Heterotrimeric G-Protein Interactions Are Conserved Despite Regulatory Element Loss in Some Plants1[OPEN]

Nikita Bhatnagar and Sona Pandey2,3
Donald Danforth Plant Science Center, St. Louis, Missouri 63132
ORCID ID: 0000-0001-7832-1347 (N.B.); 0000-0002-5570-3120 (S.P.)

Heterotrimeric G-proteins are key modulators of multiple signaling and development pathways in plants and regulate many agronomic traits, including architecture and grain yield. Regulator of G-protein signaling (RGS) proteins are an integral part of the G-protein networks; however, these are lost in many monocots. To assess if the loss of RGS in specific plants has resulted in altered G-protein networks and the extent to which RGS function is conserved across contrasting monocots, we explored G-protein-dependent developmental pathways in *Brachypodium distachyon* and *Setaria viridis*, representing species without or with a native RGS, respectively. Artificial microRNA-based suppression of Ga in both species resulted in similar phenotypes. Moreover, overexpression of *Setaria italica* RGS in *B. distachyon* resulted in phenotypes similar to the suppression of BdGa. This effect of RGS overexpression depended on its ability to deactivate Ga, as overexpression of a biochemically inactive variant protein resulted in plants indistinguishable from the wild type. Comparative transcriptome analysis of *B. distachyon* plants with suppressed levels of Ga or overexpression of RGS showed significant overlap of differentially regulated genes, corroborating the phenotypic data. These results suggest that despite the loss of RGS in many monocots, the G-protein functional networks are maintained, and Ga proteins have retained their ability to be deactivated by RGS.

The growth and development of living organisms entail proper integration of responses to a multitude of environmental and endogenous cues by several intersecting signaling modules. One such module composed of heterotrimeric G-proteins plays a vital role in all eukaryotic species, from yeast to humans and plants (Gilman, 1987; Cabrera-Vera et al., 2003; Siderovski and Willard, 2005; Stateczny et al., 2016; Pandey, 2019). The core of heterotrimeric G-proteins consists of three dissimilar proteins Ga, Gb, and Gy, which remain in an inactive trimeric conformation when the Ga is GDP bound. Signal-dependent exchange of GTP for GDP on Ga results in the dissociation of the trimeric complex. The resulting GTP-Ga and the freed GbGy dimer both can transduce the signal by interacting with downstream effectors (McCudden et al., 2005; Oldham and Hamm, 2008; Pandey, 2019). The inherent GTPase activity of Ga causes hydrolysis of bound GTP to regenerate Ga-GDP, which associates with the GbGy dimer to reconstitute the inactive trimeric complex, which is available for the next round of activation (Siderovski and Willard, 2005; Pandey, 2017). The activation and deactivation steps of this switch-like mechanism need to be synchronized for effective signal transduction. However, the inherent GTPase activity of Ga is significantly slower than the GDP-to-GTP exchange rates (Siderovski and Willard, 2005; Pandey, 2017). Therefore, the roles of proteins, such as the regulator of G-protein signaling (RGS), which act as GTPase-activity accelerating proteins (GAPs), are central in maintaining the optimal output of signal transduction (Siderovski and Willard, 2005).

G-proteins modulate a variety of critical growth and development processes in plants, encompassing responses to both environmental and endogenous signals (Urano and Jones, 2014; Pandey, 2019; Wang et al., 2019; Zhong et al., 2019). While the definition and interaction specificities of the three core subunits of G-proteins are conserved across eukaryotes, the broader networks in which they are placed seem to differ, even among different plant lineages. For example, Arabidopsis (*Arabidopsis thaliana*) and maize (*Zea mays*), representing model dicot and monocot species, respectively, possess one canonical Ga and one GbGy protein each, with considerable sequence similarities (Hackenberg et al., 2017; Gao et al., 2019; Wu et al., 2020). However, the loss of function of these genes has distinct effects in each species. The Arabidopsis Ga knockout mutant (*gpa1*) exhibits several subtle developmental and stress-responsive phenotypes but maintains its overall architecture, growth rate, and fertility (Perfus-Barbeoch et al., 2004;
Choudhury et al., 2020). In contrast, the maize Ga knock-out mutant (ct2) is dwarf, with an overall change in plant architecture and exhibits delayed growth and reduced yield (Bommert et al., 2013; Wu et al., 2018). Similarly, knockout of the sole Gβ gene (AGB1) in Arabidopsis results in altered development and stress-related phenotypes, but the plants grow to complete their life cycle and produce viable seeds (Roy Choudhury et al., 2020). In contrast, the complete loss of the Gβ gene function in maize or rice (Oryza sativa) results in seedling lethality (Utsunomiya et al., 2011, 2012; Gao et al., 2019; Wu et al., 2020).

In the context of G-protein signaling, rice and maize differ from eudicots by the absence of an RGS gene in their genome (Hackenberg et al., 2017). In fact, RGS has been lost from many monocot species, without an apparent phylogenetic pattern (Hackenberg et al., 2017). Even within the grass family, species differ in the presence or absence of an RGS gene. However, when present, RGS does play important roles in the regulation of G-protein signaling in plants. Consistent with its role as a deactivator of Ga, reduced expression of RGS results in phenotypes similar to those from overexpression of Ga and vice versa (Chen et al., 2003; Chen and Jones, 2004; Fan et al., 2008; Roy Choudhury and Pandey, 2015, 2017). Moreover, precise regulation of Ga activity is important for its physiological roles, as has been shown by expressing proteins with altered biochemical characteristics in Arabidopsis, maize, and rice (Oki et al., 2005; Zhang et al., 2009; Wu et al., 2018). In maize, complementation of ct2 mutants with a native versus a constitutively active version of CT2 (CT2CA) results in distinct phenotypes (Wu et al., 2018). Similar results were observed in rice (Oki et al., 2005) and Arabidopsis (Ullah et al., 2003; Urano et al., 2016b). These observations confirm that accurate fine-tuning of the activation/deactivation rates of the G-protein cycle is crucial for their roles in affecting plant development and productivity.

This apparent need for RGS in most organisms and its absence in many monocots raises the question of to what extent G-protein function, particularly that of Ga, is affected by the presence or absence of RGS. One possibility is that in plants lacking RGS, the entire G-protein signaling apparatus is remodeled in a way that Ga’s function has altered to compensate for this change. An alternative is that the signaling network is robust to loss of RGS such that the downstream network is minimally disrupted.

While rice and maize differ from Arabidopsis in the absence of RGS, the grasses and Brassicaceae are separated by more than 100 million years of evolution and differ in many other respects. To undertake a comparison with better phylogenetic control, we instead focused on the grass Setaria viridis, which has a native RGS gene, and compared it with Brachypodium distachyon, which does not (Hackenberg et al., 2017). We hypothesized that if loss of RGS function leads to compensatory changes or rewiring of the Ga networks, then mutations in Ga should have different phenotypes in these two plant types. In addition, overexpressing RGS in S. viridis should create phenotypes opposite to those of the Ga mutations, but overexpressing RGS in B. distachyon should have either no effect or an effect different from the S. viridis overexpression plants.

Accordingly, we suppressed Ga in B. distachyon (amiR-BdGa) and S. viridis (amiR-SvGa) with artificial microRNA technology and performed detailed phenotypic and molecular characterization of the transgenic plants to compare the G-protein phenotypes in the context of the presence/absence of a native RGS gene. We also generated gain-of-function transgenic plants expressing the RGS gene from Setaria italica, which is the domesticated form of S. viridis (Hu et al., 2018), in B. distachyon (Bd-SiRGS-OE) and in S. viridis (Sv-SiRGS-OE) to elucidate its effect on Ga-dependent development and molecular phenotype.

Our results are surprising. As shown here, despite the lack of a native RGS in B. distachyon, suppression of Ga results in similar phenotypes in B. distachyon and S. viridis. Moreover, the B. distachyon plants over-expressing the SiRGS transgene exhibited phenotypes similar to amiR-BdGa plants, suggesting that the functional modules involving Ga and RGS are intact in plants, even when the native RGS is lost. RNA sequencing (RNA-seq) transcriptomic analysis showed extensive overlap between transcripts differentially expressed in amiR-BdGa and Bd-SiRGS-OE plants, which include many major development- and hormone-related genes. Overall, our results show that the functional interaction and the intracellular networks of Ga:RGS proteins remain conserved in plant species despite the loss of a native RGS gene.

**RESULTS**

**Suppression of Ga in B. distachyon and S. viridis Results in Similar Developmental Phenotypes**

To study whether Ga-affected development in grasses depends on the presence of a native RGS protein, we generated transgenic plants with reduced levels of Ga in B. distachyon and S. viridis as contrasting species models for the absence and presence of an RGS in their genome, respectively. S. viridis has two Ga genes (Sevir.9G524500 and Sevir.3G399300), which show ~96% similarity in their amino acid sequences (Supplemental Fig. S1). The artificial microRNA sequences were selected to target both these genes (Supplemental Fig. S2). Appreciable reduction (<70%) in transcript level was observed in both amiR-BdGa and amiR-SvGa plants compared with their respective controls (Supplemental Fig. S3, A and B).

In both B. distachyon and S. viridis, plants expressing reduced levels of Ga exhibited altered architecture. The amiR-BdGa plants were shorter and exhibited delayed growth throughout their life, based on plant height from the 2-week-old to the 6-week-old stage (Table 1; Supplemental Fig. S4). The plants maintained a dwarf
Table 1. Morphological characteristics of amiR-BdGa, amiR-SvGa, Bd-SiRGS-OE, Bd-SiRGS\(^{E319A}\)-OE, and SiRGS-OE

| Sample                  | Plant Height | Leaf No. | Leaf Length | Tiller No. | Internode Length | Seed Size | Root Length |
|-------------------------|--------------|----------|-------------|------------|------------------|-----------|-------------|
| Bd wild type            | 7.54         | 4        | 5.46        | 2.6        | 1.76             | 6.62      | 13.5        |
| amiR-BdGa-L1            | 4.88**       | 3**      | 3.66**      | 2**        | 0.43**           | 4.42**    | 10.42**     |
| amiR-BdGa-L2            | 4.93**       | 2**      | 3.66**      | 1**        | 0.46**           | 4.32**    | 10.33**     |
| Bd-SiRGS-OE-L1         | 5.12**       | 2.6**    | 3.7**       | 1.8**      | 0.76**           | 5.49**    | 10.2**      |
| Bd-SiRGS-OE-L2         | 5.08**       | 2.8**    | 3.56**      | 1.8**      | 0.51**           | 5.84**    | 11.2**      |
| Bd-SiRGS\(^{E319A}\)-OE-L1 | 6.76     | 3.2      | 5.24        | 2**        | 0.68**           | 7         | 15.5        |
| Bd-SiRGS\(^{E319A}\)-OE-L2 | 7.68       | 3.8      | 4.92        | 2**        | 1.14             | 5.81*     | 15.24       |
| Sv empty vector        | 7.82         | 6.4      | 7.91        | 2.6        | 1.26             | 1.71      | 17.54       |
| amiR-SvGa-L1           | 5.86**       | 3.8**    | 6.17**      | 1.2**      | 0.94**           | 1.57**    | 14.6**      |
| amiR-SvGa-L2           | 4.2**        | 3.6**    | 4.14**      | 1**        | 0.56**           | 1.52**    | 10.8**      |
| Si empty vector        | 6.26         | 4.6      | 8.22        | 1.8        | 1.14             | 1.58      | 15          |
| SiRGS-OE-L1            | 5.8**        | 3.4      | 6.39**      | 1**        | 1**              | 1.54**    | 11.96**     |
| SiRGS-OE-L2            | 5.7**        | 3.2      | 6.89**      | 1**        | 0.93**           | 1.56      | 13.6**      |

stature at full maturity (Fig. 1, A and B). The amiR-BdGa plants also had fewer and smaller leaves (Fig. 1C) and tillers compared with the control plants. Tiller development in these plants was delayed, and the first tiller emerged −6 to 7 d later than in control plants. The overall reduction in amiR-BdGa plant height was primarily due to the reduction in internode length, as shown for the second internode (Fig. 1D; Table 1). Additionally, almost all tissues showed reduced longitudinal expansion (e.g. the plants also had shorter and broader leaves and seeds). Reduced length and fewer tillers made the amiR-BdGa plants appear less bushy at maturity. Mature seeds of amiR-BdGa plants were not only smaller and wider but also had abundant, large trichomes on the lemma not seen in the control plants (Fig. 1E; Table 1). Along with the size difference, the amiR-BdGa florets had a whitish palea compared with the brown palea of the seeds from the control plants (Fig. 1E).

Suppression of Ga in S. viridis (amiR-SvGa) resulted in similar phenotypes, with reduced height (Fig. 1, F and G), reduced longitudinal expansion of most organs, including leaves and seeds, fewer panicles, slower growth, delayed tiller emergence, and dwarf stature at maturity compared with the control plants (Fig. 1, H and I; Table 1).

Altered Architecture of Plants Expressing Reduced Levels of Ga Is Primarily due to Reduced Longitudinal Cell Expansion

The overall dwarf stature of the amiR-Ga plants throughout development suggested an effect on cell expansion. To examine this at the cellular level, we analyzed cell size in the epidermis of 2-week-old leaves. The epidermal cells of the Ga-suppression lines in both B. distachyon and S. viridis were shorter in length and wider as compared with their respective controls. This asymmetric expansion of cells resulted in a significant difference in the ratio of length to width as compared with the control plants. Along with cell size, the cell number on the leaf surface was also significantly reduced in both amiR-BdGa and amiR-SvGa plants (Fig. 2). The overall shorter stature of amiR-BdGa and amiR-SvGa plants, their slower growth, and altered developmental phenotypes are likely results of the altered cellular expansion as well as changes in cell division, similar to what has been reported for Arabidopsis and Camelina sativa G-protein mutants (Ullah et al., 2001; Oki et al., 2005; Roy Choudhury et al., 2020). These data demonstrate that the Ga-dependent developmental phenotypes in grasses with or without a native RGS are similar and the overall wiring of G-protein-dependent networks that control development is not altered in these two contrasting plant types.

Overexpression of a Nonnative RGS in B. distachyon Phenocopies amiR-BdGa Plants

As another approach to assess whether the absence of RGS in specific monocot species has altered the inherent G-protein functional networks, we generated a true gain-of-function RGS plant by overexpressing the S. italica gene in B. distachyon, which has no RGS of its own (Hackenberg et al., 2017). To compare the effect of native versus nonnative RGS overexpression, we also generated S. viridis plants overexpressing its native RGS gene. Furthermore, to ascertain that the in planta effects of RGS are truly due to its effect on the activity of Ga, we generated B. distachyon plants overexpressing a point mutant version of the S. italica RGS protein (RGS\(^{E319A}\)) that exhibits no GAP activity (Oki et al., 2005; Hackenberg et al., 2017). Overexpression of each transgene was confirmed by reverse transcription quantitative PCR (RT-qPCR), where ~80-fold increased expression was observed in both native and mutant RGS as compared with controls (Supplemental Fig. S3C).

Similar to what was observed for the amiR-BdGa and amiR-SvGa plants, the Bd-SiRGS-OE and Sv-SiRGS-OE
plants exhibited several developmental phenotypes different from the control plants. The RGS-overexpressing plants showed delayed growth, shorter stature, fewer tillers, and shorter leaves throughout development. Internode lengths were considerably reduced, and the seeds also showed phenotypes similar to what was seen for the amiR-BdGα and amiR-SvGα plants (Fig. 3). At maturity, the RGS overexpression plants maintained a dwarf stature. Importantly, plants overexpressing the point mutant version of the RGS protein (RGS3E319A) did not
exhibit any of these traits and were indistinguishable from the control plants (Fig. 3).

The results of suppression of Ga or overexpression of RGS levels were similar at the cellular level as well. As observed with amiR-BdGa plants, leaf epidermal cell length in RGS overexpression plants was significantly reduced and the cell width increased, altering the overall cell length-to-width ratio. Along with the size, the number of cells was also reduced significantly, similar to the amiR-BdGa plants (Fig. 4).

Collectively, these data confirm that the regulation of the classical G-protein activity influences a range of plant growth and developmental phenotypes and that the signaling networks controlled by Ga/RGS proteins are conserved, even in plants that have lost the native RGS. The Ga protein of these plants is able to functionally interact with and be affected by the nonnative RGS protein.

Suppression of Ga or Overexpression of RGS Results in Overlapping Transcript Level Changes

Phenotypic similarities between the Bd-SiRGS-OE and amiR-BdGa plants led us to explore the extent to which transcriptional networks are shared between these plants, i.e. are there gene expression networks that are similarly affected by overexpression of a nonnative RGS gene and loss of Ga in B. distachyon? As the developmental phenotypes were most obvious at 2 weeks after germination, we chose 14-d-old whole seedlings for this analysis. Suppression of Ga and overexpression of RGS resulted in 8,103 and 7,022 differentially expressed genes (DEGs), respectively, compared with the wild-type plants (Supplemental Table S1). Over 60% of these DEGs were shared between amiR-BdGa and Bd-SiRGS-OE plants (Fig. 5A; Supplemental Table S1). Within these overlapping DEGs, a large majority (~90%) showed similar changes, with 2,905 and 1,025 transcripts showing higher and lower expression, respectively, in both sets of transgenic plants compared with the control plants (Fig. 5B; Supplemental Table S1). Gene enrichment analysis of common up-regulated genes identified integral component of membranes, oxidation-reduction processes, metabolic pathways, and cell periphery as some of the most enriched categories. The down-regulated genes showed significant enrichment in integral component of membrane followed by several nucleotide-binding categories and kinase activity groups (Supplemental Fig. S5). Overall, these analyses suggest that the overexpression of a nonnative RGS gene in B. distachyon or the suppression of a native Ga gene affects similar transcriptional networks.

To gain further insight into the types of transcripts affected by Ga suppression or RGS overexpression, we queried the publicly available B. distachyon eFP browser (http://bar.utoronto.ca/efp_brachypodium/cgi-bin/efpWeb.cgi) for tissue-specific expression of overlapping DEGs from our data set. A total of 2,314 up-regulated genes showed significantly enriched expression in shoot-specific samples, with few gene clusters identified in internode, leaf, and coleoptiles (Fig. 5C; Supplemental Table S2). A similar trend was observed in the 701 down-regulated genes. The specific expression of DEGs identified in our data set in multiple developing tissues (Fig. 5D; Supplemental Table S2) relates to the altered vegetative development of these plants.

Identification of tissue-specific expression clusters led us to further examine these data using Weighted
Correlation Network Analysis (WGCNA), which generated 11 clusters for up-regulated genes (Supplemental Fig. S6; Supplemental Table S3) and 10 clusters for down-regulated genes (Supplemental Fig. S7; Supplemental Table S3). Three tissue-specific clusters corresponding to internode (133 genes), shoot (1,162 genes), and leaf (177 genes) in the up-regulated DEGs (Fig. 6A) and three tissue-specific clusters in the down-regulated DEGs (i.e. coleoptile [52 genes], shoot [208 genes], and leaf [72 genes; Fig. 6B]) were chosen for gene enrichment analysis using ShinyGO.v4. Each cluster had genes with well-established roles in controlling specific developmental phenotypes in other grasses such as maize or rice. For example, the internode cluster showed enrichment of integral component of membranes followed by transporter activity (Supplemental Fig. S8). This cluster contains Bradi1g301610, which is homologous to maize thick tassel dwarf1 (TD1) and Arabidopsis CLAVATA1. Maize Ga functions via the CLAVATA signaling pathway to control meristem development (Bommert et al., 2005). Similarly, the up-regulated shoot-specific cluster showed enrichment of cell wall biogenesis as the most enriched category (Supplemental Fig. S9). This cluster contains homologs of rice Expansin-A13 (Bradi5g19340) that has a role in cell expansion and hormonal responses (Lee et al., 2001). A few PMR5-N-terminal domain genes were also listed in the shoot cluster that play important roles in the maintenance of cell wall composition (Chiniquy et al., 2019). The up-regulated leaf-specific cluster was enriched in chloroplast and plastids (Supplemental Fig. S10). The down-regulated gene clusters showed significant enrichment of several classes of protein kinases, including receptor-like kinases and wall-associated kinases (Supplemental Figs. S11–S13).

To identify the protein interaction network of BdGa, the genes present in the above-mentioned six tissue-specific clusters were screened using the STRING database (Supplemental Figs. S14 and S15; Supplemental Table S4). From the entire network, 12 proteins were identified as direct interactors of BdGa, along with some of its established interaction partners: BdGb (the Gb protein, which is its cognate signaling partner), BdTD1 (homolog of Arabidopsis CLAVATA1), and RACK1 (reported to modulate gene expression of AtGPA1 and AtAGB1 in Arabidopsis; Fig. 6C; Supplemental Table S4; Guo et al., 2009). Additional proteins identified in this primary network have not been characterized, to date, in any plant system, but given their similar scores as bona fide interactors, these are expected to play an important role in Ga-mediated signaling.

Due to the known role of G-proteins in regulating hormone signaling pathways and the altered developmental phenotypes of the mutants, we explored the comparative transcript levels of corresponding genes in amiBdGa and Bd-SiRGS-OE plants. Expression levels of the core abscisic acid signaling component homologs, such as the pyrabactin resistant1 (PYR1)/PYR1-like (PYL) receptors, protein

Figure 4. Alteration in cell size and expansion due to overexpression of native and mutant versions of SiRGS in B. distachyon. A, Epidermal peels of 2-week-old leaves of Bd-SiRGS-OE and Bd-SiRGS<sup>E319A</sup>-OE plants as compared with the peels from control plants. One representative transgenic line of Bd-SiRGS-OE and Bd-SiRGS<sup>E319A</sup>-OE is shown. Yellow outlines mark the cell size boundaries in control and transgenic plants. Bars = 100 μm. B, Quantification of cell length-to-width ratio of Bd-SiRGS-OE and Bd-SiRGS<sup>E319A</sup>-OE epidermal cells as compared with control cells (Con). All values are means with ±se represented by error bars (n = 50). C, Quantification of cell number in Bd-SiRGS-OE and Bd-SiRGS<sup>E319A</sup>-OE epidermal cells as compared with control plants. All values are means with ±se represented by error bars (n = 30). Asterisks indicate significant differences using Student’s t test (*P < 0.05). L1 and L2, Transgenic lines.
phosphatase2C (PP2C) family members, kinases of the SNF1-related protein kinase2 (SnRK2) family, and a number of bZIP transcription factors (Pri-Tal et al., 2017), were significantly altered in both sets of transgenic plants compared with the control plants (Fig. 7). Similarly, transcript levels of the key homologs of gibberellic acid (GA) signaling pathway genes (Niu et al., 2019), such as GID1 (GA-insensitive dwarf1), SLY2 (Sleepy2), DELLA, SCR (Scarecrow), SCL (SCR-like), and SHR (Short root), differed from the control plants similarly in both sets of transgenic plants. Similar patterns of expression were observed for transcripts of known homologs of auxin (Shirley et al., 2018; Yu et al., 2018). Similarly, transcripts coding for proteins such as the homologs of proteins involved in cell expansion, cell size regulation, and key developmental processes, such as expansins, COBRA, and TCP transcription factors, were also significantly altered in both Bd-SiRGS-OE and amiR-BdGa plants (Fig. 8). We independently confirmed the expression levels of different transcripts by RT-qPCR analysis. In total, 12 genes were selected: BdSLC3 (Scarecrow-like3), BdTCP5/7 (TCP transcription factor5/17), CLAVATA1 precursor, BdEXP4/9/16 (Expansin4/9/16), BdGID1L2 (GA receptor), BdCOBL4 (Cobra-like4), BdBZR1 (Brassinazole-resistant1), BdSHR (Short-root), BdGPCR (G-protein coupled receptor), BdLectin-like RLK7, BdLectin-like RLK, and BdReceptor protein kinase, based on their known or predicted roles in plant growth and development. The selected genes showed similar increased or decreased transcript abundance patterns as observed in RNA-seq analysis (Fig. 9). Strikingly similar patterns of these transcript changes, either due to the suppression of Ga or higher expression of RGS, suggest their involvement in classical G-protein signaling pathways. These data also confirm that the RGS-dependent inactivation mechanisms of Ga proteins are maintained, even at the level of global transcriptional regulation, despite the absence of a native RGS gene in specific grasses.

DISCUSSION

Despite the well-established functional roles of RGS and the existence of its highly networked interaction with other proteins of the G-protein complex, the genomes of many monocot species do not possess a gene encoding a canonical RGS protein. Our extensive
evolutionary analysis has confirmed that the gene was indeed lost in the monocot lineage multiple times (Hackenberg et al., 2017). This observation has led to several interesting questions, for example: is the G-protein cycle regulated differently in plants that possess RGS proteins versus those that do not? Do RGS proteins have a role in the regulation of the G-protein cycle in plants that have lost it? And what is, if any, the significance of the regulation of G-protein activity in plants?

The nearly identical developmental differences observed in both amiR-BdGa and Bd-SiRGS-OE plants (Fig. 1; Table 1). In fact, at the cellular level, suppression of Ga in both B. distachyon and S. viridis led to the alteration of longitudinal cell expansion (Fig. 2), which has also been reported for G-protein mutants in dicots (Ullah et al., 2001; Chen et al., 2006; Roy Choudhury et al., 2019). Additionally, the overall similar phenotypes of amiR-BdGa and amiR-SvGa clarify that the relatively severe phenotypes of monocot plants with suppressed Ga levels compared with those of the corresponding dicot mutants are not due to the absence of RGS proteins in specific monocot species.

The overexpression of RGS in both B. distachyon and S. viridis also allowed us to test the extent to
which RGS protein is functional in monocot species, i.e. does it have a biological role or is it an evolutionary leftover? We envisioned three possible scenarios. One, the overexpression of RGS has no effect on G-protein-dependent phenotypes in either B. distachyon or S. viridis, suggesting that the Ga protein in monocots has evolved to function independently of an RGS protein and the gene present in specific plants has likely lost its function. Alternatively, overexpression of RGS leads to G-protein-dependent phenotypes in S. viridis (native species) but not in B. distachyon. This would suggest that some monocot Gαs, including those of B. distachyon, have lost their functional interaction with the RGS proteins in planta. Finally, a more likely possibility, also supported by our extant in vitro data (Hackenberg et al., 2017), is that overexpression of RGS has a similar effect on both B. distachyon and S. viridis. This would suggest that RGS proteins do act as GAPs in monocots, or at least in grasses, and that the functional interaction between Ga:RGS is conserved even in plants that do not possess the native protein. Our data support this final possibility. Overexpression of RGS in both native and nonnative species resulted in similar phenotypes, also seen by the suppression of Ga genes (Figs. 3 and 4). Because the overexpression of a variant protein version with no GAP activity (RGS$^{E319A}$) did not show any effect, our data also confirm that the developmental phenotypes seen by the suppression of Ga or overexpression of RGS are indeed linked to classical G-protein activity in plants. This is especially important because under specific conditions, G-proteins have also been reported to regulate certain plant phenotypes independent of their classical activity (Maruta et al., 2019; Roy Choudhury et al., 2019).

The substantial overlap between the transcript changes due to the suppression of Ga or overexpression of RGS corroborates the phenotypic data. In fact, the majority of genes known to be involved in hormone signaling pathways or plant development show similar patterns of differential expression in transgenic plants when compared with controls (Figs. 6 and 9). Detailed analysis of DEGs also confirms that the G-protein cycle in plants is involved in the regulation of major plant hormone signaling pathways, similar to what has been reported for dicots but remained debated in certain monocots (Fig. 7; Supplemental Table S5). Inferring the interaction network of the Ga protein identified its cognate interactors as well as many other proteins with yet unknown functions (Supplemental Figs. S14 and S15). Elucidating the
The roles of these proteins is expected to add significantly to our current knowledge of G-protein signaling networks.

The data presented here confirm that reduction of Ga level, either genetically (by amiR-mediated suppression) or biochemically (by overexpressing RGS), results in several growth- and development-related phenotypes. However, plants without a native RGS protein do not seem to have any obvious disadvantages, thus presenting a perplexing scenario: is the regulation of the GTPase activity of the Ga protein significant in the context of overall plant growth and development? The extra-large Ga proteins of plants, which function with the core trimeric proteins, do not seem to require an RGS protein and seem to display very little, if any, GTPase activity (Urano et al., 2016a). The answer to this question comes from studies in maize, where the ct2 mutants were complemented with a constitutively active version of the Ga protein. Mutants expressing CT2CA showed phenotypes distinct from those expressing the native CT2 gene, confirming that altering the GTPase activity has major consequences and thus must be regulated (Bommert et al., 2013). Similar results have been seen in Arabidopsis and rice, where the phenotypes of plants expressing a GTPase activity-deficient version of the protein were different from those expressing the native protein (Oki et al., 2005; Ferrero-Serrano and Assmann, 2016). In maize, the plants expressing CT2CA show improved yield due to higher seed number per plant, suggesting that the precise regulation of the G-protein cycle is agronomically relevant. In fact, many of the phenotypes of monocot plants lacking the Ga gene, especially dwarfism, were deemed highly desirable for breeding useful traits but remained underutilized due to the associated lower yield and significant fasciation of the ears in the mutants (Bommert et al., 2013). The maize ct2 mutants expressing CT2CA remain dwarf but show considerably reduced fasciation, erect leaves, and higher yield, making them an ideal breeding target (Wu et al., 2018).

How or why the loss of RGS proteins in specific plant lineages is inconsequential remains an open question at this point. We speculate that possibly there are yet unidentified proteins that can biochemically or functionally complement for a canonical RGS in plants.
Alternatively, there may exist parallel networks that are activated in plants without RGS to compensate for its loss. There is already evidence for the role of proteins such as phospholipases in deactivating the Gₐ in dicots (Roy Choudhury and Pandey, 2016; Roy Choudhury et al., 2019). Identification of additional plant-specific G-protein components or regulatory mechanisms will certainly help answer some of these questions.

**MATERIALS AND METHODS**

**Plant Growth Conditions and Phenotypic Analysis**

Wild-type and transgenic Brachypodium distachyon and Setaria viridis plants were grown from mature seeds collected under identical growth conditions (for each plant species). Seeds were directly planted into Berger 7% to 35% mix soil and kept at 4°C for 2 d for stratification. B. distachyon plants were grown in growth chambers with 24°C (day)/18°C (night) temperature, 20 h of light, and 50% relative humidity conditions. S. viridis plants were grown in growth chambers with 31°C (day)/22°C (night) temperature, 12 h of light, and 50% relative humidity conditions. The light and temperature conditions were maintained throughout the life cycle of the plants. For early to late growth phases and morphological phenotypic analysis of B. distachyon transgenic plants, the Phenovation CropReporter (www.phenovation.com/cropreporter) system was used. For early development analysis, 2-week-old seedlings were carefully removed from the soil without damaging the roots. Number of tillers, leaf number, leaf length, and root length were measured manually using a ruler. Additional aboveground phenotype data were compiled from the image data of the Phenovation CropReporter. Length of the second internode was measured from 4-week-old plants. A minimum of 15 plants per genotype were used for whole plant-based phenotypic measurements in each experiment. At least three independent replicates were performed for each experiment (45–50 plants).
Generation of Transgenic Plants

To generate B. distachyon plants with suppressed levels of Ga, the complete coding sequence region of BdGa (Bradi2g60330) was screened for efficient silencing regions using the P-SAMS amiRNA Designer tool (http://p-sams.carringtonlab.org/) as described (Carbonell et al., 2015). The selected regions were cloned into pMDC28B-OsMIR939a-B/c (Addgene plasmid 51776) to generate OsMIR939a-B/c/BdGa (Supplemental Fig. S2A). For the generation of B. distachyon plants overexpressing the native or mutant Setaria italica SiRGS gene, the full-length coding sequence of SiRGS (Seita.2G153100) and the mutant version of the protein (SiRGS<sub>536S</sub>) that lacks GTPase activity (Hackenberg et al., 2017) were cloned into the pMDC32 vector. Mutant SiRGS<sub>536S</sub> was generated using site-directed mutagenesis (Supplemental Fig. S2B). Constructs were transformed into Agrobacterium tumefaciens strain GV3101. Plant transformations were performed using the embryogenic calli from B. distachyon 21-3 plants using established protocols (Vogel and Hill, 2008). Confirmed transformants were screened and propagated until the T4 generation and used for phenotypic assays. Seeds from two independently transformed lines of the T4 generation, named amiR-BdGa-L1 and amiR-BdGa-L2 for BdGa suppression; Bd-SiRGS-OE-L1 and Bd-SiRGS-OE-L2 for SiRGS overexpression; and Bd-SiRGS<sub>536S</sub>-OE-L1 and Bd-SiRGS<sub>536S</sub>-OE-L2 for SiRGS<sub>536S</sub> overexpression, together with control seeds grown and collected under identical conditions, were used for all phenotypic analysis. Transgenic S. viridis plants were transformed and propagated until the T4 generation and used for transcript analysis using RT-qPCR. Homozygous plants of the T3 generation were used for phenotypic characterizations.

Cell Shape and Size Analysis

To quantify cell number, cell size, and overall organization, the second leaf of B. distachyon and the third leaf of S. viridis were covered with dentinal impression (hydrophilic vinyl polysiloxane, Cinch; Parkell). The resin was removed after it set, and the tissue impression was painted with transparent nail polish. The nail polish was peeled off, placed onto glass microscope slides, and imaged with a microscope (Leica DM 750). Fiji was used to quantify cell number and size. A minimum of six biological replicates per genotype (six leaves from six individual plants) were measured, and the experiment was repeated five times. The data presented are averaged from all experiments. Statistical significance was calculated using Student’s t test, and P < 0.05 was considered significant.

Gene Expression, RNA-Seq Analysis, and RT-qPCR

Total RNA was extracted from 2-week-old seedlings using the RNeasy Mini Kit (Qiagen). The first-strand cDNA was synthesized from total RNA using a SuperScript III cDNA synthesis kit (Invitrogen) after DNase I (Ambion) treatment as per the manufacturer’s instructions. Transcript levels of specific genes in transgenic plants were quantified using RT-qPCR with primers specific for BdGa and SiRGS genes as per previous protocols (Roy Choudhury et al., 2019). To confirm the transcript abundance seen in RNA-seq analysis, RT-qPCR analysis was performed on a selected set of genes. RNA was isolated from 2-week-old seedlings using TRIzol reagent (Thermo Fisher) and treated with DNase I. cDNA was synthesized using a SuperScript III cDNA synthesis kit. Gene-specific primers (Supplemental Table S6) were used to quantify the transcript levels. BdCAPDH and BdUb16 genes were used as controls, and fold changes were calculated by geometric averaging of the two control genes (Vandesompele et al., 2002). Three independent biological replicates of the experiment were performed, with three technical replicates per experiment, and data were averaged.

For RNA-seq analysis, RNA was isolated from the wild type and two independent transgenic lines each of amiR-BdGa and SiRGS-OE B. distachyon seedlings. RNA isolation and follow-up analysis were performed in triplicate, using three independent sets of plants of each genotype. RNA-seq library preparation, sequencing, and initial quality-control analysis were performed by Novogene. For quality control, the FastQC software was used (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Transcripts per million abundance was estimated by pseudoaligning the reads with that of the k-mer index available from the reference genome of B. distachyon from Phytozone (https://phytozone.jgi.doe.gov/pz/portal.htm?info=alas–Org_Bdistachyon) using Kallisto (https://pachterlab.github.io/kallisto/). DEGs were sorted using the Sleuth program (https://pachterlab.github.io/sleuth/) with likelihood ratio cutoff of false discovery rate < 0.05. The log fold change was calculated from the transcripts per million values derived for the wild-type and transgenic plants. DEGs were selected by both the false discovery rate cutoff and log fold change > 2 cutoff. Tissue expression analysis of the DEGs was performed using the publicly available data set on B. distachyon eFP browser (http://bar.utoronto.ca/efp/B.distachyon/cgi-bin/efpWeb.cgi). Expression values of young tissues of roots, internode, shoots, coleoptile, and leaf were selected with development age from 3 to 27 d after germination for further analysis. The expression correlation network was determined using WGCNA in R (Langfelder and Horvath, 2008). Three clusters of both up- and down-regulated DEGs were selected for further gene enrichment analysis using ShinyGO v0.4 (http://bioinformatics.sdsstate.edu/go41/). The protein-protein interaction network was determined using the STRING database for B. distachyon (https://string-db.org/cgi/input.pl?sessionId=60&dCdMnEN&input_page_show_search-on). Additional pathway-specific B. distachyon homologous genes were retrieved from Phytozone (https://phytozone.jgi.doe.gov/pz/portal.htm?info=alas–Org_Bdistachyon).

Accession Numbers

The sequences of genes used in the study are available at Phytozone (https://phytozone.jgi.doe.gov/pz/portal.htm?info=alas–Org_Bdistachyon) with the following accession numbers: Bradi2g60330 (BdGa), Sevir.9G524500 (SiRGS), Seira.3G399300 (SiRGS2), and Seita.2G153100 (SiRGS5). The raw and processed files for RNA-seq analysis used in this study are submitted at the National Center for Biotechnology Information Gene Expression Omnibus repository with accession number GSE153188.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Coding sequence alignment of full-length Ga genes of Arabidopsis, S. viridis, and B. distachyon.

Supplemental Figure S2. Construct design for the generation of transgenic plants.

Supplemental Figure S3. Relative transcript levels of Ga and RGS in B. distachyon and S. viridis transgenic plants.

Supplemental Figure S4. Developmental phenotypes of amiR-BdGa and Bd-SiRGS-OE compared with control plants.

Supplemental Figure S5. Gene enrichment analysis of DEGs common between amiR-BdGa and Bd-SiRGS-OE plants.

Supplemental Figure S6. Correlation network analysis using WGCNA of common up-regulated genes between amiR-BdGa and Bd-SiRGS-OE.

Supplemental Figure S7. Correlation network analysis using WGCNA of common down-regulated genes between amiR-BdGa and Bd-SiRGS-OE.

Supplemental Figure S8. Gene enrichment analysis of internode-specific common up-regulated DEGs.

Supplemental Figure S9. Gene enrichment analysis of young shoot tissue-specific common up-regulated DEGs.

Supplemental Figure S10. Gene enrichment analysis of leaf-specific common up-regulated DEGs.

Supplemental Figure S11. Gene enrichment analysis of coleoptile-specific common down-regulated DEGs.

Supplemental Figure S12. Gene enrichment analysis of shoot-specific common down-regulated DEGs.

Supplemental Figure S13. Gene enrichment analysis of leaf-specific common down-regulated DEGs.

Supplemental Figure S14. Protein-protein interaction network of three up-regulated gene clusters.
Supplemental Figure S15. Protein-protein interaction network of three down-regulated gene clusters.

Supplemental Table S1. List of DEGs.

Supplemental Table S2. Tissue-specific expression of common up- and down-regulated DEGs.

Supplemental Table S3. Expression correlation network analysis of common up- and down-regulated genes.

Supplemental Table S4. Details of the protein-protein interaction network of BdGa.

Supplemental Table S5. List of development- and hormone-related DEGs common between amiR-BdGa and Bd-SiRGS-OE plants.

Supplemental Table S6. List of primers used for RT-qPCR analysis.

ACKNOWLEDGMENTS

We thank our former lab members Dr. Dieter Hackenberg and Dr. Biswa Acharya for the generation of some of the constructs used in this study and Dr. Anitha Vijayakumar for the preliminary characterization of some of the plant phenotypes. We also thank Dr. Todd Mockler and his group (Donald Danforth Plant Science Center) for help with RNA-seq data analysis, and Dr. Elizabeth Kellogg (Donald Danforth Plant Science Center) for help with some of the experiments and useful comments on the article.

Received September 25, 2020; accepted October 9, 2020; published October 20, 2020.

LITERATURE CITED

Bommert P, Je BI, Goldshmidt A, Jackson D (2013) The maize Ga gene COMPACT PLANT2 functions in CLAVATA signalling to control shoot meristem size. Nature 502: 555–558

Bommert P, Lunde C, Nardmann J, Vollbrecht E, Running M, Jackson D, Hake S, Werr W (2005) thick sassel dwarf rice mutant d1. J Exp Bot 56: 1694–1698

Cabrera-Vera TM, Vanhauwe J, Thomas TO, Medkova M, Preining A, Mazzoni MR, Hamm HE (2003) Insights into G protein structure, function, and regulation. Endocr Rev 24: 765–781

Carroll E, Fehringer N, Mitchell S, Cox KL Jr., Reilly KC, Mockler TC, Carrington JC (2015) Highly specific gene silencing in a monocot species by artificial microRNA derived from chimeric miRNA precursors. Plant J 82: 1061–1075

Chakraborty N, Sharma P, Kanyuka K, Pathak RR, Choudhury D, Hooley D, Chakravorty D, Urano D, Assmann SM, Botella JR (2019) Nucleotide exchange-dependent and nucleotide exchange-independent functions of plant heterotrimeric GTP-binding proteins. Sci Signal 12: eaax9526

McCudden CR, Hains MD, Kimpel RJ, Siderovski DP, Willard FS (2005) G-protein signaling: Back to the future. Cell Mol Life Sci 62: 551–577

Hsu N, Chen S, Li J, Liu Y, Ji W, Li H (2019) Genome-wide identification of GRAS genes in Brachypodium distachyon and functional characterization of BdSLR1 and BdSLR1. BMC Genomics 20: 635

Oki K, Fujisawa Y, Kitoh H, Iwasaki Y (2005) Study of the constitutively active form of the alpha subunit of rice heterotrimeric G-proteins. Cell Physiol 4: 381–386

Oldham WM, Hamm HE (2008) Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 9: 60–71

Pandey S (2017) Heterotrimeric G-protein regulatory circuits in plants: Conserved and novel mechanisms. Plant Signal Behav 12: e1325983

Pandey S, Assmann SM (2004) The Arabidopsis putative G protein-coupled receptor GCR1 interacts with the G protein alpha subunit GPA1 and regulates abscisic acid signaling. Plant Cell 16: 1616–1632

Perfus-Barbeoch L, Jones AM, Assmann SM (2004) Plant heterotrimeric G protein function: Insights from Arabidopsis and rice mutants. Curr Opin Plant Biol 7: 719–731

Pri-Tal O, Shaar-Moshe I, Wiseglass G, Peleg Z, Mosquina A (2017) Non-redundant functions of the dimeric ABA receptor BdPYL1 in the grass species Brachypodium. Plant J 92: 774–786

Roy Choudhury S, Li M, Lee V, Nandeyt RS, Mysore KS, Pandey S (2020) Flexible functional interactions between G-protein subunits contribute to the specificity of plant responses. Plant J 102: 207–221

Roy Choudhury S, Marlin MA, Pandey S (2019) The role of Gβ protein in controlling cell expansion via potential interaction with lipid metabolic pathways. Plant Physiol 179: 1159–1175

Roy Choudhury S, Pandey S (2015) Phosphorylation-dependent regulation of G-protein cycle during nodule formation in soybean. Plant Cell 27: 3260–3276

Roy Choudhury S, Pandey S (2016) The role of PDLs in providing specificity to signal-response coupling by heterotrimeric G-protein components in Arabidopsis. Plant J 86: 50–61

Roy Choudhury S, Pandey S (2017) Phosphatic acid binding inhibits RGS1 activity to affect specific signaling pathways in Arabidopsis. Plant J 90: 466–477

Shirley NJ, Aubert MK, Wilkinson LG, Bird DC, Lora J, Yang X, Tucker MR (2019) Translating auxin responses into ovules, seeds and yield: Insight from Arabidopsis and the cereals. J Integr Plant Biol 61: 310–336

Siderovski DP, Willard FS (2005) The Gαs, Gβs, and Gδs of heterotrimeric G-protein alpha subunits. Int J Biol Sci 1: 51–66

Fox AR, Soto GC, Jones AM, Casal JJ, Muscietti JP, Mazella MA (2012) cry1 and GPA1 signaling genetically interact in hook opening and anthocyanin synthesis in Arabidopsis. Plant Mol Biol 80: 315–324

Gao Y, Gu H, Lebrun M, Li X, Wang Y, Sheng J, Fang H, Gu M, Liang G (2019) The heterotrimeric G protein subunit RGB1 is required for seedling formation in rice. Rice (N Y) 12: 53

Gilman AG (1987) G proteins: Transducers of receptor-generated signals. Annu Rev Biochem 56: 615–649

Guo J, Wang S, Wang J, Huang WD, Liang J, Chen JG (2009) Dissection of the relationship between RACK1 and heterotrimeric G-proteins in Arabidopsis. Plant Cell Physiol 50: 1681–1694

Hacketten D, McKain RM, Lee SG, Roy Choudhury S, McCann T, Schreier S, Harkess A, Pires JC, Wong GK, Jez JM, et al (2017) Ga and regulator of G-protein signaling (RGS) protein pairs maintain functional compatibility and conserved interaction interfaces throughout evolution despite frequent loss of RGS proteins in plants. New Phytol 216: 562–575

Hu H, Mauro-Herrera M, Doust AN (2018) Domestication and improvement in the model C4 grass, Setaria. Front Plant Sci 9: 719

Ishida T, Tabata R, Yamada M, Aida M, Mitsuhashi K, Fujimasa W, Yamaguchi K, Shimogubu S, Higuchi M, Tsuji H, et al (2014) Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in Arabidopsis. EMBO Rep 15: 1202–1209

Langfelder P, Horvath S (2008) WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics 9: 559

Lee Y, Choi D, Kende H (2001) Expanders: Expanding versions and functions. Curr Opin Plant Biol 4: 527–532

Maruta N, Trusov Y, Chakravorty D, Urano D, Assmann SM, Botella JR (2019) Nucleotide exchange-dependent and nucleotide exchange-independent functions of plant heterotrimeric GTP-binding proteins. Sci Signal 12: eaax9526

Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

Downloaded on February 11, 2021. - Published by https://plantphysiol.org

Conserved Interactions of Ga/RGS Proteins

Plant Physiol. Vol. 184, 2020 1953
Stateczny D, Oppenheimer J, Bomhoff A (2016) G protein signaling in plants: Minus times minus equals plus. Curr Opin Plant Biol 34: 127–135
Tsai YC, Weir NR, Hill K, Zhang W, Kim HJ, Shiu SH, Schaller GE, Kieber JJ (2012) Characterization of genes involved in cytokinin signaling and metabolism from rice. Plant Physiol 158: 1666–1684
Ullah H, Chen JG, Temple B, Boyes DC, Alonso JM, Davis KR, Ecker JR, Jones AM (2003) The beta-subunit of the Arabidopsis G protein negatively regulates auxin-induced cell division and affects multiple developmental processes. Plant Cell 15: 393–409
Ullah H, Chen JG, Young JC, Im KH, Sussman MR, Jones AM (2001) Modulation of cell proliferation by heterotrimeric G protein in Arabidopsis. Science 292: 2066–2069
Urano D, Jones AM (2014) Heterotrimeric G protein-coupled signaling in plants. Annu Rev Plant Biol 65: 365–384
Urano D, Maruta N, Trusov Y, Stoian R, Wu Q, Liang Y, Jaiswal DK, Thung L, Jackson D, Botella JR, et al. (2016a) Saltational evolution of the heterotrimeric G protein signaling mechanisms in the plant kingdom. Sci Signal 9: ra93
Urano D, Miura K, Wu Q, Iwasaki Y, Jackson D, Jones AM (2016b) Plant morphology of heterotrimeric G protein mutants. Plant Cell Physiol 57: 437–445
Utsunomiya Y, Samejima C, Fujisawa Y, Kato H, Iwasaki Y (2012) Rice transgenic plants with suppressed expression of the β subunit of the heterotrimeric G protein. Plant Signal Behav 7: 443–446
Utsunomiya Y, Samejima C, Takayanagi Y, Izawa Y, Yoshida T, Sawada Y, Fujisawa Y, Kato H, Iwasaki Y (2011) Suppression of the rice heterotrimeric G protein β-subunit gene, RGB1, causes dwarfism and browning of internodes and lamina joint regions. Plant J 67: 907–916
Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3: research0034-0031
Vogel J, Hill T (2008) High-efficiency Agrobacterium-mediated transformation of Brachypodium distachyon inbred line Bd21-3. Plant Cell Rep 27: 471–478
Wang Y, Wang Y, Deng D (2019) Multicafetted plant G protein: Interaction network, agronomic potential, and beyond. Planta 249: 1259–1266
Warphe KM, Lateef SS, Lapik Y, Anderson M, Lee BS, Kaufman LS (2006) G-protein-coupled receptor 1, G-protein Galphai-subunit 1, and prephenate dehydratase 1 are required for blue light-induced production of phenylalanine in etiolated Arabidopsis. Plant Physiol 140: 844–855
Warphe KM, Upadhyay S, Yeh J, Adamiak J, Hawkins SI, Lapik YR, Anderson MB, Kaufman LS (2007) The GCR1, GPA1, PRN1, NF-Y signal chain mediates both blue light and abscisic acid responses in Arabidopsis. Plant Physiol 143: 1590–1600
Wu Q, Regan M, Furukawa H, Jackson D (2018) Role of heterotrimeric Ga proteins in maize development and enhancement of agronomic traits. PLoS Genet 14: e1007374
Wu J, Xu F, Liu L, Char SN, Ding Y, Je BI, Schmelz E, Yang B, Jackson D (2020) The maize heterotrimeric G protein β subunit controls shoot meristem development and immune responses. Proc Natl Acad Sci USA 117: 1799–1805
Yu Y, Assmann SM (2018) Inter-relationships between the heterotrimeric Gβ subunit AGB1, the receptor-like kinase FERONIA, and RALF1 in salinity response. Plant Cell Environ 41: 2475–2489
Yu Y, Chakravorty D, Assmann SM (2018) The G protein β-subunit, AGB1, interacts with FERONIA in RALF1-regulated stomatal movement. Plant Physiol 176: 2426–2440
Zhang L, Wei Q, Wu W, Cheng Y, Hu G, Hu F, Sun Y, Zhu Y, Sakamoto W, Huang J (2009) Activation of the heterotrimeric G protein alpha-subunit GPA1 suppresses the ftsH-mediated inhibition of chloroplast development in Arabidopsis. Plant J 58: 1041–1053
Zhong CL, Zhang C, Liu JZ (2019) Heterotrimeric G protein signaling in plant immunity. J Exp Bot 70: 1109–1118