Plant Signaling & Behavior 7:10, 1357-1359; October 2012; © 2012 Landes Bioscience

Roles of the Arabidopsis G protein γ subunit AGG3 and its rice homologs GS3 and DEP1 in seed and organ size control

Shengjun Li, Wuxia Liu, Xiaoqing Zhang, Yaju Liu, Na Li and Yunhai Li*

State Key Laboratory of Plant Cell and Chromosome Engineering; Institute of Genetics and Developmental Biology; Chinese Academy of Sciences; Beijing, China.

The size of seeds and organs is coordinately determined by cell proliferation and cell expansion, but the mechanisms that set final seed and organ size are largely unknown in plants. In a recent study, we have demonstrated that the plant specific G protein γ subunit (AGG3) promotes seed and organ growth by increasing the period of proliferative growth in Arabidopsis. AGG3 is localized in plasma membrane and interacts with the G protein β subunit (AGB1). Homologs of AGG3 in rice (GS3 and DEP1/qPE9-1) have been identified as important quantitative trait loci for seed size and yield. However, rice GS3 and DEP1 influence seed and organ growth by restricting cell proliferation. Here, we discuss the possible molecular mechanisms by which Arabidopsis AGG3 and its rice homologs GS3 and DEP1 control seed and organ size.

Keywords: AGG3, GS3, DEP1, seed and organ size, cell proliferation

Submitted: 07/24/12

Accepted: 07/25/12

http://dx.doi.org/10.4161/psb.21620

*Correspondence to: Yunhai Li;
Email: yhli@genetics.ac.cn

Addendum to: Li S, Liu Y, Zheng L, Chen L, Li N, Corke F, et al. The plant-specific G protein γ subunit AGG3 influences organ size and shape in Arabidopsis thaliana. New Phytol 2012; 194:690–703; PMID:22380792; http://dx.doi.org/10.1111/j.1469-8137.2012.04083.x.

G protein signaling is involved in a variety of growth and developmental processes in plants and animals.[1,2] Heterotrimeric G-proteins consisting of Gα, Gβ, and Gγ subunits are signal transducers in all metazoans. G protein-coupled pathways transmit a signal, via a membrane-bound receptor and heterotrimeric G proteins, to downstream enzymes known as effectors.[3] Mammals possess 17 Gα, five Gβ, and 12 Gγ genes,[4] while the Arabidopsis genome contains one G protein α subunit (*GPA1*), one G protein β subunit (*AGB1*), and three G protein γ subunit (*AGG*) genes.[1,5,6] Thus, plants have a simple repertoire of canonical heterotrimeric G-protein complexes despite what their possible involvement is in many signal pathways.

Mutations in Arabidopsis *GPA1* or *AGB1* caused defects in plant growth and development,[7,8] whereas mutations in Arabidopsis *AGG1* or *AGG2* did not obviously affect organ growth.[9] An atypical heterotrimeric G-protein γ-subunit AGG3 was recently identified by BLAST searches using AGG1 and AGG2 as queries.[5] AGG3 has been proposed to be involved in guard cell K^+ channel regulation and ABA responses in Arabidopsis.[5] In a recent study, we discovered a role of Arabidopsis AGG3 in seed and organ size control through a genetic screen for mutations that reduced seed and organ size.[6] Loss-of-function mutations in *AGG3* caused small seeds and organs, whereas overexpression of *AGG3* significantly increased seed and organ size of wild-type plants,[6] indicating that AGG3 is an important regulator of seed and organ size. Our results further indicate that AGG3 controls organ size by increasing the duration of proliferative growth.[6] AGG3 interacts with the Arabidopsis G protein β subunit AGB1 in yeast,[5] although their interactions in planta remain to be proved. Our genetic analyses also show that the role of AGG3 in organ size control is dependent on functional GPA1 and AGB1.[6] The N-terminal region of AGG3 shares similarity with AGG1 and AGG2, and interacts with AGB1.[5] Interestingly, the N-terminal region of AGG3 showed higher affinity for AGB1 than either AGG1 or AGG2,[5] suggesting that the Gβ1γ3 might exist in Arabidopsis cells more frequently than Gβ1γ1 or Gβ1γ2 dimer, and AGG3

might play a predominant role in seed and organ size control. Consistent with this, *agg3* mutants exhibited stronger seed and organ size phenotypes than *agg1* and *agg2* mutants.[6,10]

The localization of G proteins to the cytoplasmic face of plasma membrane is important for their signal function in animal cells.[11] Arabidopsis AGB1 is localized to the cytoplasmic face of plasma membrane by interacting with Gγ subunits.[12] Arabidopsis AGG3 contains a transmembrane domain and two putative CaaX motifs that are potential targets for protein prenylation and S-acylation, which serve as an important signal to target proteins to the plasma membrane.[5,6,12] The GFP-AGG3 fusion protein is localized to the plasma membrane.[5,6] Protein domain analysis shows that the transmembrane domain and the putative CaaX motifs jointly affect the plasma membrane localization of AGG3.[6] The transmembrane domain might play a predominant role in the regulation of AGG3 subcellular localization.[6]

Arabidopsis AGG3 shares significant similarity with rice GRAIN SIZE 3 (GS3) and DENSE AND ERECT PANICLE 1 (DEP1/qPE9-1).[6,13-17] However, rice GS3 was identified as a negative regulator of grain length and weight, and loss of *GS3* function resulted in large seeds with more cells.[13,14,17] Interestingly, the N-terminal OSR (organ size regulation) domain is both necessary and sufficient to repress seed size in rice.[17] By contrast, the N-terminal region or the C-terminal fragment of AGG3 could not promote seed and organ growth in Arabidopsis.[6] A recent report has found a new allele in the *GS3* gene, resulting in a truncated protein containing the N-terminal region and the putative transmembrane domain, but lacking most of the C-terminal domains.[17] A rice varity (Chuan 7) with this mutation produced super short seeds.[17] Thus, the C-terminal domains of GS3 has been proposed to have an inhibitory effect on the function of the N-terminal OSR domain.[17] The DEP1 QTL is responsible for erect panicle, high seed number per panicle and dense panicle. The gain-of-function allele (*dep1*) increased seed yield,[16] although the *dep1* allele formed small seeds and organs.[15,16] The mutant protein encoded by the *dep1* allele is a truncated protein containing the N-terminal region and the predicted transmembrane domain, but lacking most of the C-terminal region.[15,16] DEP1 shares significant similarity with GS3,[6,16] suggesting that DEP1 and GS3 might have overlapped functions. Thus, it is reasonable to assume that the C-terminal region of DEP1 might also inhibit the function of the N-terminal region in seed and organ size control. Further identification and characterization of the null allele will help understand the role of the wild-type *DEP1*. Transient expression of the DEP1/qPE9-1-GFP fusion protein in rice protoplasts was shown to locate in the membrane,[15] whereas GFP signals were observed in the nuclei in *DEP1/qPE9-1-GFP* transgenic rice plants.[16] Both GS3 and DEP1 contain the predicted transmembrane domains,[6,13,15,17] suggesting that GS3 and DEP1 are most likely localized in the plasma membrane. It is still possible that subcellular localization of GS3 and DEP1 might be related to cell types or growth conditions.

The AGG3 has a γ-like region in its N-terminus, which has a remote overall similarity to the OSR domain of GS3 or the N-terminal region of DEP1/qPE9-1.[5,6] The N-terminuses of GS3 and DEP1 also possess several conserved residues that are critical for Gβ binding,[5] suggesting that GS3 and DEP1 could interact with the rice G protein β subunit RGB1. *RGB1* knock-down lines produced small seeds,[18] indicating that RGB1 is a positive regulator of seed size. However, GS3 and DEP1 are negative factors of seed size control, suggesting that GS3 and DEP1 might repress the function of RGB1 in seed growth. It is possible that GS3 and DEP1 might compete with RGG1 and RGG2 for RGB1 binding, resulting in decreased Gβ1γ1 and Gβ1γ2 signaling. It will be interesting to determine whether GS3 and DEP1 function as G-protein γ subunits to interact with RGB1.

Plant heterotrimeric G proteins are associated with large complexes: a 400 kDa complex in rice[19] and a 700 kDa complex in Arabidopsis.[20] The divergent functions of AGG3 and its rice homologs GS3 and DEP1 might be related to their regulators or effectors in these large complexes. Interestingly, the rice genome does not encode any obvious candidate for an RGS protein (regulator of G protein signaling), while there is a single RGS protein in Arabidopsis,[4] suggesting that divergent functions of AGG3 and its rice homologs might be due to the absence of RGS in rice. Therefore, further identification of their upstream regulators and downstream targets will help understand why Arabidopsis AGG3 and its rice homologs have distinct functions in seed and organ size control.

Acknowledgments

This work was supported by National Basic Research Program of China (2009CB941503), the National Natural Science Foundation of China (91017014; 30921003; 31000531).

References

1. Temple BR, Jones AM. The plant heterotrimeric G-protein complex. Annu Rev Plant Biol 2007; 58:249-66; PMID:17201690; http://dx.doi.org/10.1146/annurev.arplant.58.032806.103827.
2. Mizuno N, Itoh H. Functions and regulatory mechanisms of Gq-signaling pathways. Neurosignals 2009; 17:42-54; PMID:19212139; http://dx.doi.org/10.1159/000186689.
3. New DC, Wong JT. The evidence for G-protein-coupled receptors and heterotrimeric G proteins in protozoa and ancestral metazoa. Biol Signals Recept 1998; 7:98-108; PMID:9629461; http://dx.doi.org/10.1159/000014535.
4. Jones AM, Assmann SM. Plants: the latest model system for G-protein research. EMBO Rep 2004; 5:572-8; PMID:15170476; http://dx.doi.org/10.1038/sj.embor.7400174.
5. Chakravorty D, Trusov Y, Zhang W, Acharya BR, Sheahan MB, McCurdy DW, et al. An atypical heterotrimeric G-protein γ-subunit is involved in guard cell K^+-channel regulation and morphological development in Arabidopsis thaliana. Plant J 2011; 67:840-51; PMID:21575088; http://dx.doi.org/10.1111/j.1365-313X.2011.04638.x.
6. Li S, Liu Y, Zheng L, Chen L, Li N, Corke F, et al. The plant-specific G protein γ subunit AGG3 influences organ size and shape in Arabidopsis thaliana. New Phytol 2012; 194:690-703; PMID:22380792; http://dx.doi.org/10.1111/j.1469-8137.2012.04083.x.
7. Ullah H, Chen JG, Young JC, Im KH, Sussman MR, Jones AM. Modulation of cell proliferation by heterotrimeric G protein in Arabidopsis. Science 2001; 292:2066-9; PMID:11408654; http://dx.doi.org/10.1126/science.1059040.
8. Trusov Y, Zhang W, Assmann SM, Botella JR. Ggamma1 + Ggamma2 not equal to Gbeta: heterotrimeric G protein Ggamma-deficient mutants do not recapitulate all phenotypes of Gbeta-deficient mutants. Plant Physiol 2008; 147:636-49; PMID:18441222; http://dx.doi.org/10.1104/pp.108.117655.
9. Trusov Y, Rookes JE, Tilbrook K, Chakravorty D, Mason MG, Anderson D, et al. Heterotrimeric G protein gamma subunits provide functional selectivity in Gbetagamma dimer signaling in Arabidopsis. Plant Cell 2007; 19:1235-50; PMID:17468261; http://dx.doi.org/10.1105/tpc.107.050096.

10. Thung L, Trusov Y, Chakravorty D, Botella JR. Gγ1+Gγ2+Gγ3=Gβ: the search for heterotrimeric G-protein γ subunits in Arabidopsis is over. J Plant Physiol 2012; 169:542-5; PMID:22209167; http://dx.doi.org/10.1016/j.jplph.2011.11.010.
11. Magalhaes AC, Dunn H, Ferguson SS. Regulation of G protein-coupled receptor activity, trafficking and localization by Gpcr-interacting proteins. Br J Pharmacol 2011; PMID:21699508.
12. Zeng Q, Wang X, Running MP. Dual lipid modification of Arabidopsis Ggamma-subunits is required for efficient plasma membrane targeting. Plant Physiol 2007; 143:1119-31; PMID:17220359; http://dx.doi.org/10.1104/pp.106.093583.
13. Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, et al. GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 2006; 112:1164-71; PMID:16453132; http://dx.doi.org/10.1007/s00122-006-0218-1.
14. Takano-Kai N, Jiang H, Kubo T, Sweeney M, Matsumoto T, Kanamori H, et al. Evolutionary history of GS3, a gene conferring grain length in rice. Genetics 2009; 182:1323-34; PMID:19506305; http://dx.doi.org/10.1534/genetics.109.103002.
15. Zhou Y, Zhu J, Li Z, Yi C, Liu J, Zhang H, et al. Deletion in a quantitative trait gene qPE9-1 associated with panicle erectness improves plant architecture during rice domestication. Genetics 2009; 183:315-24; PMID:19546322; http://dx.doi.org/10.1534/genetics.109.102681.
16. Huang X, Qian Q, Liu Z, Sun H, He S, Luo D, et al. Natural variation at the DEP1 locus enhances grain yield in rice. Nat Genet 2009; 41:494-7; PMID:19305410; http://dx.doi.org/10.1038/ng.352.
17. Mao H, Sun S, Yao J, Wang C, Yu S, Xu C, et al. Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proc Natl Acad Sci U S A 2010; 107:19579-84; PMID:20974950; http://dx.doi.org/10.1073/pnas.1014419107.
18. Utsunomiya Y, Samejima C, Takayanagi Y, Izawa Y, Yoshida T, Sawada Y, et al. Suppression of the rice heterotrimeric G protein β-subunit gene, RGB1, causes dwarfism and browning of internodes and lamina joint regions. Plant J 2011; 67:907-16; PMID:21585570; http://dx.doi.org/10.1111/j.1365-313X.2011.04643.x.
19. Kato C, Mizutani T, Tamaki H, Kumagai H, Kamiya T, Hirobe A, et al. Characterization of heterotrimeric G protein complexes in rice plasma membrane. Plant J 2004; 38:320-31; PMID:15078334; http://dx.doi.org/10.1111/j.1365-313X.2004.02046.x.
20. Wang S, Assmann SM, Fedoroff NV. Characterization of the Arabidopsis heterotrimeric G protein. J Biol Chem 2008; 283:13913-22; PMID:18326491; http://dx.doi.org/10.1074/jbc.M801376200.