The role of G-proteins in plant immunity

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Keywords: plant immunity, G-protein, elicitor, PAMP, stomatal closure

Heterotrimeric G-proteins play an important regulatory role in multiple physiological processes, including the plant immune response, and substantial progress has been made in elucidating the G-protein-mediated defense-signaling network. This mini-review discusses the importance of G-proteins in plant immunity. We also provide an overview of how G-proteins affect plant cell death and stomatal movement. Our recent studies demonstrated that G-proteins are involved in signal transduction and induction of stomatal closure and defense responses. We also discuss future directions for G-protein signaling studies involving plant immunity.

Unlike animals, plants do not have specified cells and an adaptive immune system to defend themselves against pathogens. Instead, every living plant cell is generally equipped with the components necessary to detect invading pathogens and produce systemic signals at the infection site. The plant basal immune system (PTI) is triggered by microbe-/pathogen-associated molecular patterns (MAMPs/PAMPs)1 and elicitors.2,3 PTI provides the first level of induced defense against an invading pathogen. This non-race-specific basal resistance, together with constitutive physical and chemical barriers, successfully prevents most infections from becoming established. To overcome PTI, pathogens have evolved a repertoire of virulence effector proteins that are delivered into hosts to suppress PTI.4 In turn, plants have evolved Resistance (R) proteins, each of which recognizes the action of specific virulent effector(s) as a signal of invasion to trigger plant resistance through effector-triggered immunity (ETI).5 Natural selection drives the evolution of new pathogen effector proteins and plant R proteins. This tug-of-war between plants and pathogens is represented as a zig-zag model.6,7 Both PTI and ETI induce stomatal closure and a hypersensitive response (HR), a programmed host cell death (PCD), to limit pathogen development.4 Recent studies have advanced our understanding of plant immunity, and a key player was found to be the heterotrimeric G-protein (G-protein).

In this review, we focus on recent studies that have expanded our understanding of the role of G-proteins in plant immunity with a particular emphasis on G-protein-mediated cell death signaling and G-protein-mediated stomatal closure.

Heterotrimeric G-proteins: The players

The regulatory mechanisms of plant immunity have been extensively explored in yeast, metazoans, and plants. G-proteins are highly conserved in eukaryotes, and yeast also contains a major G-protein sensor. The heterotrimeric G-protein complex is minimally composed of γ, β, and γ subunits. Classically, the G-protein heterotrimer is activated by cell-surface receptors (G-protein-coupled receptors, GPCRs) that trigger the Gα subunit of the heterotrimer to release GDP, thus enabling the Gα subunit to bind GTP. GTP binding is accompanied by structural rearrangements that disengage the Gβγ interaction and result in heterotrimer dissociation.9-11 The free subunits then relay signals by interacting with downstream proteins collectively called effectors because they “effect” the cellular changes discussed above. G-protein signaling is terminated after the Gα subunit hydrolyzes GTP to GDP and the heterotrimer reassociates. Gα proteins provide specificity between GPCRs and effectors, amplify signal transduction, serve as a point of signal modulation, and act as timing devices that control signaling life spans. Metazoans have hundreds of GPCRs as well as dozens of effectors, modulators, and scaffold proteins that interact with the heterotrimer or its dissociated subunits.12 However, few of these are encoded in plant genomes sequenced to date.13 Our understanding of G-protein signaling in plants depends on understanding the proteins operating in this network and the relationship between each effector. Currently, some conserved plant immunity regulators such as MAPKKKα, BcLCB2, CaMBL1, NtLRP1, CaPK1, CaRING1, HSP90, 14-3-3, CaMsrB2, MKK1 and VPE have been identified in Nicotiana benthamiana and other plants.14-24

In addition to the conserved counterparts of mammalian immunity regulators, unexpected plant immunity mediators have also been identified in plants. However, G-protein signaling is poorly characterized in Arabidopsis and other plants relative to information regarding yeast and animal systems, and little is known about processes downstream of Gα, β, and γ.

Heterotrimeric G-protein: Activators of Plant Cell Death

Heterotrimeric G-proteins are involved in transducing extracellular signals from cell surface receptors into intracellular effectors.25 Although plants have only a small number of genes encoding the G-protein subunits, they have been implicated in a wide variety...
of biological processes.\textsuperscript{13} The higher plant Arabidopsis has only one canonical G-protein \( \alpha \)-subunit (G\( \alpha \)), one \( \beta \)-subunit (G\( \beta \)), and three \( \gamma \)-subunits (G\( \gamma \)).\textsuperscript{26} However, only two G\( \gamma \) were found in monocot rice species.\textsuperscript{27} Similarly, based on \textit{N. benthamiana} sequence information, it contains a single candidate G\( \alpha \) gene and possibly two G\( \beta \)-subunit genes. We previously used virus-induced gene silencing (VIGS) to investigate the role of G\( \alpha \), G\( \beta 1 \), and G\( \beta 2 \) in \textit{N. benthamiana} responses to elicitors from fungi, bacteria, and oomycetes.\textsuperscript{28} In the canonical heterotrimeric protein in \textit{N. benthamiana}, both the G\( \alpha \) and G\( \beta 2 \) subunits are related to hydrogen peroxide (H\(_2\)O\(_2\)) accumulation triggered by elicitors. Neither boehmerin nor the Nep1-induced hypersensitive response requires a functional heterotrimeric G-protein. Only harpin-triggered cell death is compromised in \textit{G\( \alpha \)}- and G\( \beta 2 \)-silenced plants, suggesting that G\( \alpha \) and G\( \beta 2 \) play a role in harpin-induced cell death. This suggests that H\(_2\)O\(_2\) plays different roles in cell death induced by various elicitors. Although not all G-protein subunits are involved in elicitor-induced cell death, all gene-silenced plants show decreased PR2b, EDS1, Nibrboh4, and NibrhoB expression, indicating that G-proteins positively regulate defense-related genes in plant defense signaling. Both the G\( \alpha \) and G\( \beta \) subunits are necessary for the rapid initial component of the biphasic oxidative burst, but only the G\( \alpha \) protein is required for ozone (O\(_3\))-induced cell death in Arabidopsis.\textsuperscript{29} The Arabidopsis G\( \beta \) is involved in unfolded protein response-associated cell death.\textsuperscript{30} All three regular subunits of the Arabidopsis G-protein play roles in disease resistance. The Arabidopsis gpa1 mutant exhibited enhanced resistance to several necrotrophic fungal pathogens, including \textit{Plectosphaerella cucumerina}, \textit{Alternaria brassicicola}, and \textit{Fusarium oxysporum}.\textsuperscript{31,32} In contrast, mutations in AGB1 and AGG1 enhanced plant susceptibility to necrotrophic fungal pathogens.\textsuperscript{31,33} These results suggested that the G\( \alpha \) subunit is a negative regulator, while G\( \beta \) and G\( \gamma \) are positive regulators of disease resistance. However, the rice G\( \alpha \) loss-of-function mutation (the \( \text{d1} \) mutation) compromises resistance against an avirulent strain of the biotrophic fungal pathogen \textit{Magnaporthe grisea}, which causes rice blast. The \( \text{d1} \) mutant is impaired in the hypersensitive response, H\(_2\)O\(_2\) production, and defense gene induction triggered by pathogens.\textsuperscript{34} In Arabidopsis, neither the G\( \alpha \) nor the G\( \beta \) mutation alters resistance to virulent or avirulent strains of \textit{Pseudomonas syringae}, a biotrophic bacterial pathogen.\textsuperscript{35} These studies suggest that the role of G-proteins in plant disease resistance depends on the type of pathogen (necrotrophic vs. biotrophic and bacterial vs. fungal) and that the different G-protein subunits may have different functions. The molecular mechanism by which the various subunits of G-proteins affect disease resistance remains unclear.

Heterotrimeric G-proteins were identified as early mediators of stress signaling.\textsuperscript{29,34} Arabidopsis plants deficient in the G\( \beta \) subunit of the heterotrimeric G-protein were more susceptible to O\(_3\) damage and displayed higher rates of leaf cell death than plants deficient in G\( \alpha \).\textsuperscript{29} In contrast, Arabidopsis plants lacking the G\( \beta \) subunit displayed greater resistance to leaf cell death triggered by tunicamycin, whereas mutants lacking the G\( \alpha \) subunit were as susceptible as wild type.\textsuperscript{30} This difference in response may be due to differential localization and unique functions of the G\( \alpha \) and G\( \beta \) subunits. While the G\( \alpha \) protein was present at approximately equal amounts in the plasma membrane and endoplasmic reticulum (ER), the G\( \beta \) protein was more abundant in the ER. Tunicamycin induces ER stress, and G\( \beta \) may specifically play a role in control of the ER stress response.\textsuperscript{30} And pea G\( \beta \) subunit plays an important role in stress signal transduction and development pathways via interacting with a wide range of proteins of multiple functions.\textsuperscript{35,36} In addition, the PAMP derived from bacterial flagellin, flg22-triggered PTI involves K\(^+\) channel regulation, and this regulation is dependent on signaling via cognate PAMP receptors and a heterotrimeric G-protein.\textsuperscript{37}

In Arabidopsis seedlings, phytochrome-dependent cell death is mediated by the heterotrimeric G-protein. In hypocotyls of far-red grown seedlings that were subsequently exposed to white light, a heterotrimeric G-protein played a role in phytochrome A-mediated signaling, leading to far-red irradiation preconditioned cell death.\textsuperscript{38} The gpa1 mutant showed decreased cell death compared with the wild type, while in the G\( \beta \) mutant \textit{agb1}, cell death increased, suggesting that G\( \alpha \) and G\( \beta \) play antagonistic roles in this cell death pathway. In addition, reactive oxygen species (ROS) mediated this cell death pathway. And GPA1 inhibits guard cell photostimulation and promotes the availability of reactive oxygen species (ROS) in guard cells.\textsuperscript{39} Moreover, \textit{agb1} was more sensitive to H\(_2\)O\(_2\) than wild-type seedlings, indicating that G-protein may play a role in seedling susceptibility to H\(_2\)O\(_2\) stress. Heterotrimeric G-proteins regulate cell death, and their diverse roles have been studied in various plant species. However, no information is available on the functions of G-protein genes that are differentially expressed in epidermal cells above adventitious roots. Their role in cell death, cell type specification, or other cellular processes remains unknown.

Epidermal cell death in rice is induced by ethylene and H\(_2\)O\(_2\) and accompanied by transcriptional regulation in response to pro-death signals. Previous studies have identified G-protein signaling through G\( \alpha \) (D1) as an essential step in epidermal cell death signaling.\textsuperscript{40} Since no genes encoding G-proteins or G-protein regulatory proteins were transcriptionally controlled in dying epidermal cells after treatment with ethylene or H\(_2\)O\(_2\), we conclude that heterotrimeric G-protein activity is regulated posttranscriptionally. While cell death rates were strongly reduced in \textit{d1}, some gene regulation was still observed in response to ethylene or H\(_2\)O\(_2\), indicating that D1 may act downstream of transcriptional regulation.

\section*{Heterotrimeric G-proteins: Mediators of Stomatal Closure Signaling}

Regulation of the stomatal aperture in plants controls photosynthesis and water status.\textsuperscript{41,42} Since mature guard cells lack plasmodesmata, all solute uptake and efflux must occur via the plasma membrane and vacuole.\textsuperscript{43} Historically, stomata were considered a passive portal for the entry of pathogenic bacteria.\textsuperscript{43} But recent studies have suggested that the stomata play an active role in the plant innate immune system.\textsuperscript{44,45} Stomatal closure restricts bacterial invasion, although plant pathogenic bacteria can secrete specific virulence factors to reopen stomata, which is an important
pathogenec strategy.\textsuperscript{44} Moreover, some fungal phytopathogens, in addition to bacteria, force entry through closed stomata, whereas others require open stomata to successfully penetrate plants.\textsuperscript{37} Some fungi and oomycetes can manipulate stomata to facilitate sporulation.\textsuperscript{48,49} Stomatal closure can be triggered by biotic (e.g., pathogens, PAMPs, elicitors) and abiotic stresses [e.g., water deficiency, cold, light, abscisic acid (ABA)]. Thus, identifying the G-protein subunits that control stomatal closure during PTI is important. All three subunit types are expressed in guard cells. Ubiquitous expression of GPA1 throughout plants was confirmed using northern blotting, and promoter::GUS analyses and reverse transcription-polymerase chain reaction (RT-PCR) results also supported guard cell expression.\textsuperscript{50-52} AGGI is ubiquitously expressed throughout the plant, and its promoter::GUS transgenic lines show strong expression in guard cells.\textsuperscript{33,53,54} For Gγ subunits, RNA gel blots show AGGI and AGG2 expression throughout the plant; however, reporter gene analyses have shown guard cell expression of AGG2 but not AGGI.\textsuperscript{33,53,55} The guard cell expression of G-protein subunits suggests that G-proteins play a role in guard cell signaling and stomatal movement regulation.

Using electrophysiological and pharmacological methods, G-proteins were found to regulate stomatal movements in beans (\textit{Vicia faba}). With the sequencing of the Arabidopsis genome and identification of G-protein encoding genes, the acquisition and characterization of mutants lacking functional heterotrimeric G-proteins facilitated direct examination of the roles of heterotrimeric G-proteins in the regulation of stomatal movements. Overall, ABA is the best-studied regulator of stomatal movements.\textsuperscript{57} ABA inhibits stomatal opening and promotes stomatal closure, suggesting that both the G\textalpha and G\textbeta subunits are involved in stomatal closure triggered by elicitors. Furthermore, in guard cells of G\textalpha, G\textbeta, and G\textbeta silenced plants, elicitor-triggered NO accumulation was abolished. This suggested that G-protein signaling was required to activate the intracellular sources of NO and contributes to the elicitor-induced stomatal closure in \textit{N. benthamiana}.

\textbf{Perspectives}

Heterotrimeric G-proteins play a central role in plant signal transduction. Recently, the direct involvement of G-proteins in defense responses has been shown using elegant biochemical and genetic analyses, although the role of G-proteins in plant defense signaling remains unknown and a systematic analysis of the genes involved in the pathway has yet to be completed. Therefore, combining various genetic, reverse genetic, and biochemical techniques is important to identify receptors, scaffold proteins, negative regulators, and to target effectors that will increase our understanding of these plant defense mechanisms.

Recent studies suggest that PCD and stomatal closure are integral components of plant immunity. Pathogens can produce virulence proteins to suppress PCD and stomatal closure, and plants in turn evolve disease-resistance proteins to recognize these virulence effectors. In the future, elucidating how different pathogen virulence factors inhibit PCD and stomatal closure will be important. An in-depth investigation will greatly increase our understanding of innate immune signaling and facilitate the discovery of methods to manage plant disease.

\textbf{Disclosure of Potential Conflicts of Interest}

No potential conflicts of interest were disclosed.

\textbf{Acknowledgments}

This work was supported in part by the National Natural Science Foundation of China (Grant No. 30871605 and 31071645), the Fundamental Research Funds for the Central Universities (KYZ201105).

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