The Roles of SUS and TCP in Arabidopsis G-Protein Signaling Pathway

Danielle Schmidt

UNC Department of Biology

INTRODUCTION

In animals, a seven-transmembrane cell surface receptor works in conjunction with a heterotrimeric G protein complex in which the relayed signal depends on the activation of the GCPR by the ligand. Plant G proteins work in an opposite manner of mammalian G proteins. Arabidopsis G proteins are self-activating, spontaneously binding GTP and separating into subunits. A negative regulator of the G protein (RGS), however, inhibits these subunits from acting on effector proteins. It does this by accelerating GTP hydrolysis. When a ligand binds the G protein, the ligand inhibits this inhibitor, allowing WNK kinase to phosphorylate RGS for endocytosis. G proteins are then free to activate effector proteins to elicit sugar signaling and proliferation responses. When RGS ceases to be phosphorylated, the "foot on the brake" is removed, and GTP hydrolysis is again accelerated, disallowing G protein complexes from acting on effector proteins.[3]

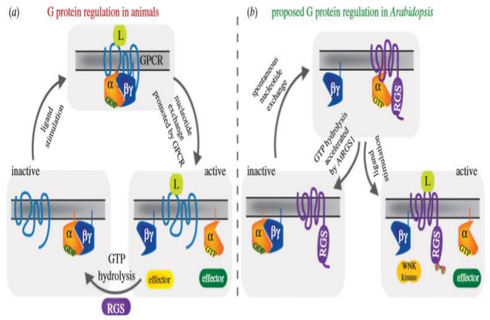


Figure 1: *Heterotrimeric G protein signaling in the plant kingdom*, Open Biology, March 2013

Effectors are targets of activated Gα subunits and Gβγ dimers. While animal G pathway effectors are well known, plants effector proteins are less understood. In Klopffleisch et al, researchers aimed to identify regulators and effectors of heterotrimeric G-protein subunits in Arabidopsis. They performed yeast 2 hybrid screening to discover a new interactome, including interactions of SUS 1 and SUS 4 with RGS. In addition to SUS interactions with RGS, 4 TCP factors were found to interact with GPA1. [1] Additional experiments, such as split luciferase assays confirmed interactions between these proteins.

SUS serves to catalyze sucrose reactions in plants, producing UDP-glucose from SUS which may be necessary for RGS endocytosis and is vital to G signaling.[6] The function of SUS as a sucrose synthase has been long known, though its regulation and possible role in the G signaling pathway is less understood. Oppositely, TCP regulation through the Gα protein, GPA1, is well documented in Y2H screens and in split luciferase assays in tobacco leaves. Yet the way these proteins affect plant phenotype and response are still largely unknown.

OBJECTIVES

Researchers sought to understand the function and relation of SUS in the G signaling pathway by investigating the phenotypic effects (including rosette leaf diameter, flowering time, and ROS response) of SUS mutants, wildtype (Col-0) and G protein mutants (gpa1, agb1, rgs1, double mutant, xlgs). It was hypothesized that the RGS1 and SUS complex represses G-signaling in plants since RGS has been shown to be endocytosed through SUS.

Additionally, the way TCP proteins affect plant phenotype and response by interacting with G proteins was investigated by observing the phenotypic effects (including flowering time, rosette leaf diameter, ROS response) of TCP mutants against wildtype (Col-0) and G protein mutants (gpa1, agb1, rgs1, xlg1, double mutants). It was hypothesized that TCPs are effectors of GPA1 in regulation of Plant physiological responses.

MATERIALS AND METHODS

Three to twelve plants of each genotype were planted using seed sterilization techniques and cultured in a short-day chamber. Plants were watered once a week or when the soil was dry for 45 days. 45 days after planting, each rosette diameter was measured and recorded. Data from each genotype was averaged and displayed in figure 2. Flowering-time rates in G protein and sus mutants were also analyzed. 35 wildtype seeds and 9 of each mutant genotype were planted using seed sterilization techniques and attended to in a short day chamber. Plants were watered once a week or when the soil was dry. Plants were observed for flowering and recorded when they first displayed a bud (Figure 3). Flowering-time by rosette leaf number was also performed, marking the smallest rosette leaf once the plant reached 2 cm. Rosette leaf number was not counted, however, due to the COVID-19 lab closures. Lastly, ROS response experiments were performed to indicate plant immune response (Figure 4). A summary table of all physiological responses was created to compare mutants.

Sus mutant lines include:

sus1 (SALK1) Sucrose synthase knockout At4g20830 - SALK_014303;
sus2 (SALK2) Sucrose synthase knockout At5g49190 - SALK_076303;
sus3 (SALK3) Sucrose synthase knockout At4g02280 - SALK_019405;
sus4 (Arab-4) Sucrose synthase knockout At3g43190.

Researchers also sought to investigate rosette leaf diameter phenotypes of TCP mutants against wildtype and G protein mutants in order to understand their function and relation in G signaling pathways. Three to twelve plants of each genotype were planted using seed sterilization techniques and cultured in a short-day chamber. Plants were watered once a week or when the soil was dry for 45 days. 45 days after planting, each rosette diameter was measured and recorded. Data from each genotype was averaged and displayed in Figure 5.

RESULTS

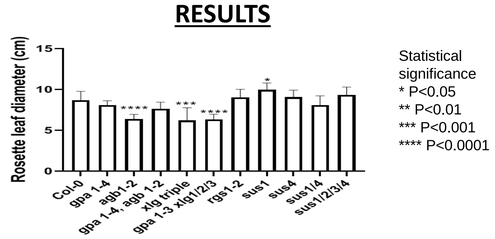


Figure 2: Bar graphs displaying the average rosette diameters of various genotypes 45 days after planting. Displays wildtype and G protein mutants against sus mutants. Stars indicate genotypes statistically different from wildtype.

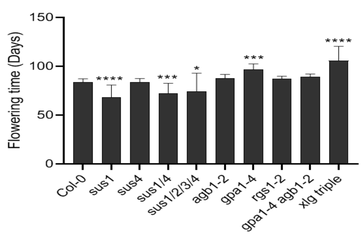
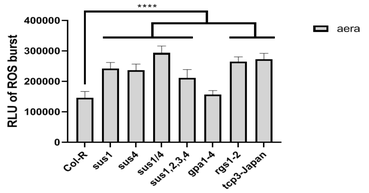


Figure 3: Bar graph measuring the average days past planting that each genotype first displayed a bud. Wildtype, G protein, tcp, and sus genotypes are investigated. Stars indicate genotypes statistically different from wildtype. Flowering time by rosette leaf number not counted due to the break.

Figure 4: Bar graph measuring the average days past planting that each genotype first displayed a bud. Wildtype, G protein, tcp, and sus genotypes are investigated. Stars indicate genotypes statistically different from wildtype.



RESULTS Cont.

	G mutant (gpa1-4, agb1-2, xlgs)	*rgs1-2*	*sus1*	*sus4*
Rosette leaf diameter	Smaller	larger	larger	larger (not signigicant)
Flowering time	delayed	rgs1 mutant late flowering or no difference with Col-0; Overexpression RGS1, cause late floweing	early	no difference
ROS burst	reduced	increased	increased	increased
all compare to Wild type Col-0				

Table 1: Summary table of sus mutant responses compared to G mutants and rgs mutant.

Investigation of mutants revealed early flowering in sus and similar to late flowering as wildtype in rgs. G protein mutants however showed delayed flowering. Increased immune response occurred in both sus and rgs mutants, while G mutants showed reduced immune response. Lastly, sus revealed larger rosette leaves while G mutants showed smaller leaf diameters than wildtype.

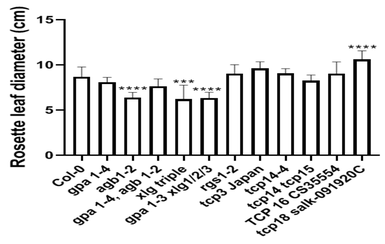


Figure 5: Bar graphs displaying the average rosette diameters of various genotypes 45 days after planting. Stars indicate genotypes statistically different from wildtype.

In tcp mutant experiments, G mutants demonstrate smaller leaves, as expected, while tcp 18 mutants reveal larger leaf size.

DISCUSSION

Sus mutants displayed similar physiological responses as rgs1-2 mutants, which were opposite to G mutants. From this data, researchers concluded that the RGS-SUS complex works to repress G signaling in plants. While G signaling may lead to a larger and earlier flowering plant with increased immune response, RGS and SUS work together to repress these functions.

Without proper G-signaling in G mutants, plants were smaller with delayed flowering time and decreased immune response. Without RGS or SUS, however, the plants were even larger than wildtype with increased flowering and immune response. This contrast supports the theory that the RGS-SUS complex works together to suppress G signaling. Though the rgs mutant did not reveal different flowering than wildtype, overexpression of RGS1 produced late flowering that is consistent with G-signaling suppression. Future experimentation may investigate rgs1/sus1/sus4 mutant's physiological responses to determine if RGS1 can enhance the SUS phenotype.

In tcp mutant experiments, G mutants revealed smaller leaves, consistent with lack of G-signaling, while tcp18 mutants revealed larger leaf diameter. This indicates that TCP18 negatively inhibits rosette leaf diameter. Researchers hypothesized that TCP18 is negatively regulated by G-signaling, such that if G-signaling is not present, TCP18 will be in excess and cause small leaf diameters. If TCP18 is not present, however, plant growth won't be stunted at all, causing abnormally large plants.

Future tcp experiments include investigation into the role TCP may have in flowering. Hypothesized effector pathways have targeted TCP as a promoter of flowering through SOC1 that is negatively regulated by G-signaling.

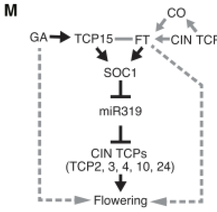


Figure 6: Hypothesized TCP Effector Pathway for plant flowering

Lucero, L. *Class I and Class II TCP Transcription Factors Modulate SOC1-Dependent Flowering at Multiple Levels.* Mol Plant. 2017 Dec.

CONCLUSION

Researchers sought to investigate the phenotypic effects, including rosette leaf diameter, flowering time, and ROS response, of sus mutants, tcp mutants, wildtype (Col-0) and G protein mutants (gpa1, agb1, rgs1, double mutant, and xlgs) in order to understand their function and relation in the G signaling pathway. From these findings, it is concluded that SUS1 and RGS1 work in complex to suppress G signaling, leading to smaller rosette leaf diameter, delayed flowering time, and reduced ROS burst. It is also concluded that G proteins, AGB1 and GPA1, inhibit TCP 18, and that TCP 18 inhibits rosette leaf diameter. In future experiments, researchers will also test flowering time and ROS response of tcp mutants. Future plans additionally include creating a sus1/sus4/rgs1 triple mutant to determine if RGS1 can enhance SUS phenotype that was found.

Much thanks to Dr. Haiyan Jia for all her facilitation of experiments and her mentorship!

SIGNIFICANCE

This study's conclusions worked to increase the knowledge of effector proteins' function and regulation in the Arabidopsis G-protein pathway. Expanding knowledge of these cell-signaling pathways is vital to understanding the mechanisms by which plants elicit sugar reactions, produce immune response, and grow. Understanding plant cell-signaling pathways lends itself to more efficient crop growth, as well as to targeted response toward pathogenic plants.

REFERENCES

[1]Klopffleisch K et al. Published September 27, 2011. Arabidopsis G-protein interactome reveals connections to cell wall carbohydrates and morphogenesis. https://www.ncbi.nlm.nih.gov/pubmed/21952135.

[2]Jia, Haiyan. Jones Lab. University of North Carolina at Chapel Hill Biology Department.

[3]Urano, Daisuke; Chen, Jin-Gui; Ramon Botella, Jose; Jones, Alan. Published 27 March 2013. Heterotrimeric G protein signaling in the plant kingdom. file:///C:/Users/des1199/Desktop/395%20Research/395%20Project/Hiyan%20Papers/2.%20UranoJonesOpenBiology2013.pdf.

[4]Stein, Ofer; Granot, David. Published February 8, 2019. An Overview of Sucrose Synthase in Plants. ncbi.nlm.nih.gov/pmc/articles/PMC6375876/.

[5]Ross-Elliott, Timothy, et al. Published June26, 2019. Biased Signaling: Distinct Ligand-directed Plasma Membrane Signalosomes Using a Common RGS/ G protein Core. https://www.biorxiv.org/c ontent/10.1101/666578v2.article-info.

[6]Cabral Oliveira, Celio. Jones Lab. University of North Carolina at Chapel Hill Biology Department.

[7]Fangfang Ji, Yun Chen; Liang, Jiansheng. Published 2006. Overexpression of the regulator of G-protein signaling protein enhances ABA-mediated inhibition of root elongation and drought tolerance in Arabidopsis. https://www.semanticscholar.org/pa per/Overexpression-of-the-regulator-of-G-protein-of-and-Chen-Ji/9df8575295 2b40589778d769e5e66 25645a473e9.