Posttranslational lipid modifications are important for proper localization of many proteins in eukaryotic cells. However, the functional interrelationships between lipid modification processes in plants remain unclear. Here we demonstrate that the two heterotrimeric G-protein γ-subunits from Arabidopsis (*Arabidopsis thaliana*), AGG1 and AGG2, are prenylated, and AGG2 is S-acylated. In wild type, enhanced yellow fluorescent protein-fused AGG1 and AGG2 are associated with plasma membranes, with AGG1 associated with internal membranes as well. Both can be prenylated by either protein geranylgeranyltransferase I (PGGT-I) or protein farnesyltransferase (PFT). Their membrane localization is intact in mutants lacking PFT activity and largely intact in mutants lacking PGGT-I activity but is disrupted in mutants lacking both PFT and PGGT-I activity. Unlike in mammals, Arabidopsis Gγs do not rely on functional Ga for membrane targeting. Mutation of the sixth to last cysteine, the putative S-acylation acceptor site, causes a dramatic change in AGG2 but not AGG1 localization pattern, suggesting S-acylation serves as an important additional signal for AGG2 to be targeted to the plasma membrane. Domain-swapping experiments suggest that a short charged sequence at the AGG2 C terminus contributes to AGG2’s efficient membrane targeting compared to AGG1. Our data show the large degree to which PFT and PGGT-I can compensate for each other in plants and suggest that differential lipid modification plays an important regulatory role in plant protein localization.

Heterotrimeric guanine nucleotide-binding proteins (G proteins) are important components of signal transduction pathways and are conserved in all eukaryotic organisms examined (Jones, 2002; Jones and Assmann, 2004; Perfus-Barbeoch et al., 2004). Heterotrimeric G proteins consist of three different subunits, Ga, Gβ, and Gγ. Gβ and Gγ are tightly associated as a functional unit under physiological conditions, while Ga can signal independently or through Gβγ. In the human genome, there are a total of 17 Ga genes that encode 23 Ga-subunits, five Gβ genes, and at least 12 Gγ genes (Cabrera-Vera et al., 2003), while in the Arabidopsis (*Arabidopsis thaliana*) genome, only one canonical Ga (GPA1, At2G26300), one Gβ (AGB1, At4G34460), and two Gγ genes (AGG1, At3g63420 and AGG2, At3g22942) have been identified (Ma et al., 1990; Mason and Botella, 2000; Lease et al., 2001; Mason and Botella, 2001). The same number of G-protein components exists in the rice (*Oryza sativa*) genome (Kato et al., 2004). Studies of plant Ga and Gβ mutants have implicated G proteins in a wide range of developmental and phytohormone-mediated cellular processes (Jones, 2002; Jones and Assmann, 2004; Perfus-Barbeoch et al., 2004), including cell division (Ullah et al., 2001, 2003; Warpeha et al., 2006), leaf shape, internode elongation (Ueguchi-Tanaka et al., 2000), seed germination (Ullah et al., 2002), stomata function (Wang et al., 2001; Coursol et al., 2003; Fan et al., 2004; Mishra et al., 2006), and pathogen susceptibility (Suharsono et al., 2002; Trusov et al., 2006). Arabidopsis *gpa1* and *agb1* mutants share some common features, such as rounder rosette leaves, but, consistent with independent roles in particular signaling events have many distinct phenotypes. *agb1* mutants produces excessive lateral roots and are more sensitive to auxin promotion of lateral roots, while *gpa1* forms fewer lateral roots and is less sensitive to auxin (Ullah et al., 2003). *agb1* mutants are more hypersensitive to abscisic acid (ABA) than *gpa1* (Pandey et al., 2006), and *GPA1* and *AGB1* play opposite roles in regulating cell proliferation in roots (Chen et al., 2006).

Critical to their signaling function is the localization of G proteins to the cytoplasmic face of plasma membrane in animal cells (Casey, 1995). This subcellular localization is often facilitated by lipid modifications of the Ga- and Gγ-subunits. In mammalian and yeast (*Saccharomyces cerevisiae*) cells, Ga is usually modified by attachment of a 14-carbon myristate to an N-terminal Gly via an amide bond and/or by linking a 16-carbon palmitate to a nearby Cys through a thioester bond (Milkman et al., 1995). Gγ is usually modified by prenylation, attachment of a 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoid via a thioether

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bond to a Cys in the CaaX motif at the C terminus, where a is preferentially a small aliphatic amino acid and X significantly affects the substrate specificity (Wedegaertner et al., 1995; Lane and Beese, 2006). Extensive structural studies have been conducted to understand the molecular basis of substrate specificity and mechanisms of the mammalian protein prenyltransferase activities (Lane and Beese, 2006). Mammalian protein geranylgeranyltransferase I (PGGT-I) and protein farnesyltransferase (PFT) have distinct but overlapping substrate specificity. PGGT-I prefers a Leu and Phe residue in the X position, while PFT can recognize several other amino acids but prefers Met, Ser, Gln, or Ala (Zhang et al., 1994; Zhang and Casey, 1996; Lane and Beese, 2006). Up to 11 residues in the linker region proximal to the CaaX motif appear to affect the efficiency of prenylation (Maurer-Stroh and Eisenhaber, 2005). In general, PGGT-I substrates have a higher number of Lys residues in the proximal linker region (Maurer-Stroh and Eisenhaber, 2005). With a proximal polylsine domain, K-RasB, which has a CVIM motif, was shown to be a good substrate not only for PFT but for PGGT-I as well (James et al., 1995). Following prenylation, proteolysis of the last three amino acids and methylesterification of the prenyl Cys are closely coupled in vivo to promote the hydrophobicity of the modified protein (Hancock et al., 1991; Rodriguez-Concepcion et al., 2000; Crowell and Kennedy, 2001; Bracha et al., 2002; Narasimha Chary et al., 2002). Lipid modifications of Gy in mammalian cells are not required for Gβγ dimerization but are essential for their plasma membrane association and interaction with Gα and effector proteins (Wedegaertner et al., 1995; Dietrich et al., 2003), and Gβγ complex formation was shown to stabilize the Gγ-subunit (Prorn and Gautam, 1993).

Palmitoylation of a Cys adjacent to the prenyl Cys (-CCTLM) in yeast Gy (STE18) is required for its full function in pheromone response, and its palmitoylation seemed to depend on prenylation (Hirschman and Jenness, 1999; Manahan et al., 2000). In contrast, none of the human Gy has an adjacent Cys, or the polylbasic domain proximal to the CaaX box, as in K-RasB (Takida and Wedegaertner, 2003). Consequently, Gβ1'y2 requires the presence of Gα to target the plasma membrane rather than to the endoplasmic reticulum (ER; Takida and Wedegaertner, 2003). Interestingly, when a palmitoylation site is introduced into Gy2, the dependence on Gα for correct plasma membrane localization is abolished. No defined motifs are known that predict palmitoylation (Roth et al., 2006). A number of proteins were shown to be palmitoylated in vitro simply by incubating with palmitoyl-CoA in the absence of an S-acyltransferase (Veit, 2000, 2004; Lavy et al., 2002). However, at physiological conditions, spontaneous acylation is thought not to be a significant process, considering the inhibitory effects of acyl-binding proteins and a low concentration of acyl-CoA (Dunphy et al., 2000). A dynamic process of protein acylation and deacylation in vivo is catalyzed by S-acyltransferase and palmitoyl-protein thioesterase, and a family of Asp-His-His-Cys; Cys-rich domain (DHHC-CRD) proteins show protein S-acyltransferase activities in yeast, but the enzymology is not clearly understood (Roth et al., 2002, 2006; Mitchell et al., 2006). TIP GROWTH DEFECTIVE1 of Arabidopsis was recently reported to be a DHHC-CRD S-acyltransferase (Hemsley et al., 2005). Arabidopsis tip1 mutants show pleiotropic growth defects, including short and deformed root hairs, pale leaves, and poorer growth.

In Arabidopsis, the two Gy genes, AGG1 and AGG2, were identified through their interaction with AGB1 in yeast two-hybrid experiments (Mason and Botella, 2000, 2001). AGG1 has a CRCLIL and AGG2 has a CGCSIL motif at the C terminus, making them potential targets for both protein prenylation and S-acylation (Fig. 1). Arabidopsis genes encoding PFT and PGGT-I subunits have been cloned, and their respective loss-of-function mutants have been identified. pluripetala (plp-I) mutants disrupt the a-subunit shared between PFT and PGGT-I (Running et al., 2004). enhanced response to abscisic acid1 (era1) and ggb are mutants of the β-subunit of PFT and PGGT-I, respectively (Cutler et al., 1996; Ziegelhoffer et al., 2000; Johnson et al., 2005). plp-1 mutants lack detectable PFT and PGGT-I activity and show many morphological phenotypes, including extra petals, much enlarged meristems, extreme fasciation, and much slower growth, though the plants remain viable and fertile (Running et al., 2004). plp-1 mutants also exhibit G-protein mutant phenotypes such as rounder leaves and seed hypersensitivity to ABA shown in both gpa1 and gbg1, and shorter siliques and bigger inflorescences in gbg1 (Lease et al., 2001; Ullah et al., 2001, 2003; Pandey et al., 2006). era1 mutants have a much weaker morphological phenotype but are more hypersensitive to ABA inhibition of seed germination than plp (Running et al., 1998; Bonetta et al., 2000; Yalovsky et al., 2000a), and ggb-2

Figure 1. Comparison of AGG2 and AGG1 protein sequences. Both AGG2 and AGG1 have three Cys residues that are underscored by an asterisk. AGG2 has three putative N-glycosylation motifs, NSSG, NASA, and NATW, which are underlined. AGG1 lacks these motifs. AGG1m2 and AGG2m1 were generated to determine the effect of preceding sequence of the CAAAX motif on lipid modification efficiency and protein stability.
mutants have no apparent growth defects and are slightly ABA hypersensitive (Johnson et al., 2005), despite the large number of putative PGGT-I targets in plants. This is in contrast to orthologous mutants in yeast, which are lethal (He et al., 1991). eral-4 ggb-2 double mutants exactly resemble plp, indicating that PLP, ERA1, and GGB do not function independently of each other. The mild morphological phenotypes of eral and ggb compared to plp indicate that there is likely to be great functional redundancy between plant PFT and PGGT-I, possibly much more so than in mammals and yeast (Johnson et al., 2005). This redundancy is also shown genetically by the partial rescue of yeast (Fig. 2A). The β-subunits of PFT and PGGT were copurified with the α-subunit fused in frame with FLAG epitope at the C terminus. Incubation of purified GST-AGG2 and GST-AGG1 with different combinations of enzyme and substrate showed that the two γ-subunits are not only geranylgeranylated by PGGT-I but also farnesylated by PFT, while the mutated forms are not prenylated (Fig. 2B). The results also show that Arabidopsis PGGT-I can utilize farnesyl pyrophosphate (FPP), albeit with a much weaker signal detected. That signals were not detected when no PGGT-I was added, or when GST-AGG2C97S or GST-AGG1C95S was incubated with the purified PGGT-I, confirmed that prenylation of AGG1 and AGG2 requires the conserved fourth to last Cys, and the signals from the other reactions are not due to nonspecific substrate affinity but due to enzymatic activities.

**RESULTS**

**AGG1 and AGG2 Are Prenylated in Vitro**

AGG1 and AGG2 contain a CaaL motif for prenylation (Fig. 1). To determine if AGG1 and AGG2 serve as prenylation targets, glutathione S-transferase (GST)-fused proteins GST-AGG2 and GST-AGG1, as well as mutant proteins GST-AGG2C97S and GST-AGG1C95S, in which the prenyl Cys was mutated to Ser, were induced and purified. Arabidopsis prenyltransferases PFT and PGGT-I were expressed and purified from yeast (Fig. 2A). The β-subunits of PFT and PGGT were copurified with the α-subunit fused in frame with FLAG epitope at the C terminus. Incubation of purified GST-AGG2 and GST-AGG1 with different combinations of enzyme and substrate showed that the two γ-subunits are not only geranylgeranylated by PGGT-I but also farnesylated by PFT, while the mutated forms are not prenylated (Fig. 2B). The results also show that Arabidopsis PGGT-I can utilize farnesyl pyrophosphate (FPP), albeit with a much weaker signal detected. That signals were not detected when no PGGT-I was added, or when GST-AGG2C97S or GST-AGG1C95S was incubated with the purified PGGT-I, confirmed that prenylation of AGG1 and AGG2 requires the conserved fourth to last Cys, and the signals from the other reactions are not due to nonspecific substrate affinity but due to enzymatic activities.

**Figure 2.** In vitro prenylation of AGG1 and AGG2. A, Arabidopsis recombinant PFT (lane 2) and PGGT-I (lane 3) were expressed and purified from yeast using Anti-Flag antibody (Sigma). Numbers to the left of lane 1 indicate positions of molecular marker (Bio-Rad) in kilodaltons. Positions of protein bands are indicated by the arrows to the left of lane 1 indicate positions of molecular marker (Bio-Rad) in kilodaltons. Positions of protein bands are indicated by the arrows to the left of lane 1 indicate positions of molecular marker (Bio-Rad) in kilodaltons. Positions of protein bands are indicated by the arrows to the left of lane 1 indicate positions of molecular marker (Bio-Rad) in kilodaltons. Positions of protein bands are indicated by the arrows to the left of lane 1 indicate positions of molecular marker (Bio-Rad) in kilodaltons. Positions of protein bands are indicated by the arrows to the left of lane 1 indicate positions of molecular marker (Bio-Rad) in kilodaltons.

B. Purified GST-AGG1, GST-AGG2, GST-AGG1C95S, and GST-AGG2C97S (in which the fourth to last Cys are replaced by Ser) were incubated with (+) or without (−) the purified protein prenyltransferase and tritium-labeled prenyl substrate as indicated in each lane, resolved by SDS-PAGE, and visualized by fluorography.
Prenylation Is Critical for the Proper Subcellular Localization of AGG1 and AGG2

To demonstrate that the lack of prenylation affects the localization of AGG1 and AGG2, AGG1, AGG1C95S, AGG2, and AGG2C97S were fused to EYFP at the N terminus under control of the 35S promoter of Cauliflower mosaic virus. Stable transgenic Arabidopsis plants for all the constructs as well as the EYFP vector alone were assessed by fluorescence confocal microscopy. Without other constraints, nonnuclear proteins within about 70 kD have been shown to freely enter into the nucleus through the nuclear pore complex (Galy et al., 2003). EYFP (27 kD) and its variant fluorophores are cytosolic proteins that can go into the nucleus but not vacuoles (Dixit et al., 2006). For the EYFP vector alone, a bright signal was detected in the nuclei, perinuclear cytoplasm, cytoplasmic strands, and the cortical cytoplasm that was pressed by the large vacuole against the cell wall (Fig. 3A). After plasmolysis, EYFP signal was detected in the condensed cytoplasm and nucleus, as shown in a guard cell for its easily recognizable shape in an overlay of differential interference contrast (DIC) and confocal image (Fig. 3B). Fluorescent signals of EYFP-AGG2 were detected as sharp, thin lines surrounding the cell periphery, suggesting that AGG2 is associated with the plasma membrane (Fig. 3C). Plasmolysis further confirmed that signals are confined to the plasma membrane (Fig. 3D).

In EYFP-AGG1 plants, the fluorescence pattern is different from both AGG2 and EYFP vector alone. While most of the EYFP-AGG1 fluorescence is detected in the plasma membrane, there is considerable signal associated with more internal structures, especially some bright, small, and motile particles (Fig. 4B). The dynamic feature of these brightly fluorescing particles is visible in a supplemental movie (Supplemental Movie S1). After plasmolysis, EYFP-AGG1 displays aggregates of bright spots and endomembrane localization (Fig. 4C). To see if these bright dots are Golgi or Golgi-associated structures, we crossed EYFP-AGG1 plants with plants containing the Golgi marker GmMan1 fused to cyan fluorescent protein (CFP; Nebenfuhr et al., 1999). GmMan1-CFP plants alone exhibit weaker growth. Seeds from crosses between EYFP-AGG1 plants and GmMan1-CFP can germinate on hygromycin plates but cannot initiate true leaves. Nevertheless, using the expanded cotyledons, EYFP-AGG1 was indeed detected associated with Golgi (Fig. 4D, closeup in inset). A consistent pattern of the merged yellow color between the red (EYFP-AGG1) and green (GmMan1-CFP), indicating that EYFP-AGG1 colocalizes with GmMan1-CFP but does not overlap completely. GmMan1-CFP was shown to localize at the cis/medial side of the Golgi stacks (Nebenfuhr et al., 1999). Thus, it appears that EYFP-AGG1 is most likely associated with the trans side of the Golgi and possibly with the trans-Golgi network, suggesting AGG1 is transported to the plasma membrane.
through the vesicular membrane trafficking pathway like the human G proteins (Michaelson et al., 2002).

Both EYFP-AGG2<sup>C97S</sup> (Fig. 3, E and F) and EYFP-AGG1<sup>C95S</sup> (Fig. 4E) showed similar localization patterns to that of EYFP-vector alone (Figs. 3, A and B, and 4A), indicating that prenylation is critical for membrane localization of AGG2 and AGG1.

We crossed EYFP-AGG2 and EYFP-AGG1 plants to <i>ggb</i>-2, <i>plp</i>-1, and <i>era1</i>-4 mutants to see if the AGG1 and AGG2 distribution pattern is altered in prenylation mutants. Localization of AGG2 does not change in <i>era1</i>-4 (Fig. 3G) or AGG1 (Fig. 4F), suggesting they are primarily prenylated by PGGT-I in vivo and loss of PFT function does not affect their prenylation. In <i>ggb</i>-2, AGG2 (Fig. 3H) and AGG1 (Fig. 4G) still retain most of their membrane association, indicating that loss of PGGT-I function is largely compensated by PFT, in agreement with the results from the in vitro assay. However, weakly fluorescing nuclei can be detected in the projections of multiple stacks for both AGG2 and AGG1, and for AGG1, there are fewer fluorescing Golgi stacks, indicating that a portion of AGG1 and AGG2 is not prenylated and not membrane associated in the absence of PGGT-I, further suggesting that AGG1 and AGG2 are preferably prenylated by PGGT-I but not by PFT in vivo. In <i>plp</i>-1 mutants, cytoplasmic strands and nuclei were clearly visualized for AGG2 (Fig. 3I) and AGG1 (Fig. 4H), indicating that AGG2 and AGG1 is significantly distributed in the cytosol when both PFT and PGGT-I activities are abolished.

To show the membrane versus cytosol partitioning of the Arabidopsis G-protein γ-subunits, we prepared total, membrane, and soluble fractions of protein from leaves of the above-mentioned overexpression plants, resolved on SDS-PAGE and visualized by immunoblotting with antibodies specific for green fluorescent protein (GFP; Fig. 5). Consistent with confocal imaging, EYFP-AGG2 is detected in the membrane fraction in both wild-type and <i>era1</i>-4 mutants, and EYFP-AGG2<sup>C97S</sup> is present in the cytosolic fraction.

![Figure 4](image_url)

Figure 4. Subcellular localization of EYFP tagged AGG1. Projection of multiple stacks taken from the lower epidermis of cotyledon or leaf is presented for every section except A and D. The images for the guard cells are an overlay of DIC (gray) and EYFP (green). A, EYFP-vector is cytosolic as evidenced by the bright nuclei and cytoplasmic strands. B, EYFP-AGG1 is associated with plasma membrane and internal membranes, with bright spots apparent. C, A guard cell from EYFP-AGG1 after plasmolysis displays aggregates of bright spots and endomembrane localization. D, EYFP-AGG1 (red) colocalizes with the Golgi marker GmMan1-CFP (green) in the middle of the Golgi stack as indicated by the merged yellow. The inset at the top left corner is from the arrow pointed at Golgi, showing the yellow merged color between the green and red. GmMan1 has been reported to locate at the cis/medial Golgi (Nebenfuhr et al., 1999). AGG1 is possibly located at the medial/trans Golgi. E, EYFP-AGG1<sup>C95S</sup> is cytosolic. F, The localization pattern of AGG1 is not altered in <i>era1</i>-4. G, In <i>ggb</i>-2, EYFP-AGG1 can be detected in the nuclei, but some bright spots still remain as indicated by the arrow. H, In <i>plp</i>-1 mutants, AGG1 is largely detected in the cytosol as evidenced by the bright nuclei. I, The localization pattern of AGG2 is not altered in <i>gpa1</i>-1. J, The localization pattern of AGG2 is not altered in <i>agb1</i>-1. K, The localization pattern of AGG1 is not altered in <i>gpa1</i>-1. L, The localization pattern of AGG1 is not altered in <i>agb1</i>-1. M, Nucleus; CW, cell wall; G, Golgi. All scale bars = 10 μm.
Supplemental Fig. S2). A similar pattern was observed for AGG1. A band in the cytosol was shown with longer exposure (as shown in EYFP-AGG2 was detected in the membrane fraction, and a weaker soluble and membrane fractions. In the era1 mutant, EYFP-AGG2 was detected in both soluble and membrane fractions. In the ggb-2 mutant, the majority of EYFP-AGG2 was detected in the membrane fraction, and a weaker band in the cytosol was shown with longer exposure (as shown in Supplemental Fig. S2). A similar pattern was observed for AGG1.

The observed lower fluorescence intensity in the ggb-2 and plp-1 mutant background, 2 times more protein was loaded for AGG2 ggb-2 and 3 times more protein was loaded for AGG2 plp-1 (see “Methods and Materials”). In ggb-2 mutants, the majority of EYFP-AGG2 remains in the membrane fraction, demonstrating that the loss of PGGT-I function does not severely alter its localization. However, in a blot with longer exposure, weak signal of EYFP-AGG2 is detected in the soluble fraction in ggb-2 but not in wild type or in era1-4 mutants. Intact EYFP-AGG2 in the wild type, as well as in era1-4, was detected in the membrane fraction. EYFP-AGG2 was in the cytosol. In the plp-1 mutant, EYFP-AGG2 was detected in both soluble and membrane fractions. In the ggb-2 mutant, the majority of EYFP-AGG2 was detected in the membrane fraction, and a weaker band in the cytosol was shown with longer exposure (as shown in Supplemental Fig. S2). A similar pattern was observed for AGG1.

AGG2 Localization Is Disrupted by Mutation of a Potential Acylation Acceptor Cys Two Residues Preceding the Prenyl Cys

In yeast Gy, an adjacent Cys to the prenyl Cys has been shown to be palmitoylated, and palmitoylation occurs after prenylation (Hirschman and Jenness, 1999; Manahan et al., 2000). In Arabidopsis, there are two additional Cys to the prenyl Cys in AGG1 and AGG2, and one of them is separated by one amino acid upstream of the prenyl Cys (Fig. 1). To see if this Cys is subject to acylation, we conducted point mutation analysis by mutating the Cys at the 93rd position for AGG1 (AGG1C93S) and at the 95th position for AGG2 (AGG2C95S) to Ser. Because farnesylated but nonacylated human Gy2 was only sufficiently targeted to the ER (Takida and Wedegaertner, 2003), binary vectors containing EYFP-AGG1, EYFP-AGG2, GFP-AGG1C93S, and GFP-AGG2C95S were either infiltrated alone or co-infiltrated with an ER localization marker CFP-HDEL into Nicotiana benthamiana leaf epidermal cells. As also shown in stable transgenic plants, wild-type EYFP-AGG1 in transient assays shows many bright spots (Fig. 6G). These bright spots were embedded within the ER network but do not colocalize with the ER marker (Fig. 6, A–C). The weakly fluorescing signal in the background suggests that, unlike farnesylated human Gy2, geranylgeranylated EYFP-AGG1 does not significantly accumulate in the ER, and its delivery from ER to trans-Golgi is a rapid process. GFP-AGG1C93S showed a similar localization pattern to that of wild-type EYFP-AGG1 (Fig. 6, D–F). Stable transformed Arabidopsis plants with GFP-AGG1C93S (Fig. 6H) and the one that was crossed with GmMann1-CFP (Fig. 6I) also showed a similar pattern to that of EYFP-AGG1 (Fig. 4, B and D), suggesting the mutated Cys is not required for AGG1’s proper targeting.

In contrast, AGG2C95S displayed a distinct pattern from that of wild-type AGG2. GFP-AGG2C95S is associated with internal membranes in both transient assays (Fig. 7, A–C) and stable transformed Arabidopsis plants (Fig. 7E), while wild-type EYFP-AGG2 presents only sharp lines of signal circumventing the plasma membrane (Figs. 3C and 7D). Like EYFP-AGG1 and GFP-AGG1C93S, the GFP-AGG2C95S Arabidopsis plants with GmMann1-CFP are extremely unhealthy when grown on hygromycin plates and not every cell

AGG1 and AGG2 Do Not Depend on GPA1 or AGB1 for Their Membrane Localization

The farnesylation of mammalian Gy2 was shown to be critical but not sufficient to target Gβγ2 to the plasma membrane. Farnesylation is sufficient to target Gβγ2 to the ER, but it requires the presence of Goα to target to the plasma membrane (Takida and Wedegaertner, 2003). To see if Go is necessary for Arabidopsis Gβγ to localize to the plasma membrane, we crossed Arabidopsis plants expressing EYFP-AGG1 and EYFP-AGG2 with both gap1-1 and agb1-1. gap1-1 is a T-DNA insertion that results in no detectable protein in western blots, and agb1-1 is a splice site mutation that introduces a stop codon early in the transcript, suggesting both are null alleles (Lease et al., 2001; Ullah et al., 2001). No alteration of localization pattern was observed for either EYFP-AGG2 or EYFP-AGG1 in gap1-1 or agb1-1 mutants (Fig. 4, I–L), suggesting that AGG1 and AGG2 can localize to the membrane solely through their lipid modification.

Figure 5. Immunoblotting using GFP-specific monoclonal antibody. Total (T), soluble (S), and total membrane (M) fraction of protein was prepared from equivalent amounts of Arabidopsis leaf tissue within each sample expressing EYFP-AGG2, AGG2C97S, AGG1, and AGG1C93S as well as EYFP-AGG2 and AGG1 in the background of plp-1, ggb-2, and era1-4 mutants. Intact EYFP-AGG2 in the wild type, as well as in era1-4, was detected in the membrane fraction. EYFP-AGG2 was in the cytosol. In the plp-1 mutant, EYFP-AGG2 was detected in both soluble and membrane fractions. In the ggb-2 mutant, the majority of EYFP-AGG2 was detected in the membrane fraction, and a weaker band in the cytosol was shown with longer exposure (as shown in Supplemental Fig. S2).
showed both signals. Within those cells that do detect both signals, the bright spots in the GFP-AGG2\textsubscript{C55S} were also shown to be associated with Golgi (Fig. 7F) in the same manner as that observed in EYFP-AGG1 (Fig. 4D) and GFP-AGG1\textsubscript{C55S} (Fig. 6I).

The fatty acid analog 2-bromo-palmitate (2-BP) is known as a palmitoylation inhibitor both in vivo and in vitro. 2-BP blocks the synthesis of acyl-CoA and also interferes with the transfer of fatty acids to the thiol group (Resh, 2006b). When EYFP-AGG2 were infiltrated into \textit{N. benthamiana} along with 2-BP, a similarly disrupted pattern to that of GFP-AGG2\textsubscript{C55S} was displayed (Fig. 7H), while plasma membrane localization of EYFP-AUX1 that contains transmembrane domains (Yang et al., 2006) was not altered by the same treatment (Fig. 7I), nor was the EYFP-AGG2 mock treatment with 10\% dimethyl sulfoxide (DMSO) alone (Fig. 7G). It appears that 2-BP treatment does not alter AGG1’s localization, consistent with the results from the point mutation (data not shown). The disruption of the plasma membrane localization of AGG2 by both mutation of the putative S-acylated Cys and S-acylation inhibitor suggests that wild-type AGG2 is S-acylated, and S-acylation serves as an important second signal for plasma membrane targeting of the prenylated protein.

Charged Residues Preceding the CSIL Motif of AGG2 Are Indispensable for Its Correct Localization

Many prenylation targets, including CaM53 (Caldelari et al., 2001) and K-RasB (Hancock et al., 1989), have a polybasic proximal domain that either promotes prenylation efficiency or serves as a second plasma membrane targeting signal. A polybasic region with a net positive charge of four or more is required to function as a plasma membrane targeting motif (Hancock et al., 1990; Michaelson et al., 2001). A polybasic sequence with six Lys was reported to be able to facilitate palmitoylation without requiring previous prenylation (Booden et al., 1999), suggesting the polybasic domain indeed serves as a membrane-targeting signal. A polybasic domain with 11 Arg and three Lys residues in CaM53 has been shown to increase PGGT-I prenylation efficiency by an order of magnitude in vitro (Caldelari et al., 2001). Neither AGG1 nor AGG2 has a long proximal polybasic domain as does CaM53 (Fig. 1). However, AGG2 does have three more charged amino acid residues in the preceding hypervariable region than AGG1 (KEAKRCG versus NGGEGCR). We suspected this might be responsible for the observed different localization patterns between AGG1 and AGG2. We swapped the hypervariable regions of AGG1 and AGG2 proximal to the CaaX boxes (Fig. 1) and observed subcellular localization patterns of the resulting GFP-tagged proteins, AGG2m1 and AGG1m2, in \textit{N. benthamiana} leaf epidermal cells and Arabidopsis transformants. AGG2m1 was associated with Golgi and other internal membranes as seen in S-acylation diminished GFP-AGG2\textsubscript{C55S} (Fig. 8, A–C), while wild-type AGG2 was only detected on the plasma membrane, strongly suggesting that the basic residues contribute to the efficient targeting of AGG2. Stable transformation

Figure 6. Mutation of the putative S-acylation acceptor Cys of AGG1 does not change AGG1’s subcellular localization pattern. Agrobacterium C58 containing binary vector EYFP-AGG1 and GFP-AGG1\textsubscript{C55S} were coinfiltrated with ER localization marker CFP-HDEL into \textit{N. benthamiana}. Projection of multiple stacks taken from the lower epidermal of cotyledon or leaf cells is presented for every section except I. A to C, Similar to that shown in the stable transformants, transient assays of AGG1 show bright spots and weak signals with other internal membranes. The inset in C was from the spot designated by an asterisk. D to F, Transient assay of GFP-AGG1\textsubscript{C55S} with a mutation of the putative S-acylation acceptor Cys, shows a similar pattern to that of wild-type EYFP-AGG1. G, Transient expression pattern of EYFP-AGG1 alone is similar to that of A, suggesting that coinfiltration of CFP-HDEL does not alter EYFP-AGG1’s localization pattern. H, GFP-AGG1\textsubscript{C55S} in stable Arabidopsis transformants has a similar fluorescing pattern to wild-type EYFP-AGG1, \textit{i}, Colocalization pattern of GFP-AGG1\textsubscript{C55S} and GmMan1-CFP. All scale bars = 10 \textmu m.
of GFP-AGG2m1 (Fig. 8D) also displays more fluorescing internal signals than AGG2 (Fig. 8F). In contrast, the localization pattern of AGG1m2 in stable transformants (Fig. 8E) is very different from that of wild-type AGG1 (Fig. 4B) but very similar to that of AGG2 (Fig. 8F), with GFP signal mainly detected in the plasma membrane, suggesting that the added short basic sequence possibly promotes both prenylation and S-acylation process.

DISCUSSION

The importance of G protein in signal transduction processes and some phenotypic similarities between the protein prenyltransferase mutant plp-1 and the Go mutant gap1 and GB mutant gab1 led us to choose the potential dual lipid modification targets AGG1 and AGG2 as models for the study of lipid modification mechanisms in Arabidopsis and to delineate PLP’s physiological function. AGG1 and AGG2 share a CaaX box with Gg from mammals and yeast but show unique proximal sequence domains. We showed that both AGG1 and AGG2 are prenylated, and prenylation is essential but not sufficient for their proper plasma membrane association. S-acylation functions as an important second signal to efficiently target AGG2 to the plasma membrane.

Arabidopsis PFT can largely compensate for loss of function of PGGT-I, as shown both biochemically and genetically. This may help explain the lack of visible phenotypes of ggb mutants. The result that PFT can efficiently farnesylate the ideal PGGT-I targets AGG1 and AGG2 is largely consistent with earlier reports that the recombinant tomato (Lycopersicon esculentum) farnesyltransferase can farnesylate mutated APETALA1 with a Caal box, GST-ap1mL, with [3H]FPP (Yalovsky et al., 1997, 2000b). The change of localization patterns of wild-type AGG1 and AGG2 in the plp-1 background provides strong evidence that AGG1 and AGG2 are prenylated in vivo.

Although prenylation is critical for the membrane association of many target proteins, there is always a question if prenylation is sufficient for plasma membrane association. The membrane affinity provided by prenylation alone, particularly farnesylation, is thought not enough to stably anchor the associated protein to the plasma membrane as a sticky finger, but rather provides a greasy handle to interact with the other membrane-bound proteins (Magee and Seabra, 2003). Prenyl groups are also thought to be too bulky and too rigid to associate with the lipid rafts, where sphingolipid and cholesterol is enriched and receptor-mediated signaling events are organized (Dunphy et al., 2001). In contrast, protein palmitoylation is well suited to this role, and mammalian Goi palmitoyltransferase activity was determined to be enriched in the lipid rafts (Dunphy et al., 2001). Dual lipid modification was required for the yeast Gy-subunit Ste18p (-CCTLM) for a fully functional pheromone...
response, and its palmitoylation seemed to depend on its farnesylation (Manahan et al., 2000). Unlike Ste18, which has two adjacent Cys, both AGG1 and AGG2 have two Cys interspersed by another amino acid residue at their C terminus (Fig. 1). Similar CxCxXx structures exist in H-Ras and R-Ras, both of which have been shown to be prenylated and palmitoylated (Hancock et al., 1989). K-RasB does not have an extra Cys for palmitoylation, but it has a long stretch of basic amino acids that offers an alternative second signal to facilitate plasma membrane targeting (Hancock et al., 1989). Although AGG2 has a Lys and Arg right next to the -CGCSIL motif, neither AGG1 nor AGG2 has a long stretch of polybasic domain like in K-RasB. The disrupted fluorescence pattern resulting from mutation of the potential S-acyl acceptor Cys indicates that AGG2 is likely subject to S-acylation in vivo and also suggests that the proximal basic residues of AGG2 do not serve as a sufficient second plasma membrane targeting signal. Unlike AGG2, AGG1 is possibly either not S-acylated or at least not efficiently S-acylated based on the fact that no dramatic change was observed among wild-type AGG1 and the putative S-acylation acceptor mutant form in both transient assay and stable transformants. However, we cannot exclude other factors that might prevent AGG1 from efficient plasma membrane targeting or promote retrograding from plasma membrane to the trans-Golgi network. Recent studies from yeast on the role of Gα on pheromone signaling revealed that a new branch of G-protein signaling occurs at the endosome rather than plasma membrane (Slessareva et al., 2006). AGG1’s internal membrane association implies that Arabidopsis Gβγ1 might activate effectors at these domains as well.

The complete cytosolic distribution of the prenyl Cys mutants AGG2C97S and AGG1C95S suggests that initial prenylation is required for the S-acylation of their adjacent Cys. S-acyltransferase is found to be associated with ER (Lobo et al., 2002), Golgi (Resh, 2006a), plasma membrane (Dunphy et al., 2001), or vacuolar membranes (Veit et al., 2003). Thus for S-acylation to occur under physiological conditions, a mechanism is required to bring the target proteins close to the membranes where the S-acyltransferase resides. In contrast, protein prenyltransferases are cytosolic, and, thus, protein prenylation can occur simultaneously once the protein is translated. The reported dependence of palmitoylation of the second Cys on the farnesylation of the prenyl Cys in Ste18 and Ras2P from yeast cells agrees with this primary requirement of protein membrane association prior to S-acylation (Manahan et al., 2000; Lobo et al., 2002; Dong et al., 2003). Alternatively, the membrane attachment can be facilitated by a polybasic domain that interacts electrostatically with the head groups of negative-charged phospholipids in the cytoplasmic leaflet of the membrane (Magee and Marshall, 1999). It has been shown that requirement of farnesylation for palmitoylation of H-Ras can be replaced by a polybasic domain with six Lys (Booden et al., 1999). A nonprenylated but palmitoylated type II RAC, AtRAC10 (Lavy et al., 2002), also has a long stretch of charged residues with many Lys residues that is required for palmitoylation (Lavy and Yalovsky, 2006). Unsurprisingly, proteins with one or more intrinsic transmembrane domains have been identified to be palmitoylated through a global analysis approach (Roth et al., 2006). Proteins with none of the membrane association mechanisms mentioned above have also been identified to be palmitoylated in yeast, and possibly these types of targets were brought to the peripheral of membranes through interacting with membrane-associated proteins.

Interestingly, no human Gγ has an adjacent Cys, as in the yeast Gγ, or the preceding polybasic domains to the -CaaX box, as in the K-RasB (Takida and Wedegaertner, 2003). Gβγ2 requires the presence of Gα to be targeted to the plasma membrane rather than to the

![Figure 8](image-url)

Figure 8. The proximal basic residues of AGG2 are important for AGG2’s efficient plasma membrane targeting. A to C, GFP-AGG2m1, swapped with AGG1’s proximal sequence, was coinfiltrated with ER localization marker CFP-HDEL into N. benthamiana. Internal membrane association of GFP-AGG2m1 is detected. D, Cells from stable transformants of GFP-AGG2m1 also show internal fluorescing signals. E, GFP-AGG1m2, AGG1 with AGG2’s basic proximal sequence, is primarily detected associated with the plasma membrane as shown in stable Arabidopsis transformants, with a similar pattern to that of EYFP-AGG2 (F). All scale bars = 10 μm. [See online article for color version of this figure.]
ER (Takida and Wedegaertner, 2003). It was also shown that when an S-acylation site was introduced into the Gy2, the dependence on Ga for correct plasma membrane localization is not required. Using gpa1, we showed that AGG1 and AGG2 do not rely on Ga for their correct targeting. On the other hand, the cytosolic distribution of the prenylation-depleted AGG1<sup>Δ355</sup> and AGG2<sup>Δ755</sup> indicates that, as in humans, Arabidopsis Ga is not sufficient to bring GBY to the membrane.

In our EYFP tagging experiments, AGG2 appears to be more abundant and better targeted to the plasma membrane than AGG1. Although we found AGG2 has extra putative N-glycosylation motifs that AGG1 lacks using the Motif Scan program (http://myhits.isb-sib.ch/cgi-bin/motif_scan; Falquet et al., 2002; Fig. 1), AGG2 does not exhibit the smear pattern on SDS-PAGE as glycosylated proteins usually do. Thus, glycosylation probably does not account for the observed localization pattern and signal intensity difference between AGG2 and AGG1. AGG1 and AGG2 have very similar but not the same CaaX motif (CSIL versus CLIL) and the same location of a potential palmitoylation Cys (CGCISL versus CRCLIL). However, among the five preceding amino acids of AGG2, there are four charged residues, three of which are basic, while AGG1 has only one charged amino acid, an acidic amino acid. Results from our swapping experiment demonstrate that the hypervariable sequence in AGG2 facilitates the prenylation process and makes AGG2 target better to the plasma membrane. AGG1 containing the proximal domain from AGG2 changes the localization of AGG1 to be more similar to AGG2. While the differences between AGG1 and AGG2 localization is clear, we cannot rule out the possibility that overexpression of these lines may cause localization patterns that differ from the native proteins. We obtained one T-DNA insertion mutant line in the intron region (FLAG-208A03) for AGG1 from the Institut National de la Recherche Agronomique Biological Resource Centre in Versailles and all of the T-DNA insertion lines for AGG2 from Salk (Alonso et al., 2003), but we have not observed a developmental phenotype in these lines (data not shown). It will be interesting to see the degree to which various prenylation- and palmitoylation-deficient mutant forms rescue phenotypes that may be seen in the double mutant agg1 agg2.

In the plp-1 background, where both PFT and PGTT-I functions are lost, we still detected some AGG2 remaining in the membrane fraction, although the total signal in plp-1 is already much less than that in the wild type. We are not certain what accounts for the remaining membrane association, though there are several possibilities. One is that the third type of prenyltransferase, PGTT-II or Rab geranylgeranyltransferase, known to exclusively prenylate Rab GT-Pases in mammalian cells (Seabara et al., 1992), may compensate to some degree for loss of both PFT and PGTT-I in plants. However, prenylation studies using either PFT or PGTT-I targets and the crude extract from plp-1 or ggb mutant did not show extra prenylation activity (Running et al., 2004; Johnson et al., 2005), indicating either there is no cross specificity between PGTT-I and PGTT-II or the activity under physiological conditions is too low to detect the crude extract under a condition not favored by PGTT-II. Further experiments using the purified PGTT-II enzyme are needed to see if AGG1 and AGG2 are possibly prenylated by PGTT-II at low efficiency. A second possibility is that dual acylation can compensate for the loss of prenylation. It is possible that the charged residues unambiguously allow AGG2 access to the internal membrane where the S-acyltransferase likely resides.

### MATERIALS AND METHODS

#### Expression of GST-Fusion Arabidopsis PFT and PGTT-I in Yeast

The full-length c-DNA of PLP (At5g59380) was amplified with forward primer QZI1 (CCGGAATTCTCGACTTTGGATCGGAGTC) and QZI2 (TTCGAATTCATGCCAGTAGTAACCCG), digested with HI/Pl and subcloned into the pEMBL vector pESC-HIS (Stratagene), and verified by sequencing in frame with the GAL10 promoter of the yeast (Saccharomyces cerevisiae) expression vector pESC-HIS (Stratagene). Then the transformed cells were selected for their ability to grow on medium lacking His.

Expression of Recombinant Arabidopsis PFT and PGTT-I in Yeast

The full-length c-DNA of PLP (At5g59380) was amplified with primer QZI1 (CCGGAATTCTCGACTTTGGATCGGAGTC) and QZI2 (TTCGAATTCATGCCAGTAGTAACCCG), digested with HI/Pl and subcloned into the pEMBL vector pESC-HIS (Stratagene), and verified by sequencing in frame with the GAL10 promoter of the yeast (Saccharomyces cerevisiae) expression vector pESC-HIS (Stratagene). Then the transformed cells were selected for their ability to grow on medium lacking His.

Induction of protein expression was performed as described (Casbon and Kinney, 2004) except for the following modifications. For medium scale protein induction, single colonies from the transformed cells were grown for 3 d with shaking (300 rpm) at 30°C in 5 mL medium consisting of 0.08% (w/v) complete supplement mixture without His (CSM-HIS, BIO101), 0.17% (w/v) yeast nitrogen base without amino acids (Difco), 0.5% (w/v) ammonium sulfate, and 2% (w/v) raffinose. Then the 5-mL culture was added into 50 mL of the same medium and continued growing for 2 d at 30°C. Cells were then collected, washed, and resuspended in 250 mL of the same medium except that the raffinose was replaced with 2% (w/v) Gal for induction of protein expression for 24 h. Spheroplast and crude protein were prepared using CelLytic Y Plus (product no. PCY1-P, Sigma). PFT and PGTT-I were purified using EzViewRed ANTI-FLAG M2 Affinity gel (product no. F2426, Sigma) and eluted with 3× FLAG peptide (product no. F4799, Sigma) according to the manufacturer’s instructions.
manufacturer’s instructions. The eluted enzyme was stored at −80°C for further use.

In Vitro Protein Prenylation Assays

In vitro prenylation assays were performed as described (Randall et al., 1993; Zhang et al., 1994; Yalovsky et al., 2008b; Johnson et al., 2005). The reactions consisted of 2 μg of purified GST-AGG2, GST-AGG2/C95S, GST-AGG1, or GST-AGG1/C95S, 10 ng of purified recombinant Arabidopsis (Arabidopsis thaliana) PFT or PGGT-I, 50 mM Tris, pH 8.0, 5 mM MgCl2, 50 μM ZnCl2, 0.2% (w/v) glucosepyranoside, 5 mM dithiothreitol, and 1 μL of either [1-3H]FPP (1 μCi/mL) or [1-14C]GPP (1 μCi/mL), both from American Radiological Company) in a total volume of 20 μL. The reactions were pre-equilibrated at 30°C for 5 min before adding prenyl substrates and incubated for 90 min afterward, resolved by 10% SDS-PAGE, and analyzed by fluorography using Autofluor fluorographic reagent (National Diagnostics) and Kodak XAR film (Eastman Kodak).

Construction of Plant Expression Vectors and Transformation

The AGG1 cDNA was amplified using primer sets QZ41 (ATACATAGCTTCTGACAGAACTCAGT) and QZ43 (CGTGTGCTTCTAGATCAAAGTATGACCTCCGACA) and reverse primer QZ6e (CTGGTCTCTAGATCATCTAAACAA). The corresponding AGG1 was amplified by QZY43 (CACCATGGAAGCGGGTAGCTCCAATTCGTC). The AGG1 cDNA was amplified using QZ41 (ATACATAGCTTCTGACAGAACTCAGT) and QZ43 (CGTGTGCTTCTAGATCAAAGTATGACCTCCGACA) and reverse primer QZ6e (CTGGTCTCTAGATCATCTAAACAA). The corresponding AGG2 was amplified by QZY42 (TCAAAGTATTAAGCATCTAGAGCCTTCTCCGACA) and QZY41 (ATACATAGCTTCTGACAGAACTCAGT) was used to change the fourth to last Cys in AGG1/C93S to create AGG1/C95S.

In Vitro Protein Prenylation Assays

The reactions consisted of 2 μg of purified GST-AGG2, GST-AGG2/C95S, GST-AGG1, or GST-AGG1/C95S, 10 ng of purified recombinant Arabidopsis (Arabidopsis thaliana) PFT or PGGT-I, 50 mM Tris, pH 8.0, 5 mM MgCl2, 50 μM ZnCl2, 0.2% (w/v) glucosepyranoside, 5 mM dithiothreitol, and 1 μL of either [1-3H]FPP (1 μCi/mL) or [1-14C]GPP (1 μCi/mL), both from American Radiological Company) in a total volume of 20 μL. The reactions were pre-equilibrated at 30°C for 5 min before adding prenyl substrates and incubated for 90 min afterward, resolved by 10% SDS-PAGE, and analyzed by fluorography using Autofluor fluorographic reagent (National Diagnostics) and Kodak XAR film (Eastman Kodak).

Microscopy Imaging

Cotyledons or leaf lower epidermal cells of transgenic plants or transiently infiltrated N. benthamiana with EYFP, GFP, or GFP signal were imaged using a Zeiss LSM 510 confocal/multiphoton microscope equipped with a 40× DIC lens that allows simultaneous acquisition of confocal, multiphoton, and transmitted light images. Colocalization of EYFP or GFP with CFP was taken under multitrack options with one channel set up for EYFP or GFP and another channel set up for CFP. To observe the signal distribution after plasmolysis, leaf sections were immersed in 30% Suc for 2 h. Images were finalized by Adobe Photoshop 7.0.

Protein Preparation and Immunoblotting

Proteins were prepared according to Preuss et al. (2004). A total of 0.2 g of leaf tissue was ground in 0.6 mL of cold buffer containing 20 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM MgCl2, 1 mM dithiothreitol, and 1× complete protease inhibitors (Roche Applied Science). The homogenate was transferred to a 2-mL tube and spun at 6,000×g for 5 min at 4°C, and 0.4 mL of postnuclear supernatant (PNS) was collected as total protein and 0.2 mL of PNS was fractionated at 100,000×g for 1 h. The supernatant (soluble fraction) was collected. The pellet was resuspended in 200 μL of 1× loading buffer. An equal volume of 2× loading buffer was added to both the saved PNS (total protein) and soluble fraction. Samples were boiled for 10 min and spun for 2 min. Five microliters of the above sample from YFP-vector control, 10 μL from YFP-AGG1, YFP-AGG2/C95S, and YFP-AGG2 era1-4, 15 μL from EYFP-AGG1, and 30 μL from EYFP-AGG2/1 was loaded onto 4% to 15% SDS-PAGE gradient gel. Signals were detected by exposing Kodak XAR film (Eastman Kodak) to the membrane after applying ECL western Blotting Substrate (Pierce).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Immunoblotting using GFP-specific monoclonal antibody.

Supplemental Movie S1. EYFP-AGG1 is detected associated with motile subcellular organelles.

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LITERATURE CITED

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen HM, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, et al. (2003) Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science 301: 653–657

Bonetta D, Baylis P, Sun S, Sage T, McCourt P (2000) Farnesylation is involved in meristem organization in Arabidopsis. Planta 211: 182–190

Boonen MA, Baker TL, Solski PA, Der CJ, Punke SG, Buss JE (1999) A non-farnesylated Ha-Ras protein can be palmitoylated and trigger potent differentiation and transformation. J Biol Chem 274: 1423–1431

Bracha K, Lavy M, Yakovsky S (2002) The Arabidopsis AtSTE24 is a CAAX protease with broad substrate specificity. J Biol Chem 277: 29856–29861

Cabrera-Vera TM, Vanhauwe J, Thomas TO, Medkova M, Preininger A, Boisson-Dernier A, von Heijne G (2002) A novel motif is important for targeting prenylated proteins to membrane domains. Proc Natl Acad Sci USA 99: 12393–12398

Casey PJ (1995) Protein lipidation in cell signaling. Science 268: 221–225

Darnell J, Green R (1990) Molecular cloning and characterization of an Arabidopsis thaliana G protein gamma-subunit cDNA. Proc Natl Acad Sci USA 87: 3015–3019

Dawes J, Martienssen R (1991) The nuclear pore proteins Nup153 and Nup93 and Nup205 determine the limit of nuclear pore complex size exclusion in vivo. Mol Biol Cell 14: 5104–5115

Gally V, Mattaj IW, Askjaer P (2003) Caenorhabditis elegans nucleoporins Nup93 and Nup205 determine the limit of nuclear pore complex size exclusion in vivo. Mol Biol Cell 14: 5104–5115

Gietz RD, Woods RA (2002) Transformation of yeast by the LiAc/SS carrier DNA/PEG method. Methods Enzymol 358: 87–96

Hancock JE, Cadwallader K, Marshall C (1991) Methylation and proteolysis are essential for efficient membrane binding of prenylated p21K-ras(B). EMBO J 10: 641–646

Hancock JE, Magee AI, Childs JE, Marshall CJ (1989) All ras proteins are polyisoprenylated but only some are palmitoylated. Cell 57: 1167–1177

Hancock JF, Paterson H, Marshall CJ (1990) A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize p21ras to the plasma membrane. Cell 63: 133–139

He B, Chen P, Chen S, Vancura K, Michaelis S, Powers S (1991) RAM2, an essential gene of yeast, and RAM1 encode the two polypeptide components of the farnesyltransferase that prenylates a-factor and Ras proteins. Proc Natl Acad Sci USA 88: 11373–11377

Hemsley PA, Kemp AC, Grierson CS (2005) The TIP GROWTH DEFICIENT S-acyl transferase regulates plant cell growth in Arabidopsis. Plant Cell 17: 2554–2563

Hirschman JE, Jenness DD (1999) Dual lipid modification of the yeast G protein subunit Ste18p determines membrane localization of Gf3y. Mol Cell Biol 19: 7705–7711

Huang Y, Chien K (1993) Polylysine and CVIM sequences in the prenylation signal can substitute for a CAAX motif in targeting of a heterotrimeric G protein. J Biol Chem 268: 22838–22842

Jones AM, Assmann SM (2004) Plants: the latest model system for studying Ras signaling. Trends Plant Sci 9: 112–118

Kato C, Mizutani T, Kakeya Y, Kato H, Iwaski Y (2003) Characterization of the Dictyostelium G alpha subunit. Biochem J 370: 63–70

Kawahara H, Matsuoka K, Kageyama H, Harada H (1993) Molecular cloning and characterization of G protein gamma subunits from various mammalian cells. J Biol Chem 268: 13423–13429

Khan FE, Saleem S, Ghozlan A, Assmann SM (2002) RNAi and molecular genetics reveal that GProtein-coupled receptor signal transduction in Arabidopsis thaliana requires the G-protein beta subunit Gs2. Plant Cell 14: 1113–1125

Kurokawa T, Ishihara Y, Kiba T, Ikeno Y, Nakamura T, Osumi T, Takano-Yamamoto A, Okazaki Y, Kajita Y, Nakamura K (2002) Structure of G protein beta 3. Structure 10: 1061–1072

Lane KA, Smiley MD, Guarente L (1993) The G-protein beta subunit Gbg2 is required for optimal viability of Caenorhabditis elegans. Cell 75: 1133–1144

Lavoix S, Robertson A, Caspar DA, Barlow DP, Kooistra R, von Heijne G (1996) A novel motif in the amino-terminal domain of the yeast G protein subunit Ste18p determines membrane localization of Gf3y. Mol Cell Biol 16: 5104–5115

Leake KA, Benitez JC, Poulley SR, Hellewell PA, Calvo J, Clarke D, Guy EA, North RJ, Waters MR, Kwan YP, et al. (2002) Two classes of beta-gamma subunits are required for growth of Dictyostelium discoideum. Mol Biol Cell 13: 3065–3073
