnesyl-directing CaaX box (40). Most Ras proteins are palmitoylated near the C terminus (16); both of the yeast Ras proteins are palmitoylated at a cysteine immediately adjacent to the prenylated cysteine (4). Palmitoylation depends on prior prenylation (8, 16). Ste18p also contains a farnesyl-directing CaaX box and an adjacent cysteine (Fig. 1A), raising the possibility that both cysteine residues receive lipid modifications. Mutations resulting in replacement of either cysteine by serine lead to extreme defects in mating (11, 15, 48). We sought to determine how these mutations in the CaaX motif change the chemical structure and the biochemical properties of Ste18p. To assess

### TABLE 1. Strain list

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>W303-1A</td>
<td>MATα ade2 his3 leu2 trpl1 ura3 can1</td>
</tr>
<tr>
<td>HC106S</td>
<td>W303-1A ste18-C106S</td>
</tr>
<tr>
<td>HC107S</td>
<td>W303-1A ste18-C107S</td>
</tr>
<tr>
<td>BR1146</td>
<td>W303-1A ste18-URA3</td>
</tr>
<tr>
<td>809-A</td>
<td>W303-1A ram1:HIS3</td>
</tr>
<tr>
<td>810-A</td>
<td>W303-1A ram1:HIS3 ste18-URA3</td>
</tr>
<tr>
<td>811-A</td>
<td>W303-1A ram1:HIS3 ste18-C107S</td>
</tr>
<tr>
<td>814-A</td>
<td>W303-1A ram1:HIS3 ste18-URA3</td>
</tr>
<tr>
<td>816-M70p2</td>
<td>W303-1A ste18:ura3 containing plasmid M70p2</td>
</tr>
<tr>
<td>816-1/M70p2C106S</td>
<td>W303-1A ste18:ura3 containing plasmid M70p2C106S</td>
</tr>
<tr>
<td>816-1/M70p2C107S</td>
<td>W303-1A ste18:ura3 containing plasmid M70p2C107S</td>
</tr>
<tr>
<td>817-5/M70p2</td>
<td>W303-1A ste18:ura3 containing plasmid M70p2</td>
</tr>
<tr>
<td>817-5/M70p2C106S</td>
<td>W303-1A ste18:ura3 containing plasmid M70p2C106S</td>
</tr>
<tr>
<td>817-5/M70p2C107S</td>
<td>W303-1A ste18:ura3 containing plasmid M70p2C107S</td>
</tr>
<tr>
<td>W303 (diploid)</td>
<td>MATα/MATα ade2 ade2 his3/his3 leu2/-leu2 trpl1/trpl1 ura3/ura3 can1/can1</td>
</tr>
</tbody>
</table>

* Strains HC106S and HC107S were from M. S. Whiteway (48). Strain BR1146 was also kindly provided by M. S. Whiteway. All other strains were generated during the course of this study.

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changes in chemical structure, we examined the mobilities of the wild-type and mutant proteins on SDS-polyacrylamide gels. We reasoned that mutations affecting farnesylation at Cys 107 or a second modification at Cys 106 might cause detectable changes in the electrophoretic mobility of Ste18p.

Previous studies investigating the electrophoretic mobility of Ste18p were limited to fusion proteins that had been overproduced (11, 13). Under these conditions, the mobility of Ste18p was slower in the ste18-C107Y mutant (13) and in a ram1 mutant defective in farnesyltransferase activity (11). In the present study, we analyzed the mobilities and levels of accumulation of mutant forms of Ste18p that had been expressed under the control of the native promoter at the normal chromosomal location. Because the antiserum was directed against the N terminus of Ste18p (20), it was unnecessary to use an epitope-tagged form of the protein. Cleared lysates were prepared from the various mutant cells, and proteins were re-epitope-tagged form of the protein. Cleared lysates were pre-

FIG. 1. SDS-polyacrylamide gel analysis of the ste18 mutant proteins. (A) C-terminal sequence of Ste18p precursor predicted from nucleic acid sequence. (B) Immunoblotting analysis performed on cleared lysates of exponentially growing cells. The STE18 allele designations are listed across the top. Amounts of protein loaded (from left) were as follows: ste18::URA3 (null), 33 μg; STE18, 5 μg; ste18-C107S, 33 μg; STE18*, 5 μg; STE18*, 5 μg; ste18-C106S, 10 μg; and STE18*, 5 μg. Strains used were BR1426, W303-1A, HC107S, and HC106S.

The relative abundances of the Ste18p proteins in the different mutant and wild-type strains were estimated by comparing the intensities of the Ste18p bands on the immunoblot with respect to the amount of total protein analyzed (Fig. 1B). Only the ste18-C107S mutant showed a consistent reduction in the amount of total protein analyzed (Fig. 1B). Only the ste18-C107S mutant protein was consistent with the loss of prenylation at Cys 107, as proposed previously (11, 13). In contrast, the ste18-C106S mutant protein migrated more rapidly than wild-type Ste18p, suggesting that this mutation causes a defect in a processing event other than farnesylation.

The relative abundances of the Ste18p proteins in the different mutant and wild-type strains were estimated by comparing the intensities of the Ste18p bands on the immunoblot with respect to the amount of total protein analyzed (Fig. 1B). Only the ste18-C107S mutant showed a consistent reduction in the abundance of Ste18p. In four independent analyses, the ste18-C107S mutant protein was detected at a level that was 10 to 20% of the level of Ste18p from the wild-type control strain. Thus, prenylation of wild-type Ste18p may be essential for maintaining the stability of G_{1}\G_{2}; the faster-migrating minor species detected in the ste18-C107S mutant may represent a degradation intermediate. The ste18-C106S mutant protein was relatively abundant (between 25 and 60% of the level of the wild type).

The ste18-C106 mutant protein, but not the ste18-C107 mutant protein, is a substrate for farnesylation. The electro-
However, unlike the overproduced protein, relatively minor changes in mobility were observed for wild-type Ste18p from the ram1 mutant. Moreover, the Ste18p from the STE18\(^{\text{null}}\) strain was more abundant than that from the ste18\(\text{-Cl07S}\) strain, in that it did not exhibit the same degradation product, and it did not migrate to the same position. Thus, in the absence of farnesyltransferase activity, wild-type Ste18p received a modification that was not detected when the protein was overproduced; this modification required the presence of both Cys 106 and Cys 107 (Fig. 2B; compare lanes 2, 4, and 6). Possible modifying activities include either geranylgeranyltransferase type I (GGTase I) or GGTase II. Cross-specificity of the far- 

cleavage of the thioester linkage by thiol reducing agents. Wild-type cells (lane 1) gave two radioactive species that were not observed for the ste18\(-Cl06S\) and ste18\(\text{::URA3}\) control cells (lanes 2 and 3, respectively). The molecular masses of these species were consistent with Gβγ (13 kDa) and Gβγ (60 kDa) dimers. Similar labeled species were obtained with cells overproducing Ste4p and Ste18p (lane 5), but not with control cells lacking Ste4p (lane 4). Nearly all of the label was released from these species when the gel was incubated in hydroxylamine (data not shown). These results are consistent with at least some of the Ste18p molecules containing a palmitoyl modification. To determine whether the bulk of the Ste18p molecules contained a hydroxylamine-sensitive modification at Cys 106, we tested whether the electrophoretic mobilities of Ste18p and the ste18\(\text{-Cl06S}\) mutant were altered by hydroxylamine treatment (Fig. 3B). If the aberrant mobility of the mutant protein was due to its failure to receive a palmitate moiety, then the mobility of the wild-type protein, but not that of the mutant protein, should increase upon hydroxylamine treatment. Cleared lysates were incubated with neutral hydroxylamine and then processed for SDS-PAGE. The mobility of Ste18p, but not that of the ste18\(\text{-Cl06S}\) mutant protein, increased following hydroxylamine treatment; the treated wild-type protein comigrated with the mutant protein. This result indicates that essentially all Ste18p molecules contain a thioester linkage at Cys 106.

**ste18 mutants form Gβγ complexes with Ste4p.** Lipid modifications of proteins potentially influence their interaction with membranes or with other proteins. We used immunoprecipitation to test whether formation of the Gβγ complex requires lipid modification of Ste18p. Cleared lysates from the ste18 mutant and wild-type control cells were extracted with the detergent dodecyl-β-D-maltoside and then incubated with anti-Ste4p antiserum. The supernatant fractions and pellet fractions were assayed for Ste18p by immunoblotting methods (Fig. 4). Each mutant Ste18p was found in the pellet fraction after Ste4p had been immunoprecipitated with anti-Ste4p antibody, thus indicating the presence of Gβγ complexes. Quantification of the amount of Ste18p remaining in the supernatant after antibody treatment indicated that immunoprecipitation of each mutant protein was as efficient as that of the wild-type protein (about 80%). This experiment demonstrates that changes in the lipid modification of Ste18p do not prevent Gβγ complex formation. Similar results have been obtained for mammalian Gβγ subunits (22, 31, 42).

Both Cys 106 and Cys 107 are required for stable association of Gβγ with membranes. To test whether the ste18 mutations result in changes in the membrane localization of Gβγ, we fractionated membranes from the various ste18 mutants on
Renografin density gradients and assayed the fractions for Ste4p and Ste18p. Renografin density gradients resolve plasma membranes, internal membranes, and nonmembrane proteins (23, 40). Previously, we have shown that Ste4p and Ste18p from wild-type cells are associated with all three fractions (20). However, in the ste18-C106S mutant, Ste4p and Ste18p were not associated with either membrane fraction (Fig. 5B). Since the ste18-C106S mutant protein is apparently farnesylated (Fig. 2), our results indicate that farnesylation of Ste18p is not sufficient to promote stable association of Gβγ with membranes; we cannot rule out the possibility that the mutant protein is associated weakly with membranes in vivo and is released during analysis. In the ste18-C107S mutant, the Gβγ complexes were no longer associated with plasma membranes or with internal membranes (Fig. 5C). Because the ste18-C107S mutant protein was not as abundant as the wild-type Ste18p, we were unable to evaluate its distribution in the gradient. When a strain (816-1/M70p2C107S) that overproduces the ste18-C107S mutant protein was examined, Ste4p and the mutant Ste18p were detected only in the dense fractions that contained no membranes (data not shown). The ste18-C107S and ste18-C106S mutant proteins, which formed Gβγ complexes but failed to associate with membranes (Fig. 5), also failed to promote the pheromone response and mating (48).

DISCUSSION

This study explored the role that covalent lipid modifications play in the localization of the yeast Gβγ complex. Previous workers established that residues of the CaaX motif and the neighboring cysteine residue (Cys 106) are important for the activity of Ste18p (15, 48) and that Ste18p receives a farnesyl modification (11). Our results provide evidence for a thioester lipid modification at Cys 106. This lipid modification is likely to be palmitoyl since yeast Ras is palmitoylated at a similar position and since Ste18p can be labeled with [3H]palmitate in a manner that is hydroxylamine sensitive and dependent on Cys 106. As for mammalian G proteins (30, 42), prenylation of yeast Gγ is essential for membrane attachment but not for binding Gβ. Unlike other known Gγ subunits, Ste18p apparently requires an additional palmitoyl modification for localization and function.

Ste18p is similar to the yeast Ras proteins (encoded by RAS1 and RAS2) in several respects. Both proteins contain a cysteine immediately adjacent to a farnesyl-directing CaaX motif, and the palmitoyl moiety is added only after farnesylation (8). Like Ras (1, 4), palmitoylation of Ste18p is essential for localization to the plasma membrane. Both unpalmitoylated Ras (1, 4, 6) and ste18-C107S mutant protein (48) exhibit biological activity that is significantly reduced but not eliminated. Thus, the membrane localization that is afforded by the palmitoyl moiety appears to facilitate interaction with other components of the signal transduction pathway but is not absolutely required. In yeast, the same farnesyltransferase (α and β subunits, encoded by RAM2 and RAM1, respectively) operates on Ras, Ste18p, and the a-factor pheromone (11, 12, 19, 34).

Ste18p is unusual among Gγ proteins in that the C-terminal isoprenyl group is farnesyl. Although transducin γ (13) and, apparently, γ11 (36) are substrates for farnesyltransferase, all other Gγ subunits are modified by GGTase I (49). We are unaware of other Gγ subunits that contain Cys adjacent to the prenylation site. Previous genetic tests (48) suggest that farnesylation is not absolutely required for Ste18p function, since the ram1 mutant, which lacks farnesyltransferase, shows only a minor reduction in pheromone responsiveness. Moreover, mutant Ste18p proteins from these strains showed only slightly slower electrophoretic mobilities than Ste18p from wild-type cells, yet the mobility was faster than the ste18-C107S mutant protein or the Ste18p that had been overproduced in the ram1 mutant (Fig. 1 and 2). Together these results raise the possibility that an enzyme other than farnesyltransferase modifies Cys 107 and that the activity modifies a significant portion of Ste18p when overproduced. Possible modifying activities include either GGTase I or GGTase II. GGTase I potentially modifies Ste18p when overproduced. Possible modifying activities include either GGTase I or GGTase II. GGTase I potentially modifies Ste18p in the ram1 mutant, since farnesyltransferase and GGTase I exhibit some cross-specificity (32, 45, 49). However, the alternative modification that operates on wild-type Ste18p in the ram1 mutant apparently requires both Cys 106 and Cys 107 (Fig. 2B). This result suggests a role for GGTase II, since this enzyme has been shown to modify both paired cysteines within several C-terminal motifs (–XXCC, –XCXC, or –CCXX) that are found among Rab proteins (9); however, potential substrates containing the motif –CCXXX have not
been examined. The partial activity of the ste18 truncation mutant lacking the three C-terminal residues (i.e., containing –XXCC) (48) is consistent with modification by GGTTase II. Association of yeast casein kinase I (Yck1p) with the plasma membrane also depends on the motif –XXCC (46). However, a potential problem for the proposal that GGTTase II modifies Ste18p and Yck1p is that Rab proteins are substrates for GGTTase II only when they have bound the guanine nucleotide dissociation inhibitor-like protein REP1, and short peptides containing the prenylation motif are not recognized by GGTTase II (see reference 49). Conceivably, either the paired cysteine motif in Ste18p and Yck1p occurs within a context that permits GGTTase II recognition or another, unidentified enzyme or auxiliary factor operates on these substrates.

How does dual lipid modification mediate membrane association of yeast Gβγ? The failure of the ste18-C106S mutant protein to accumulate on membranes indicates that palmitoylation either provides a signal for targeting Ste18p to membranes or contributes to the affinity of Ste18p for membranes. In vitro, synthetic peptides containing both palmitoyl and farnesyl show very slow rates of intermembrane transfer (T1/2 > 50 h) compared with the singly modified peptides (41). According to the bilayer trapping mechanism (2, 38, 41), farnesylation of Ste18p may promote weak membrane interactions, and upon association with a membrane compartment containing palmitoyltransferase, Ste18p may become palmitoylated and, thus, anchored at that site. The location of the palmitoyltransferase for Ste18p is unknown. In mammalian cells, palmitoyltransferase in the plasma membrane modifies Go (7) whereas an activity in Golgi membranes palmitoylates a farnesylated form of Ras (16). Roles for palmitoylation in both membrane targeting and membrane affinity have been described. Palmitoylation of SNAP-25 is necessary for localization of newly synthesized protein at the plasma membrane but is not required for maintaining fully assembled protein at the membrane, since brefeldin A blocks both palmitoylation and membrane targeting of newly synthesized SNAP-25 and since hydroxylamine hydrolyzes the thioacyl linkage without affecting membrane attachment (14). In contrast, brefeldin A does not block assembly of mammalian Gβγ on the plasma membrane (37), and Ras protein is removed from the plasma membrane upon hydroxylamine treatment (28).

After ligand stimulation and release from Go, yeast Gβγ (26, 35) as well as many of its mammalian Gβγ isoforms (3, 5, 21, 24) are thought to stimulate the activity of effector molecules located on the plasma membrane. The role that Gβγ plays in signal transduction may be simply to promote assembly of effector molecules at the plasma membrane (35). Thus, dissociation of Gβγ from the plasma membrane provides a possible mechanism for regulating the duration of Gβγ signaling activity. Dissociation could be a consequence of depalmitoylation; evidence for reversible palmitoylation of mammalian Ras exists, and palmitoylation of Ras apparently regulates plasma membrane attachment (28). Alternatively, a carrier protein that binds the Gβγ complex may shield the lipid groups from the aqueous environment and thereby permit dissociation from the membrane. Guanine nucleotide dissociation inhibitor functions as such a carrier during recycling of geranylgeranylated Rab proteins (10, 33). An internal membrane compartment may provide a site where Gβγ can reassociate with Go before it is reinserted on the plasma membrane. If relevant, repalmitoylation could occur either in this internal compartment or at the plasma membrane. Clearly, evaluation of these models will require determination of the palmitoylation state of Gβγ and the presence of specific binding proteins in the various subcellular compartments.

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REFERENCES


