Characterization of the *Arabidopsis* Heterotrimeric G Protein

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Running head: *Arabidopsis* heterotrimeric G protein complex

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We have used fluorescence resonance energy transfer (FRET) and co-immunoprecipitation to analyze the interactions among the α, β and γ subunits of the *Arabidopsis* heterotrimeric G protein. Using CFP and YFP fusion constructs, we show that overexpressed Gγ1 localizes to protoplast membranes, but Gβ exhibits membrane localization only when the Gγ1 protein is co-overexpressed. Overexpressed Ga shows membrane localization unaccompanied by overexpression of either Gβ or Gγ1. We detect FRET between Gβ and Gγ1 in the absence of Ga overexpression and between Ga and Gγ1, but only when all three subunits are co-overexpressed. Both Ga and Gβ are associated with large macromolecular complexes of approximately 700 kD in the plasma membrane. Ga is present in both large complexes and as free Ga in plasma membranes from wildtype plants. In plants homozygous for a null allele of the Gβ gene, Ga is associated with smaller complexes in the 200-400 kD range, indicating that its presence in the large complex depends on association with Gβγ. Activation of the Ga subunit with GTPγS results in partial dissociation of Ga from the complex. Hydrogen peroxide (H2O2) promotes extensive dissociation of the Ga complex, but does not interfere with binding of GTPγS to purified recombinant Ga, suggesting that reactive oxygen species affect the stability of the large complex, but not the activity of Ga itself.

The results of pharmacological and genetic studies have provided evidence that the plant heterotrimeric G protein is involved in the transmission of light (1) and hormone signals (2), as well as in the regulation of ion channels (3). *Arabidopsis* gpa1 mutant plants, which lack the Ga protein, encoded by the GPA1 gene, exhibit reduced cell division during hypocotyl and leaf formation (4), while overexpression of GPA1 causes ectopic cell division, including meristem proliferation (5). Homozygous gpa1 mutant plants are less sensitive to ABA inhibition of stomatal opening and guard cell inward K+ currents than wildtype plants (6). By contrast, gpa1 mutant seeds exhibit hypersensitivity to ABA in inhibition of germination and in root growth and seedling gene expression. In addition, gpa1 mutant plants are hyposensitive to gibberellic acid and brassinolide (7,8). *Arabidopsis* agb1-2 mutant plants, which lack the Gβ protein encoded by the AGB1 gene, show alterations in leaf, flower and fruit development, decreased hypocotyl cell division and hypersensitivity to D-glucose (9-11). There are two Gγ subunit genes in *Arabidopsis*, AGG1 and AGG2, and mutant analysis indicates that each Gγ subunit participates in a subset of Gβ-related developmental processes (12).

Evidence is also accumulating that the heterotrimeric G protein mediates plant responses to bacterial and fungal pathogens and abiotic stress. Heterotrimeric G protein signaling to membrane-bound NADPH oxidase has been implicated in the development of disease resistance and in the apoptotic hypersensitive response in rice (13) and homozygous agb1-1 mutant plants are more susceptible to necrotrophic fungal pathogens than are wildtype plants (14,15). The Ga and Gβ subunits serve both separable and synergistic functions in signaling by reactive oxygen species in the oxidative stress response (16) and the Gβ subunit of the heterotrimeric G protein mediates cell death signaling in the *Arabidopsis* unfolded protein response (UPR) (17).

By contrast to the large number of different G protein subunits in animals, *Arabidopsis* has only one Ga subunit (18), one Gβ subunit (19), and two Gγ subunits (20, 21). The *Arabidopsis* G protein...
subunits exhibit limited homology with their animal counterparts (22). The GPA1 protein is roughly 30% identical to the mammalian Ga subfamily proteins, the AGB1 protein shows about 42% identity to mammalian Gβ subunits, and the AGGs display ~25%-35% identity with certain mammalian Gγ subunits (20,21). At the cellular level, Arabidopsis Ga has been immunolocalized to the plasma membrane and endoplasmic reticulum (23), while Arabidopsis Gβ has been detected in the plasma membrane, endoplasmic reticulum and Golgi apparatus (24,25). Interactions between Arabidopsis Gβ, Gγ1 and Gγ2 have been detected using a yeast two-hybrid system and by vitro binding assays (20,21), and there is also evidence for interactions between the rice Ga and Gβ subunits (26). Structural predictions for Ga, Gβ, and Gγ1 suggest that they can form a heterotrimer similar to that formed by mammalian G protein subunits (27). Although the heterotrimerization of Arabidopsis G protein subunits in cowpea protoplasts was recently reported (24), there is still limited information available about the structural and biochemical characteristics of the Arabidopsis heterotrimeric G protein complex.

Here we provide further structural and biochemical characterization of the Arabidopsis heterotrimeric G protein complex using transgenic plants and transiently transformed Arabidopsis protoplasts. First, we show interactions between Gβ and Gγ1, as well as between Ga and Gβγ1 in Arabidopsis mesophyll protoplasts using fluorescence resonance energy transfer (FRET) and co-immunoprecipitation. We then show that the heterotrimeric G protein is part of a large complex of roughly 700 kD using blue native (BN) gel electrophoresis. We also show that the Ga subunit is present both in the large complex and as a free monomer in the plasma membrane fraction of wildtype plants and that it is also detected in smaller complexes in agb1-2 mutant plants. We report that GTPγS binds to recombinant Ga protein, and promotes slight dissociation of the Ga monomer from the large complex. Treatment of the plasma membrane fraction with hydrogen peroxide (H2O2) promotes the extensive dissociation of Ga from this complex, but does not affect binding of GTPγS to the recombinant protein. These observations suggest that reactive oxygen species activate plant G protein signaling by promoting dissociation of the G protein macromolecular complex, rather than by acting directly on the Ga protein.

**EXPERIMENTAL PROCEDURES**

**Plant materials.** We used Arabidopsis thaliana Col-0 plants and agb1-2 and gpa1-4 null mutant homozygotes in the Col-0 background (27). Plants used to isolate plasma membrane fractions were grown in MetroMix 200 (Scotts-Sierra Horticultural Products Company, Marysville, OH) in 5-cm pots (51 per flat) at 65% humidity under fluorescent light at 150 μmol photons/m2/s with a 12 h light/12 h dark photoperiod for 5 weeks.

**Vectors for expression of CFP and YFP fusions.** The AGB and AGG1 coding sequences were cloned by PCR from a previously described A. thaliana Col-0 cDNA library (28). According to sequence information in the Arabidopsis database (http://www.arabidopsis.org), primers were designed as follows, with the underlined sequences adding restriction sites and the bold faced sequences adding a spacer (primers were purchased from Integrated DNA Technologies, Coralville, IA). The forward primer for AGB1 was: (5′-AGATCTGGAGGTTGGATTA-3′). The reverse primer for AGB1 is: (5′-TCTAGATCCCTAGTCAGATATTACATCGTC TGTCTCGAGCTCAA-3′). The reverse primer for AGB1 is: (5′-TCTAGATCCCTAGTCAGATATTACATCGTC TGTCTCGAGCTCAA-3′). The forward primer for AGG1 was: (5′-AGATCTGGAGTGGAGGTAGTAGTG TATATGCGAGAGGAAACTGTGGTT-3′). The reverse primer for AGG1 was: (5′-TCTAGATCCCTAGTCAGATATTACATCGTC TGTCTCGAGCTCAA-3′).

In order to insert CFP into GPA1, the binary vector of loop CFP-GPA1 (L-CFP-GPA1) was obtained from Dr. Alan Jones (University of North Carolina, Chapel Hill, North Carolina). The full length sequence of L-CFP-GPA1 was cloned from the binary vector. The amplified PCR fragments were cloned into pGEM-Teasy (Promega, Madison, WI) and sequences were verified. To make the pPVA321-CFP (or YFP)-Gβ and pPVA321-YFP-Gγ1 constructs, PCR products were cleaved from pGEM-Teasy (Promega) and further cloned into pPVA321-C-YFP (a kind gift of Dr. Xuemei Chen, University of California, Riverside, California) using the BglIII and XbaI sites. To make the pPVA321-L-CFP-Gα, pPVA321-Gβ and pPVA321-Gγ1 constructs,
PCR fragments of L-CFP-GPA1, AGB1 or AGG1 were cleaved from corresponding pGEM-Teasy constructs and cloned into pVAV321 with the removal of C/YFP via NcoI and XbaI. Then, the entire (35S)-Gβ or (35S)-Gγ1 expression cassette was cut by Clal and KpnI, which 5' overhangs were filled by Klenow fragment to form blunt ends and then ligated into pVAV321-YFP-Gγ1 or pVAV321-YFP-Gβ using the Clal and SmaI sites resulting in pVAV321-Gγ1-(35S)-YFP-Gβ and pVAV321-Gβ-(35S)-YFP-Gγ1. Finally, the entire (35S)-L-CFP-Gα expression cassette was further cloned into pVAV321-Gγ1-(35S)-YFP-Gβ or pVAV321-Gβ-(35S)-YFP-Gγ1 via SacI, SmaI sites and Klenow fragment as described above, resulting in pVAV321-L-CFP-Gα-(35S)-Gγ1-(35S)-YFP-Gβ and pVAV321-L-CFP-Gα-(35S)-Gβ-(35S)-YFP-Gγ1. The (35S)-L-CFP-Gα and (35S)-YFP-Gβ expression cassettes were further cloned into the pCAMBIA3300 Agrobacterium binary vector and transformed into homozygous agb1-2 and gpa1-4 mutant plants. Transformed plants that exhibited the wildtype leaf morphology phenotype on 10 µg/ml glufosinate MS plates were used for further study.

Protoplast isolation and transfection, confocal microscopy and FRET. Arabidopsis mesophyll protoplasts were prepared from fully expanded leaves of 4 to 5 week-old plants and transfected using the polyethylene glycol method developed by J. Sheen’s laboratory (29). An additional 21% sucrose gradient was applied to the isolated protoplasts to obtain healthy protoplasts, followed by transfection with plasmids containing the FRET pair to be tested. Plasmids were isolated using a Plasmid Maxi Kit (Qiagen, Chatsworth, CA). A total of ~1x10^5 cells were transfected with 50 µg plasmid for each microscopy observation, FRET measurement or immunoprecipitation experiment. The transfected protoplasts were incubated at 22°C for 12-16 h before being mounted in chambers (Molecular Probes, Eugene, OR) for microscopy. The transfected protoplasts were imaged using a Zeiss LSM 510 META laser scanning microscope (Carl Zeiss, Thornwood, NY), with a 40×NA 1.2 water objective. To monitor fusion protein expression and localization, protoplasts were excited with two Argon laser lines, 458 nm for CFP and 514 nm for YFP and emission images were collected simultaneously with a 480-520 nm filter for CFP and a 530-590 nm filter for YFP.

FRET is a method widely used to identify in vivo interactions between two proteins tagged with a donor (CFP) and an acceptor (YFP) fluorophor pair. FRET has also been used in conjunction with fluorescence lifetime imaging microscopy (FLIM) to monitor protein-protein interactions in plant cells (24,30-32). In addition to common concerns, several other factors complicating FRET analysis in plants have been identified (33,34). These are that (a) CFP and YFP excitation and emission spectra have crossover problems when a conventional epifluorescence microscope is used; (b) detection of FRET depends not only on the microscope optics, but also on the relative local concentrations of the donor CFP fusion and the acceptor YFP fusion; and (c) interference from the high background autofluorescence of chlorophyll, cell walls, and high concentrations of phenolic compounds in plant cells. (d) photobleaching, which is conventionally used to confirm FRET in animal cells (35,36), often cannot be applied to plant cells because it dramatically perturbs cellular structures (33).

To obviate these difficulties, we used a Zeiss LSM510 Meta spectral confocal fluorescence microscope equipped with a chameleon multiphoton laser, which can be modulated to a two photon laser at 820 nm without any crossover excitation of YFP (37). In addition, lambda stacks of acquired images were processed using the linear unmixing function mode of the Zeiss LSM510, allowing us to remove crossover emission from our analysis. Thus, our novel approach of two photon excitation combined with spectral image analysis allowed us to avoid the major type of artifact that plagues FRET studies performed with conventional epifluorescence microscopy (concern (a) above). To avoid concern (b) above, we ensured expression consistency between donor and acceptor molecules by transcriptionally fusing their expression cassettes into one construct, which we transfected into Arabidopsis protoplasts (Fig. 1B). The linear unmixing function mode of the Zeiss LSM510 also allowed us to remove autofluorescence signals, thus avoiding concern (c) above. We also performed extensive attempts to utilize photobleaching to confirm the FRET results reported here. However, we observed that
protoplasts deformed or burst after the photobleaching procedure and thus the photobleaching method could not validly be used in our studies.

In further support of the method we used, we performed FRET tests using ECFP-TGA5 and EYFP-TGA5 as positive controls, because these proteins were previously shown to interact (33). As negative controls, we assayed for FRET between ECFP and EYFP as well as between ECFP-TGA5 and EYFP-LexA (33). Constructs expressing the FRET fusion pairs used as positive and negative controls were kindly provided by Dr. Eric Lam. Upon CFP excitation with two photon laser at 820 nm, a YFP emission peak was detected in protoplasts expressing the positive FRET control pair, but not the negative control pairs (data not shown).

In this report, spectral color encoding was performed with the Zeiss LSM 510 software. Thus, when a FRET signal was detected, the image is displayed in a green color indicating a YFP emission spectrum at loci in protoplasts expressing positive FRET pairs, while when a FRET signal was not detected, the image is displayed in a cyan color indicating a CFP emission spectrum at loci in protoplasts expressing negative FRET pairs.

Protoplasts co-transfected with CFP and YFP fusion proteins were excited with a chameleon multiphoton laser (Coherent MRU 1000), which was modulated to 820 nm wavelength and set at 4-5% laser intensity, ideal for cross-talk free FRET analysis. The emission spectra from selected regions of interest were recorded by a connected multi-channel-spectrometer (MCS) in twelve channels, each with a 10 nm band width, from 464 to 584 nm, using a 650 KP dichroic mirror in the lambda stack acquisition mode. Emission spectra were recorded from at least 20 individual protoplasts transfected with each G protein FRET pair tested. Spectral analysis for automatic peak detection was performed with the LSM 510 software. The fluorescence spectra of the CFP- and YFP-fused G protein subunits were corrected for background fluorescence. FRET ratios were calculated as the ratio of YFP and CFP emission, where the intensities of YFP and CFP emission at 532 nm and 479 nm, respectively, are recorded by the MCS upon excitation of the specimen with the two photon laser at 820 nm.

### Immunoprecipitation and Western blotting

The catch and release reversible immunoprecipitation system (Upstate, Lake Placid, NY) was used for immunoprecipitation experiments. The transfected protoplasts were lysed with 1× wash buffer provided in the kit and then centrifuged at 15,000×g for 10 min. The clear extract was incubated with a rabbit polyclonal anti-Gα antibody (1:250) (gift of Dr. Alan M. Jones) and the affinity ligand (1:50) in the kit column at 4°C for 10-12 h. The column was washed three times by centrifugation with 1× wash buffer. 1× denaturing elution buffer in the kit was used to elute bound proteins, which were then fractionated by electrophoresis and transferred to a membrane for Western blotting. Total extract, flow through (concentrated using Stratagene resin from Stratagene, La Jolla, CA), and eluted proteins were loaded on a 12% polyacrylamide discontinuous gel (Bio-Rad mini electrophoresis system, Hercules, CA). After electrophoresis, proteins were transferred to Hybond-P PVDF membrane (Amersham, Piscataway, NJ) using a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad). Immunoblotting was performed with mouse monoclonal anti-G/C/Y antibodies (anti-G/C/Y antibody detects GFP, CFP and YFP proteins, 1:2000, BD Bioscience, Mountain View, CA). After incubation with horseradish peroxidase–conjugated anti-rabbit IgG antibodies, proteins were detected using ECL Plus protein gel blotting detection reagents (Amersham) according to the manufacturer’s instructions. The immunoprecipitation experiments were repeated 3 or more times using independent protein preparations.

### Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE)

Plasma membrane fractions for BN-PAGE were prepared using an aqueous two-phase partitioning system as described (38-41). BN-PAGE/SDS-PAGE two-dimensional gel electrophoresis was performed as described previously with some modification (28,42,43). Plasma membrane proteins were incubated with solubilization buffer (20 mM Bis-Tris-HCl, pH 7.0, 250 mM e-aminoacproic acid, 2 mM EDTA, 1.0% NP-40, 0.25% Coomassie blue G 250 and 10% glycerol) for 30 min and centrifuged twice at 15,000×g for 5 min. For GTPγS or H2O2 treatment experiments, solubilization buffer containing 100 µM GTPγS or
20 μM H2O2 was used. The supernatant was fractionated on a 5.5%-16% blue native polyacrylamide gradient gel and albumin bovine monomer (66 kD), lactate dehydrogenase (140 kD), catalase (232 kD), ferritin (440 kD), porcine thyroid (669 kD) from Amersham, were loaded alongside as marker proteins. One lane of the first dimension BN-PAGE gel was transferred to Hybond-P PVDF membrane (Amersham) and immunoblotted with anti-G/C/Y antibodies (BD Bioscience) to detect CFP tagged Gβ complex. The remaining first dimension lanes of the BN-PAGE gel were cut and incubated in denaturing solution (1% SDS, 1% β-mercaptoethanol) for 2 h and applied to a 10% SDS-PAGE gel. After electrophoresis, the proteins were transferred to PVDF membrane for immunoblotting with anti-Gα antibody. Each experiment was repeated 3 times. The blots for GTPγS or H2O2 treatment experiments were scanned and the intensity was quantified using Image J software. The percentage of the complex was calculated by the ratio of the value of the intensity of the complex to the value of the intensity of the total protein.

**Recombinant Gα expression, purification and the [35S]GTPγS binding assay.** We amplified GPA1 by PCR from an Arabidopsis cDNA library as described above using the forward primer 5′-GAGCTCATGGGTTACTCTGCAGTAGAA-3′ and the reverse primer 5′-GGTACCTCATAAAGCCAGCTCAGTAAA-3′. The amplified fragment was cloned into QIAexpression pQE-30 6×His tag vector (Qiagen) at the SacI and KpnI restriction sites (underlined in the primer sequences) and expressed in BL21-CodonPlus (DE3)-RIPL strain (Stratagene). The recombinant 6×His tagged Gα was purified according to the manufacturer’s instructions (The QIAexpressionist, Qiagen). Purification was confirmed by SDS-PAGE and the purified recombinant Gα protein was dialyzed against GTPγS binding assay buffer (50 mM Tris·HCl, pH 8.0, 5 μM GDP and 3 mM MgCl₂, 1 mM EDTA, 100 mM NaCl). The recombinant Gα was incubated with 20 nM [35S]GTPγS in the above buffer for different times and the reaction was diluted with stop solution (25 mM Tris·HCl, pH 8.0, 25 mM MgCl₂, 100 mM NaCl). The reaction mixture was applied to a 0.45 micron nitrocellulose membrane filter (Whatman, Florham Park, NJ), which was rinsed 3 times with stop solution to remove free [35S]GTPγS. The amount of recombinant Gα bound [35S]GTPγS was then quantified by liquid scintillation spectrometry. The specific binding of [35S]GTPγS with recombinant Gα was evaluated by nucleotide competition experiments with unlabeled ATP and GTP. The effect of H2O2 on the binding of [35S]GTPγS to recombinant Gα was tested as described (44).

**RESULTS**

**Over-expression and subcellular localization of Arabidopsis heterotrimeric G protein subunits.** Based on the predicted structure of the Arabidopsis G protein, we constructed genes encoding N-terminal fusions of enhanced cyan (CFP) and yellow fluorescent proteins (YFP) and Gβ and Gγ1, respectively, and expressed them from a CaMV 35S promoter (Fig. 1A and 1B). To minimize the possibility of interference of the CFP insertion with the structure and function of Gα, we inserted CFP into the second loop within the α-helical domain of Gα (24,35,45), designating the construct L-CFP-Gα (Fig. 1A and B). The effect of the C/G/YFP moiety on the function of the protein was tested for each Gα and Gβ construct by transforming it into the respective null mutant (gpa1-4 and agb1-2) and affirming its ability to complement the mutant phenotype (see Methods).

**Arabidopsis** mesophyll protoplasts were transformed separately with constructs expressing L-CFP-Gα, YFP-Gβ, or YFP-Gγ1 in order to determine sub-cellular localization of each G-protein subunit. Protoplasts with the lowest detectable expression levels were always chosen, to rule out over-expression phenotypes. Peripheral fluorescence, consistent with the localization of the fluorescent fusion protein to the plasma membrane, was observed in both wildtype and agb1-2 mutant protoplasts transfected with L-CFP-Gα (Fig. 2A.1 and Fig. 2B.1), indicating that the plasma membrane localization of Gα is independent of the presence of Gβ. Peripheral fluorescence was also observed in both wild-type and agb1-2 mutant protoplasts transfected with YFP-Gγ1 (Fig. 2A.2 and Fig 2B.2), indicating that Gγ1 localizes to the plasma membrane and this localization does not require Gβ. Peripheral localization was not observed in protoplasts
transfected only with YFP-Gβ construct; instead we observed small fluorescent bodies in the cytoplasm of protoplasts (Fig. 2A.3 and Fig. 2B.3). However, peripheral fluorescence was detected when protoplasts were co-transfected with YFP-Gβ-(35S)-γ1 (Fig. 2A.4 and Fig. 2B.4), indicating that the γ1 subunit was required for plasma membrane-localization of YFP-Gβ. Even when these constructs were transfected into wildtype protoplasts, which contain all three subunits, the endogenous level of γ1 was evidently not sufficient to detect a peripheral signal from the Gβ fusion protein unless the γ1 protein was also overexpressed. We infer that γ1 is required for plasma membrane localization of Gβ, while Ga and Gγ1 are each able to localize to the membrane independently of Gβ.

**FRET detection of in vivo interaction between the subunits of the Arabidopsis heterotrimeric G protein.** We used FRET to ask whether the Gβ and γ1 subunits are in close proximity in the membrane. Modeling predictions suggested that the Ga, Gβ, and γ1 proteins of Arabidopsis can form a heterotrimer similar to those characterized in other organisms (8). Moreover, an interaction between Gβ and γ1 has been detected in a yeast two-hybrid system and by in vitro pull-down assays (20,21). In HEK cells and Dictyostelium, FRET has been used to monitor the association of subunits of the heterotrimeric G protein and the dissociation of the Ga subunit from Gβγ dimer in response to external stimuli (35,46,47). FRET is detected as a shift in the emission spectrum of CFP (480 ± 20 nm) to that of YFP (530 ± 15 nm) upon excitation of CFP with the two photon laser at 820 nm and occurs if the tested proteins are in close juxtaposition. The emission spectra of CFP- and YFP-G protein fusions are shown in Fig. 3G.

Upon co-transfection of Arabidopsis mesophyll protoplasts with CFP-Gβ and YFP-Gγ1, we observed a FRET signal in peripheral areas of cells (Fig. 3A) and a spectral shift from 480 nm, representing the CFP spectrum, to 530 nm, representing the YFP spectrum (Fig. 3H). This result clearly shows that FRET occurs in the plasma membrane between CFP-Gβ and YFP-Gγ1. We conclude that Gβ and Gγ1 form a complex, which is required for membrane localization of the Gβ protein.

Next we used FRET to assess in vivo interactions between Ga and Gβγ1. We co-transfected Arabidopsis mesophyll protoplasts with combinations of plasmids encoding the following: L-CFP-Ga and YFP-Gβ; L-CFP-Ga and YFP-Gγ1; and L-CFP-Ga, YFP-Gβ and YFP-Gγ1. We did not detect a FRET signal and spectral shift (Fig. 3H) in cells when we co-transfected the protoplasts with constructs expressing either Gβ or Gγ1 fluorescent fusion proteins pairwise with L-CFP-Ga. (Fig. 3B and 3C). However, we were able to detect a FRET signal (Fig. 3D) and spectral shift (Fig. 3I) in cells when we co-transfected the protoplasts with constructs containing the coding sequences for all three subunits. Although the FRET construct pairs were transfected into wildtype protoplasts containing all three subunits, the endogenous levels of the third subunit were insufficient to allow FRET detection when only two of the three subunits were overexpressed. The presence of FRET between L-CFP-Ga, YFP-Gγ1 and YFP-Gβ (Fig. 3D and 3I) supports the inference that the subunits of Arabidopsis G protein form a heterotrimer in vivo.

To further determine whether the energy transfer is from L-CFP-Ga to YFP-Gβ or from L-CFP-Ga to YFP-Gγ1 in the heterotrimeric G protein complex formed in Arabidopsis protoplasts, we created single constructs expressing all three genes with two as a FRET pair and the third one untagged as follows: L-CFP-Ga-(35S)-YFP-Gβ-(35S)-Gγ1 and L-CFP-Ga-(35S)-Gβ-(35S)-YFP-Gγ1 (Fig. 1B). We detected FRET signal and a spectral shift in protoplasts transfected with L-CFP-Ga-(35S)-Gβ-(35S)-YFP-Gγ1 (Fig. 3F and 3I), but not in protoplasts transfected with the L-CFP-Ga-(35S)-YFP-Gβ-(35S)-Gγ1 construct (Fig. 3E and 3I). Thus, energy transfer occurs between L-CFP-Ga and YFP-Gγ1, but not between L-CFP-Ga and YFP-Gβ, implying that the CFP of L-CFP-Ga fusion protein is in closer proximity to the YFP of the YFP-Gγ1 fusion than to that of the YFP-Gβ in the G protein complex formed in Arabidopsis protoplasts. This observation implies that the Ga subunit does not interact with either Gβ or Gγ1 alone in protoplasts, but interacts with the Gβγ complex. Quantitative analysis of FRET ratios among the six tested FRET pairs is given in Figure 3J to further demonstrate the occurrence of FRET, where a
FRET signal is considered as positive when the ratio is greater than one. These results verify the successful detection of FRET between *Arabidopsis* heterotrimeric G protein subunits and indicate that they form a heterotrimeric complex in vivo.

We next applied an independent biochemical method to confirm that the *Arabidopsis* G-protein complex contains all 3 subunits in vivo. First, we immunoprecipitated L-CFP-Gα with Gα antibody and assessed whether YFP-Gγ1 proteins transiently expressed in protoplasts were co-immunoprecipitated. L-CFP-Gα and YFP-Gγ1 differ in size and can be detected together in the same western blot with anti-G/C/Y antibody to assess the efficiency of co-immunoprecipitation. We found that YFP-Gγ1 indeed can be co-immunoprecipitated with L-CFP-Gα from protoplasts transfected with a L-CFP-Gα-(35S)-Gβ-(35S)-YFP-Gγ1 construct (Fig. 4B), but not from protoplasts co-transfected with L-CFP-Gα and YFP-Gγ1 (Fig. 4A). Thus, our biochemical results support the conclusions from FRET analysis that the subunits of the *Arabidopsis* G protein form a heterotrimeric complex in vivo and that the interaction of Gα and Gγ1 requires Gβ.

The *Arabidopsis* heterotrimeric G protein complex. We used two-dimensional PAGE to further characterize the plasma membrane-bound heterotrimeric G protein complex. We isolated the plasma membrane fraction from rosette leaves using an aqueous two-phase partitioning system (48,49), solubilized it with NP-40, and fractionated the native complex by BN-PAGE, followed by denaturing SDS-PAGE and Western blotting. In the plasma membrane fraction, Gα antibody detects the Gα protein in a complex of approximately 700 kD, as well as in a low-molecular weight, apparently monomeric form (Fig. 5A). Approximately 30% of the Gα protein in wildtype plants is in the high molecular weight complex in the plasma membrane. Thus Gα protein appears to be part of a complex larger than anticipated for the heterotrimer, whose molecular mass is expected to be about 100 kD. A complex of about the same size (ca. 700 kD) was detected by Western blotting with anti-CFP antibodies in total extract derived from *Arabidopsis* plants expressing a CFP-Gβ fusion protein under control of the 35S promoter (Fig. 5B). However, by contrast to what was observed with Gα, all of the immunoreactive Gβ protein was associated with this large complex and none of its was detected at the expected mobility of either the heterotrimer (100 kD) or the Gβγ heterodimer (60 kD). Thus both Gα and Gβ are part of large membrane-bound complexes in *Arabidopsis*, but a significant fraction of Gα is also present in the membrane as the free monomer.

To determine whether the Gβ subunit is required for the formation of the large complex containing the Gα subunit, we isolated Gα-containing complexes from *agb1-2* mutant plants and observed that Gα was also in the plasma membrane fraction of the *agb1-2* mutant plants. This result is consistent with our observation in imaging studies that the plasma membrane localization of Gα is independent of the presence of Gβ. However, only about 8% of the Gα protein was associated with the ca. 700 kD complex in *agb1-2* mutant plants, as compared with ~30% in wildtype plants, as judged by the detection of Gα with anti-Gα antibodies after BN-PAGE and SDS-PAGE (Fig. 5C). In plants lacking the Gβ subunit, a substantial fraction of the Gα protein was associated with several complexes in the 140-400 kD range (Fig. 5C); such complexes were not observed in wildtype plants (Fig. 5A). These observations suggest that Gβ is not required for the plasma membrane localization of Gα but that Gβ promotes the association of Gα with the large complex. Alternatively, other proteins may bind to Gα in the absence of Gβ, resulting in formation of a different large complex.

Dissociation of the G protein complex. To examine whether G protein activation causes the dissociation of Gα from the complex, we used BN-PAGE to measure the change in the fraction of Gα in the high molecular weight and monomeric forms in the plasma membrane-bound fraction after incubation with GTPγS. GTPγS is a non-hydrolysable analog of GTP which binds Gα and locks it in its active state (50). It has been reported that the Gα subunit of the rice heterotrimeric G protein is fully dissociated from a large (~400 kD) complex in the presence of GTPγS (26). We isolated the plasma membrane fraction from wildtype *Arabidopsis* plants, incubated it with 100 μM GTPγS and fractionated it by BN-PAGE, followed by SDS-PAGE and...
Western blotting with Gα antibody. The fraction of Gα protein in the high molecular weight complex declined from 29% to 20% after incubation with GTPγS, a roughly 30% decrease (Fig. 6A). Given that GTPγS treatment is expected to result in heterotrimer dissociation, this dissociation may not result in extensive release of Gα from the large complex in Arabidopsis.

In view of the known involvement of reactive oxygen species (ROS) in heterotrimeric G protein signaling, we also asked whether H2O2 affects the stability of the Gα-containing high molecular weight complex or the GTP-binding capacity of recombinant Arabidopsis Gα protein. To determine whether H2O2 affects the stability of the complex, we analyzed the effect of H2O2 treatment on the fraction of Gα protein in the high molecular weight membrane-bound complex. We isolated the plasma membrane fraction from wildtype plants and treated it with physiological levels of H2O2 (20 μM) (53) prior to BN-PAGE and SDS-PAGE fractionation. We observed that H2O2 treatment promotes the extensive release of Gα protein from the high molecular weight complex (Fig. 6B). The fraction of complexed Gα protein declined from about 30% to less than 10% after H2O2 treatment (Fig. 6C).

It has been reported that the GTP-binding activity of two mammalian Gα proteins, Gαi and Gαo, are directly regulated by ROS via changes in the redox state of sulfhydryl residues in the protein (44,51). Since the Arabidopsis Gα protein contains potentially regulatory sulfhydryl residues (18), we asked whether H2O2 affects the GTP binding activity of recombinant Gα. We purified recombinant 6×His tagged Gα (Fig. 7A) and first assayed its ability to bind GTP. Recombinant Arabidopsis Gα selectively binds GTP, as determined by the ability of GTP, but not ATP, to compete for binding of GTPγS (Fig. 7B). We then asked whether H2O2 affects the ability of the recombinant Gα protein to bind GTPγS. We observed that incubation of the recombinant protein with 20 μM H2O2 has little effect on its GTP-binding activity, as judged by the binding of GTPγS. Thus 20 μM H2O2 promotes dissociation of Gα from the membrane-bound high molecular mass complex, but does not affect its GTP-binding activity (Fig. 7C).

**DISCUSSION**

It was previously reported that the subunits of the Arabidopsis G protein interact in the plasma membrane of cowpea protoplasts (24). These experiments demonstrated the utility of using transfected constructs of fluorescent fusion proteins to assess the localization and dynamics of plant G protein subunits, a strategy employed extensively in mammalian cell lines and Dictostelium (35,47). In the present study, we detected peripheral fluorescence, consistent with plasma membrane localization, in Arabidopsis wildtype and agb1-2 protoplasts transiently transfected with CaMV 35S promoter-driven cDNAs for the Gα protein carrying a CFP in the second loop of the α-helical domain. We similarly observed peripheral localization of N-terminally fused YFP-Gγ1, regardless of whether this construct was expressed in wildtype or Gβ mutant (agb1-2) protoplasts. These results are consistent with imaging studies performed by Zeng et al. (54) but different from those of Adjobo-Hermanns et al. (24). The latter performed heterologous expression studies of Arabidopsis G-protein subunits in cowpea protoplasts, and observed that co-expression of Gβ was required for plasma membrane-localization of Gγ1.

When protoplasts expressing a similar 35S promoter-driven cDNA encoding an N-terminally fused YFP-Gβ construct were imaged, peripheral fluorescence was not observed. In such protoplasts, the detected fluorescence was cytoplasmic and particulate, and in some cells, in large inclusion bodies possibly representing Gβ aggregates. However, co-expression of a Gγ1 cDNA with the YFP-Gβ cDNA promoted peripheral localization of fluorescence, indicating that Gγ1 suffices to target Gβ to the plasma membrane. This is consistent with the presence of a prenylation sequence at the C terminus of the Gγ1 protein, but not that of the Gβ protein. This observation provides an interesting contrast to the behavior of mammalian G proteins, for which localization of the Gβγ complex to the plasma membrane requires prior association of Gα and Gβγ (55).

We have reported that more than 60% of the
cellular Gα protein in wildtype Arabidopsis leaf tissue is plasma membrane-bound, while less than 40% of the Gβ protein is in the plasma membrane and the rest is in internal membranes, including the ER (17). The difference in subcellular partitioning of Gα and Gβ is likely to be attributable to differential subcellular targeting of Gβ in dimers with Gγ1 and Gγ2. Although both Gγ1 and Gγ2 associate with plasma membranes as a consequence of prenylation, Gγ1 was also detected in internal membranes (12). This observation is consistent with our report that Gβγ has a function separable from that of the heterotrimer in the unfolded protein response (UPR) (17). The Arabidopsis Gγ2 protein, which is S-acetylated as well as a prenylated, localizes to the plasma membrane independently of Gβ and more extensively than Gγ1(12,24). These observations suggest that the two Gβγ dimers of Arabidopsis, Gβγ1 and Gβγ2, have distinct functions in different subcellular compartments. Also consistent with this concept is the observation that Gγ1 (agg1) and Gγ2 (agg2) mutants share different subsets of the several phenotypes reported for Gβ (agg1) mutants (12,54).

We detected FRET between the CFP and YFP moieties of CFP-Gβ and YFP-Gγ1 fusion proteins, but only when the fusions were N-terminal for both proteins. We also tested Gβ and Gγ FRET pairs in which one or both of the fusions were C-terminal (Gβ-CFP and YFP-Gγ1, CFP-Gβ and Gγ1-YFP, Gβ-CFP and Gγ1-YFP). No FRET signal was detected if even one member of the FRET pair was a C-terminal fusion, although both CFP-Gβ and Gβ-CFP fusions were functional by the criterion that they were able to complement the agg1-2 null mutation phenotypically (data not shown). These observations are in agreement with the structure of the Arabidopsis heterotrimeric G protein predicted by molecular modeling, which places the N-terminus of Gβ and the N-terminus of Gγ1 in close proximity (27).

We detected FRET between L-CFP-Gα and YFP-Gγ1, but only when a Gβ cDNA was co-expressed with the Gα and Gγ fusion constructs in Arabidopsis mesophyll protoplasts. This result is different from what was observed in cowpea protoplasts, where FRET was also detected between L-CFP-Gα and YFP-Gβ1 with the co-expression of Gγ1, although they did not test FRET between L-CFP-Gα and YFP-Gγ1 with the co-expression of Gβ (24). Given that different expression systems were used, it is possible that the folding of YFP-Gβ1 or the conformation of the G protein complex differs in Arabidopsis and cowpea protoplasts, resulting in the apparent variation of proximity of G protein subunits in the complex formed. Whatever the source of this difference, the results of both our study and that of Adjobo-Hermanns et al. (24) support the conclusion that Arabidopsis G proteins form a heterotrimeric complex in vivo which can be detected by FRET. Thus, based on our results, we conclude that both Gα and Gγ1 can be localized to the plasma membrane and their close juxtaposition requires the presence of Gβ and therefore the formation of the heterotrimer. Since the protoplasts used were from wildtype plants, this observation suggests that the endogenous levels of the G protein subunits are insufficient to assemble enough of the heterotrimer to generate a detectable FRET signal. We did not detect FRET in the plasma membrane between L-CFP-Gα and YFP-Gβ either with or without co-expression of Gγ1 in Arabidopsis protoplasts. This result differs from those reported in mammalian studies in which FRET can be detected between L-CFP-Gα and YFP-Gβ as well as between L-CFP-Gα and YFP-Gγ1 (35) and may be attributable to slight structural differences between mammalian and Arabidopsis heterotrimeric G proteins (27). Heterotrimer formation and Gβ-dependent interaction of Gα and Gγ1 was further supported by independent biochemical approaches showing that, if Gβ was co-expressed, it was then possible to co-immunoprecipitate YFP-Gγ1 with L-CFP-Gα using Gα antibodies (Fig. 4B).

Roughly 30% of the native Gα protein in the plasma membranes of wildtype Arabidopsis plants was in a large complex of approximately 700 kD and the remainder appeared to be monomeric, judging from its mobility in a native gel (Fig. 5A). By contrast, all of the Gβ detectable in plants stably transformed with a 35S-CFP-Gβ cDNA fusion construct was associated with a large complex of roughly the same size (~700 kD) (Fig. 5B). The large Gα-containing complexes were much less abundant in agg1-2 mutant plants.
lacking the Gβ subunit. Instead, the Gα subunit was present in smaller membrane-bound complexes ranging in size from 140 to 400 kD and as free monomer. This observation suggests that the formation of the large Gα-containing complexes requires the presence of the Gβ subunit, further suggesting that the plasma membrane complexes containing Ga and Gβ are the same. A number of Gα-interacting proteins have been identified, including AtRGS (regulator of G protein signaling) (56), AtGCR1 (G protein coupled receptor) (57), AtGCR2 (58), PLDα (phospholipase D alpha) (59), PRN1 (pirin) (60), THF1 (thylakoid formation) (45), and PD1 (prephenate dehydratase) (61). It remains to be determined whether any of these proteins is a component of the ca. 700 kD complex.

It is well documented that Gα dissociates from the Gβγ complex upon binding of GTP to Gα in mammalian and Dictyostelium G heterotrimeric G proteins, with the possible exception of those containing Gαi (62). However, the evidence about whether plant heterotrimeric G proteins dissociate upon activation is somewhat contradictory. The fact that FRET still occurs between Arabidopsis Gβ and a constitutively active mutant form of the Arabidopsis Gα subunit has been interpreted as indicating that the plant heterotrimer does not dissociate upon activation (24). However, we observed that a significant fraction (~70%) of plasma-membrane localized Arabidopsis Gα is already present in the monomeric form, and that GTPγS promotes partial dissociation of the remaining Gα from the large complex (Fig. 6A). Gel filtration experiments in rice indicate that Gα, Gβ and the two Gγ subunits are in a 400 kD complex and GTPγS treatment results in almost complete dissociation of all three types of subunits from the complex (26). When a constitutively active form of rice Gα is expressed in a Gα-null background, the constitutively active subunit is predominantly found in the monomeric form (28). Because the Arabidopsis Gα exhibits slow GTPase activity, most if not all of the Arabidopsis Gα protein is proposed to be in its active GTP-bound form (54,63). We found that H2O2 promotes dissociation of Arabidopsis Gα from the ca. 700 kD complex (Fig. 6C), but does not affect its ability to bind GTPγS (Fig. 7C). In view of the evidence that Ga mediates oxidative stress signaling (18), this result suggests that reactive oxygen species affect the stability of the complex, but, unlike the situation with mammalian Gαi and Gαo (51), not the activity of Gα itself.

In conclusion, our results affirm that the Arabidopsis G-protein complex behaves similarly to mammalian G protein complexes in that it forms a heterotrimer in vivo. However, our results also reveal distinct aspects of G-protein biochemistry in plants, including a lack of dependence of Gβ and Gγ localization on Gα, an apparently closer interaction of Gα and Gγ than Gα and Gβ, a predominance of the monomeric form of Gα in the plasma membrane, and a lack of apparent regulation of Gα activity by reactive oxygen species. The question of how G protein signaling evolved in plants (64) to encompass these unique attributes will be an interesting topic for future studies.

REFERENCES

and Jones, A. M. (2006) *Molecular biology of the cell* 17(10), 4257-4269
FOOTNOTES

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FIGURE LEGENDS

Figure 1. The predicted structure of the Arabidopsis heterotrimeric G protein and the CFP and YFP fusion constructs used in the present study.
A. The predicted structure of Arabidopsis heterotrimeric G protein was redrawn from Ullah et al. (27). The sites of YFP and CFP insertion or fusion are indicated. B and C. Structure of the CFP and YFP fusion constructs with Arabidopsis G protein subunits used in this study.

Figure 2. Subcellular localization of CFP- and YFP-Arabidopsis G protein fusions.
A. Images of Arabidopsis wildtype mesophyll protoplasts transformed with the indicated constructs:
(1) L-CFP-Gα, (2) YFP-Gγ1, (3) YFP-Gβ, (4) (35S)-YFP-Gβ-(35S)-Gγ1. B. Images of Arabidopsis agb1-2 mutant mesophyll protoplasts transformed with the indicated constructs: (1) L-CFP-Gα, (2) YFP-Gγ1, (3) YFP-Gγ1, (3) YFP-Gβ, (4) (35S)-YFP-Gβ-(35S)-Gγ1.

Figure 3. Interaction of Arabidopsis G-protein subunits in vivo detected by FRET.

A-F: Images of Arabidopsis mesophyll protoplasts transformed with the indicated constructs: A. CFP-Gβ+YFP-Gγ1, B. YFP-Gβ+L-CFP-Gα, C. YFP-Gγ1+L-CFP-Gα, D. L-CFP-Gα+YFP-Gβ+YFP-Gγ1, E. L-CFP-Gα-(35S)-YFP-Gβ-(35S)-Gγ1, F. L-CFP-Gα-(35S)-Gβ-(35S)-YFP-Gγ1. CFP, YFP and FRET labels above the images indicate the individual optical filter conditions. FRET images were obtained by spectral encoding with the LSM510 META software of the series of images collected in the lambda stack acquisition mode from 464 nm to 584 nm. Upon the excitation of CFP with the two photon laser at 820 nm, the image is displayed in green when a FRET signal was detected, while the image is displayed in cyan when a FRET signal was not detected. G: Fluorescence emission spectra from the representative protoplasts shown in Fig. 2A. CFP was excited with a two photon laser at 820 nm and YFP was excited with an Argon laser at 514 nm. H-I: Fluorescence emission spectra from representative protoplasts in A to F upon excitation with the two photon laser at 820 nm. The spectra in A, D and F show an increase in the YFP emission peak, indicative of FRET between CFP-Gβ and YFP-Gγ1 or L-CFP-Gα and YFP-Gγ1. Fluorescence intensity was normalized to 480 nm, which is one of the peaks of the CFP emission spectrum. J: Quantitative analysis of the FRET ratio between tested pairs illustrated in A to F. The FRET ratio is the ratio of yellow emission (532 nm) to cyan emission (479 nm) upon excitation of CFP with the two photon laser at 820 nm. For each of A through F, black diamonds show FRET ratios in 20 individual protoplasts, observed in the FRET detection mode. If more than 1 cell has the same FRET ratio, the representative diamonds can not be distinguished so that less 20 diamonds are apparent in J.

Figure 4. Co-immunoprecipitation of Ga and Gγ1 is dependent on co-expression of Gβ, indicating formation of a heterotrimer.

Protein extracts of protoplasts transformed with A. (35S)-L-CFP-Gα and (35S)-YFP-Gγ1 and B. (35S)-L-CFP-Gα-(35S)-Gβ-(35S)-YFP-Gγ1 were immunoprecipitated with anti-Gα. Lanes 1 of A. and B.: 10% of the total extract (TE); Lanes 2 of A. and B.: the concentrated flow through (20 μl) from the column (FT); Lanes 3 of A. and B.: proteins in the 1st eluate with denaturing buffer (E1); Lanes 4 of A. and B.: proteins in the 2nd eluate with denaturing buffer (E2). Proteins were separated by SDS-PAGE gel, transferred to PVDF membrane, and probed with anti-CFP antibody. Note that in panel B, but not panel A, YFP-Gγ1 is co-immunoprecipitated with Ga (compare lanes E1 in B. vs. A.).

Figure 5. The Arabidopsis Ga protein is associated with a complex of approximately 700 kD.

A. The plasma membrane fractions from Col-0 and gpa1-4 mutant rosette leaves were fractionated by BN-PAGE in the 1st dimension and SDS-PAGE in the 2nd dimension, transferred to PVDF membrane, and probed with anti-Gα antibody. The arrowhead points to the location of Ga. B. Total protein extracts of agb1-2 plants over-expressing either CFP or Gβ-CFP were fractionated by BN-PAGE, transferred to PVDF membrane, and the blot was probed with anti-CFP antibody. The arrowhead points to the location of the Gβ complex. C. The plasma membrane fraction from agb1-2 mutant plants was fractionated by BN-PAGE and SDS-PAGE and the blots were probed with anti-Gα antibody. The sizes of protein mass standards are indicated.

Figure 6. The Arabidopsis Ga protein complex dissociates upon treatment with GTPγS or H2O2.

A and B. The isolated plasma membrane fraction from Col-0 rosette leaves was incubated with buffer containing either A. 100 μM GTPγS or B. 20 μM H2O2, diluted with solubilization buffer containing 20 mM bis-Tris-HCl, pH 7.0, 250 mM e-aminocaproic acid, 2 mM EDTA, 1.0% NP-40, and fractionated by BN-PAGE, followed by SDS-PAGE. The blots were probed with anti-Gα antibody. The sizes of protein mass standards are indicated. C. Quantification of the fraction of Gα in the large complex
in control and treated plasma membrane extracts. This experiment was repeated 3 times and the error bars shown here are SE.

**Figure 7. GTPγS binds to recombinant Arabidopsis Gα protein.**

* A. Purification of 6xHis-tagged Gα was assessed by 10% SDS-PAGE. Lane 1, total lysate of *E. coli* expressing 6xHis-tagged Gα; lane 2, flow through from nickel affinity resin; lane 3, wash, and lane 4, eluate. The 50 kD marker protein is indicated. * B. [35S]GTPγS binding to recombinant 6xHis-tagged Gα protein in the presence of increasing concentrations of unlabelled ATP or GTP (n=6). * C. [35S]GTPγS binding to recombinant 6xHis-tagged Gα in the presence of either 20 µM H₂O₂ or H₂O (control) (n=6). The radioactivity of the [35S]GTPγS added to the reaction mixture was set as maximum (100%). This experiment was repeated 3 times and the error bars shown here are SE.
Figure 1

A

B

35S  L-CFP-\( \alpha \)  NOS
35S  CFP or YFP-\( \beta \)  NOS
35S  YFP-\( \gamma 1 \)  NOS

C

35S  \( \gamma 1 \)  NOS  35S  YFP-\( \beta \)  NOS
35S  \( \beta \)  NOS  35S  YFP-\( \gamma 1 \)  NOS
35S-L-CFP-\( \alpha \)-NOS  35S-\( \gamma 1 \)-NOS  35S-YFP-\( \gamma 1 \)-NOS
35S-L-CFP-\( \alpha \)-NOS  35S-\( \beta \)-NOS  35S-YFP-\( \gamma 1 \)-NOS

Arabidopsis
Figure 2

A

L-CFP-Go  YFP-Go  YFP-Gβ  YFP-Gγ1

B

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Figure 3

A  CFP  YFP  FRET

CFP-Gβ+ YFP-Gγ1

B

YFP-Gβ+ L-CFP-Gα

C

YFP-Gγ1+ L-CFP-Gα

D

L-CFP-Gα+ YFP-Gβ+ YFP-Gγ1

E

L-CFP-Gα- YFP-Gβ- Gγ1

F

L-CFP-Gα- Gβ- YFP-Gγ1

G

Fluorescence intensity
(normalized to 480 nm)

H

Fluorescence intensity
(normalized to 480 nm)

I

Fluorescence intensity
(normalized to 480 nm)

J

YFP emission / CFP emission
Figure 4

A

<table>
<thead>
<tr>
<th>TE</th>
<th>FT</th>
<th>E1</th>
<th>E2</th>
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<tbody>
<tr>
<td>L-CFP-Gα</td>
<td>YFP-Gγ1</td>
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B

<table>
<thead>
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<tr>
<td>L-CFP-Gα</td>
<td>YFP-Gγ1</td>
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76 kD
50 kD
36 kD
29 kD

L-CFP-Gα
YFP-Gγ1
Figure 5

A

BN-PAGE

SDS-PAGE

Ga

Col

gpa1-4

B

BN-PAGE

35S-CFP

35S-Gβ-CFP

Gβ complex

C

BN-PAGE

51 kD

36 kD

51 kD

36 kD
Figure 6

A

B

C

\[ \text{Control} \quad \text{GTP} \gamma S \quad \text{Control} \quad \text{H}_2\text{O}_2 \]

\[ \text{669} \quad 440 \quad 232 \quad 140 \quad 66 \text{ kD} \]

% Ga in HMW complex

\[ \text{Control} \quad \text{GTP} \gamma S \quad \text{Control} \quad \text{H}_2\text{O}_2 \]
Figure 7

A

B

C

50 kD

1 2 3 4

GTPγS bound (% of maximum)

0 25 50 75 100

GTP

ATP

2000 200 20 2 0 (µM)

H2O

H2O2

0 2 5 10 (min)

GTPγS bound (% of maximum)
Characterization of the Arabidopsis heterotrimeric G protein
Shiyu Wang, Sarah M. Assmann and Nina V. Fedoroff

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