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A loyal "G"uard

Ryan Kenneally[1,2] and Yangnan Gu[1,2,*]
[1]Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA 94720, USA
[2]Innovative Genomics Institute, University of California, Berkeley, Berkeley, CA 94720, USA
*Correspondence: guyangnan@berkeley.edu
https://doi.org/10.1016/j.chom.2022.09.016

G proteins are conserved eukaryotic signal transducers that play crucial roles in plant development and responses to environmental stimuli. In this issue of *Cell Host & Microbe*, Ma et al. (2022) discover a plant-specific family of kinases that act as bona fide nuclear effectors for G-protein signaling during plant immune activation.

The heterotrimeric G-protein complex is one of the best-characterized cell surface signal transducers and is comprised of Gα, Gβ, and Gγ subunits. In animals, the G-protein complex is activated by plasma membrane-localized G-protein coupled receptors (GPCRs), one of the largest protein families that is intrinsically associated with numerous human diseases and thus among the most targeted molecules for drug development. GPCRs perceive changes in the environment and facilitate GDP-to-GTP (guanosine-diphosphate-to-guanosine-triphosphate) exchange in Gα protein with their guanine exchange factor (GEF) activity. While the GDP-bound Gα is in the inactive, heterotrimeric state, the activated GTP-bound Gα dissociates from the Gβγ dimer, and Gα and Gβγ subsequently work separately with their downstream G-protein effectors to regulate myriad signal transduction events. Gα signal deactivation occurs through GTP hydrolysis by Gα proteins, aided by the GTPase activity-accelerating proteins (GAPs), such as regulator of G-protein signaling (RGS) proteins.

Although plants encode most of the core components of heterotrimeric G proteins, a significant number of G protein subunits evolved unusual structures and biochemical properties compared to their animal counterparts. For example, Arabidopsis has only one canonical Gα (compared to the 23 found in humans) but developed several plant-specific extra-large G proteins (XLGs), which contain a C-terminal canonical Gα domain and a long N-terminal extension bearing a cysteine-rich domain and a nuclear localization or export signal. In addition, functional GPCRs have not been discovered in plants, and guanine nucleotide exchange appears less critical for the activation of plant Gα proteins due to their unexpectedly high GTP-binding capacity and low GTPase activity (Pandey, 2019; Ghusinga et al., 2022). Accumulating evidence supports that, instead of GPCRs, G-protein phosphorylation by receptor-like kinases (RLKs) plays a fundamental role in activating G-protein signaling in plants (Pandey, 2019; Stateczny et al., 2016). RLKs are plasma membrane-localized transmembrane proteins and comprise an expansive family of plant receptors that perceive diverse environmental signals, analogous to animal GPCRs. It was reported by Jian-Min Zhou's group that the Arabidopsis heterotrimeric G protein complex with noncanonical Gα protein XLG2, as well as the GAP protein RGS1, associate with FLAGELLIN SENSING 2 (FLS2), an immune-related RLK that acts as a pattern recognition receptor (PRR). Upon recognizing bacterial flagellin or its derivative flg22, a microbial-associated molecular pattern, FLS2 initiates a phosphorylation cascade among its coreceptor BRASSINOSTEROID INSENSITIVE 1 ASSOCIATED KINASE 1 (BAK1) and receptor-like cytoplasmic kinases, such as BOTRYTIS-INDUCED KINASE 1 (BIK1), to transduce signal across the plasma membrane and activate pattern-triggered immunity (PTI). Activated BIK1 phosphorylates RGS1, which dissociates RGS1 from the G-protein/FLS2 complex and consequently stabilizes XLG2 in the GTP-bound form (Figure 1). Meanwhile, XLG2 is also phosphorylated by BIK1 at multiple serine residues (S141/148/150/151) at its N terminus, and the phosphorylated XLG2 is required for the full activation of FLS2-mediated PTI response (Liang et al., 2016, 2018). This integrated regulation by both the phosphorylation status and the GDP/GTP binding is considered to represent a new model for

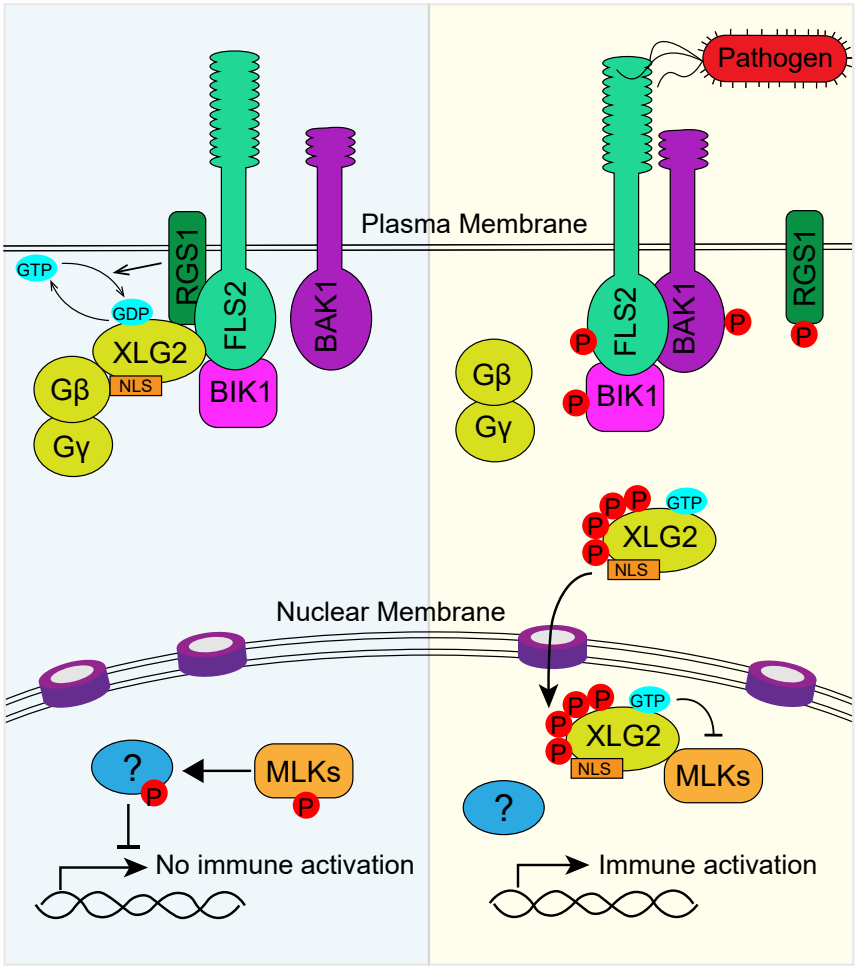

Figure 1. XLG2-mediated G-protein signaling during pattern-triggered immunity in plants
In the inactive state (left panel), the heterotrimeric G protein complex interacts with FLS2 at the plasma membrane. FLS2 also interacts with RGS1, which stabilizes the GDP-bound form of XLG2 by promoting its GTP hydrolyzing activity. In the nucleus, MLKs phosphorylate unknown downstream targets that repress immune activation. Upon recognizing pathogen flagellin (right panel), FLS2 phosphorylates and activates BAK1 and BIK1, which subsequently phosphorylate multiple targets including RGS1 and XLG2. Phosphorylated RGS1 dissociates from FLS2, which consequently results in the stabilization of GTP-bound form of XLG2. Meanwhile, XLG2 is also phosphorylated by BIK1 at multiple serine residues near its nuclear localization signal at the N terminus and dissociates from Gβγ. Phosphorylated XLG2 translocates into the nucleus, where it binds to MLK and inhibits its kinase activity, leading to de-repression of immune responses

G-protein activation in plants and reconcile existing conflicts with the paradigm established in animals (Ghusinga et al., 2022).

Despite the advances in understanding plant G-protein activation mechanism, downstream signaling of plant G proteins remains largely elusive. In this issue of *Cell Host & Microbe*, Ma et al. (2022) applied a combined molecular, biochemical, genetic, and multi-omics approach to the best-studied eudicot plant model *Arabidopsis thaliana* to reveal critical signaling components downstream of the extra-large G protein XLG2 during immune activation. The authors demonstrated that XLG2 transduces signal perception by FLS2 at the plasma membrane to the nucleus, where it interacts with a plant-specific kinase family—MUT9-like kinases (MLKs)—to derepress plant immune response.

Based on their previous work (Liang et al., 2016, 2018), Ma et al. first showed that flg22 treatment could induce nuclear accumulation of XLG2 using both transient analysis and protoplasts prepared from stable transgenic Arabidopsis. The nuclear translocation of XLG2 likely requires its phosphorylation, as the non-phosphorylatable XLG2 mutant was unable to accumulate in the nucleus upon flg22 treatment. Both the nuclear translocation and the immune activity of XLG2 depend on its nuclear localization signal, which, interestingly, locates near the multi-phosphorylation sites at the N terminus. Nevertheless, the phosphomimic mutant is not sufficient to trigger XLG2 nuclear accumulation. These data are consistent with a recent report that the XLG2 N terminus, but not its phosphorylation, is essential for its functional localization and signaling transduction downstream of another PRR CHITIN ELICITOR RECEPTOR KINASE (CERK1) (Petutschnig et al., 2022) and support that XLG2 transduces signal from multiple immune-related RLKs.

To search for potential effectors for XLG2 signaling in the nucleus, the authors performed immunoprecipitation coupled with mass spectrometry using FLAG-tagged XLG2 *in vivo* and identified three out of four homologs of MUT9-like kinases (MLK2/3/4). These MLKs specifically interact with XLG2 in the nucleus, but not with the canonical Arabidopsis Gα protein, and the interaction could be enhanced by flg22 treatment and PTI induction. Subsequently, the authors showed that the *mlk1/3/4* and the *mlk1/2/3* triple mutant displayed enhanced resistance against the bacterial pathogen *Pseudomonas syringae* (*Pst*) and overexpression of single *MLK* had an opposite effect, suggesting that these MLKs play a negative regulatory role in plant immunity, likely in a redundant manner.

Importantly, the authors found that *mlk1/2/3* could almost completely rescue the compromised PTI response and pathogen susceptibility of the *xlg2/3* mutant. RNA sequencing analysis revealed that loss of *XLG2* and overexpression of *MLK4* both resulted in reduced transcriptome response to *Pst* infection, and their responses substantially overlapped, supporting that XLG2 and MLKs function in the same pathway to regulate immunity. Further biochemical analysis demonstrated that MLK-dependent immune suppression requires its kinase activity, which could be strongly inhibited by XLG2 when co-expressed, suggesting that XLG2 may modulate the kinase activity of MLKs to boost PTI activation. Consistent with this hypothesis, the

authors found that the autophosphorylation of MLK4 is significantly reduced upon flg22 treatment, and this response is XLG2 dependent. *In silico* structure modeling and protein docking analysis allowed the authors to identify a β-sheet structure in the MLK carboxyl terminus that is necessary for the physical interaction with XLG2. More importantly, the authors showed that deletion of this β-sheet blocked signal transduction from XLG2 during PTI but did not affect MLK-mediated immune suppression. Taken together, these data exquisitely support a model in which XLG2 translocates to the nucleus upon phosphorylation by RLK signaling and inhibits the kinase activity of MLKs to derepress PTI (Figure 1). Although XLG2 has been shown necessary for resistance against multiple phytopathogens in both eudicot plant Arabidopsis and monocot plant rice (Zhu et al., 2009; Zhao et al., 2022), it is yet to be determined whether the above mechanism is conserved in higher plants.

Ma et al. (2022) and other studies have answered key questions in the plant G-protein signaling puzzle; however, more research is required to reveal the whole picture. One outstanding question is how XLG2 affects MLK kinase activity, and, furthermore, what are the downstream immune targets of MLKs? In addition, because XLGs also interact with RLKs that function in plant growth and development (Pandey, 2019), it would be interesting to determine whether different phosphorylation patterns of XLGs exist upon activation of different RLKs and whether the phosphorylation pattern plays a role in distinguishing downstream signaling pathways and in determining differential interactions with G-protein effectors yet to be discovered. G proteins have also been implicated as key signal transducing elements in abiotic stress responses such as drought, cold, and salt stress. Whether shared G-protein signaling pathways could explain the partial overlap of these different stress responses is an open question. Future studies will also shed light on if other types of posttranslational modifications may participate in fine-tuning G-protein-dependent immune signaling.

ACKNOWLEDGMENTS

The authors are thankful for funding from National Institute of Food and Agriculture (HATCH project CA-B-PLB-0243-H), National Science Foundation (2049931), Society of Hellman Fellows fund, Innovative Genomics Institute, and University of California Berkeley.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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