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Review

Emerging insights into heterotrimeric G protein signaling in plants

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ABSTRACT

Heterotrimeric guanine nucleotide-binding protein (G protein) signaling is an evolutionarily conserved mechanism in diverse eukaryotic organisms. In plants, the repertoire of the heterotrimeric G protein complex, which is composed of the Gα, Gβ, and Gγ subunits, is much simpler than that in metazoans, and the identity of typical G protein-coupled receptors (GPCRs) together with their ligands still remains unclear. Comparative phenotypic analysis in *Arabidopsis* and rice plants using gain- and loss-of-function mutants of G protein components revealed that heterotrimeric G protein signaling plays important roles in a wide variety of plant growth and developmental processes. Grain yield is a complex trait determined by quantitative trait loci (QTL) and is influenced by soil nitrogen availability and environmental changes. Recent studies have shown that the manipulation of two non-canonical Gγ subunits, GS3 (GRAIN SIZE 3) and DEP1 (DENSE AND ERECT PANICLE 1), represents new strategies to simultaneously increase grain yield and nitrogen use efficiency in rice. This review discusses the latest advances in our understanding of the heterotrimeric G protein signal transduction pathway and its application in improving yield and stress tolerance in crops.

1. Introduction

The heterotrimeric G protein complex, which is composed of the Gα, Gβ, and Gγ subunits, is an integral membrane signal transduction component that mediates intracellular responses to external stimuli in all eukaryotes (Gilman, 1987). In mammals, multiple copies of candidate genes encode the heterotrimeric G protein Gα, Gβ, and Gγ subunits. For example, the human genome encodes 23 Gα, 5 Gβ, and 12 Gγ subunits, indicating the potential for over 1380 unique combinations of heterotrimeric G proteins (Jones and Assmann, 2004; Bisht et al., 2011). These G proteins are responsible for coupling a lot of cell surface G protein-linked receptors that are activated by ligands, such as hormones, proteins, or other signaling molecules, and appropriate intracellular effectors that mediate various cellular responses (Wettschureck and Offermanns, 2005).

G protein-coupled receptors (GPCRs) are a kind of seven-transmembrane proteins that bind extracellular signaling molecules, transduce the signals into the cell and finally induce cellular responses. Inside the cell, on the plasma membrane, the Gα and Gβγ dimer classically act as two functional modules. The Gα subunit has both guanosine triphosphate (GTP)-binding and GTPase activity and acts as a bimodal molecular switch, typically with a guanosine diphosphate (GDP)-bound "off" mode and a GTP-bound "on" mode. In the absence of ligands, GDP binds to the Gα subunit, which interacts with the Gβγ dimer to form an inactive heterotrimer. When the GPCR binds to a signaling molecule, it undergoes a conformational change, leading to the exchange of GDP for GTP at the Gα subunit. This promotes dissociation of the heterotrimer into free GTP-Gα and Gβγ dimers, both of which can interact with an array of downstream effectors (Fig. 1). The Gα subunit possesses GTPase activity and can spontaneously hydrolyze the bound GTP, which in turn leads to the regeneration of the GDP-bound form and re-association with the Gβγ dimer, consequently inactivating the G proteins (Cabrera-Vera et al., 2003). In addition, G proteins transmit their signals *via* an activation-deactivation cycle during which GTPase activating proteins (GAPs), regulators of G protein signaling (RGS), are needed in some cases to increase the hydrolysis of GTP to GDP (Ross and Wilkie, 2000).

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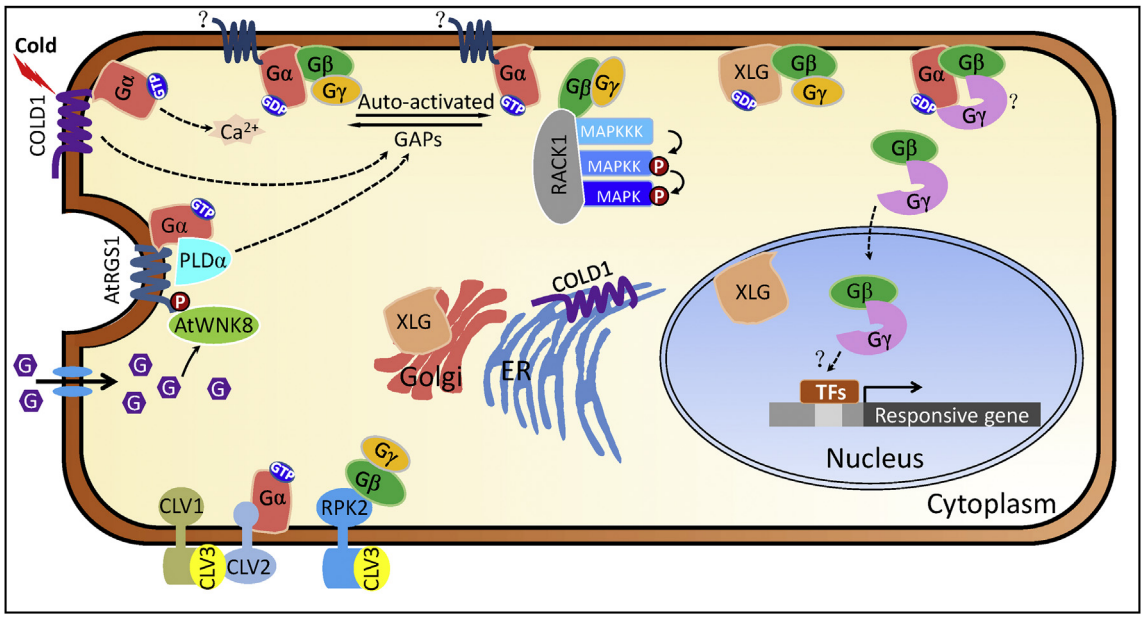

Fig. 1. Schematic representation of heterotrimeric G protein signaling in plants. In plants, the free activated Gα subunit and free Gβγ dimer classically act as two functional modules. The plant Gα protein is similar to the mammalian Gα protein, which has both GTP-binding and GTPase activities and acts as a bimodal molecular switch, typically with a GDP-bound "off" mode and a GTP-bound "on" mode. However, the plant Gα protein is self-activated, and spontaneous fluctuation initiates GDP dissociation and GDP/GTP exchange. RACK1 acts as a scaffold protein that connects three-tier MAPK cascade to the heterotrimeric G proteins. The AtRGS1 and PLDα proteins function as GAPs by enhancing the rate of GTP hydrolysis by the Gα subunit. The AtWNK8 (WITH NO LYSINE KINASE 8) kinase is activated by glucose, and consequently phosphorylates AtRGS1 and induces AtRGS1 endocytosis. The plasma membrane-localized COLD1 protein interacts with RGA1 and functions as a GAP, which in turn accelerates G-protein GTPase activity and triggers Ca^{2+} signaling to mediate chilling tolerance. The maize receptor-like kinase (RLK) FEA2, an ortholog of the *Arabidopsis* CLV2, interacts with the maize Gα subunit CT2. The *Arabidopsis* RPK2, a receptor of the CLV3 peptide, also interacts with the *Arabidopsis* Gβ subunit. In addition to the canonical Gα subunit, the *Arabidopsis* genome encodes three extra-large G proteins (XLG1, XLG2 and XLG3). The XLG proteins are reported to interact with the Gβ subunit and display differential localization to the plasma membrane, cytoplasm or nucleus. In addition, both Gβ and non-canonical Gγ subunits interact with the Gα subunit and form an inactive heterotrimer, whereas the freely released non-canonical Gβγ dimer modulates downstream effectors (i.e., transcription factors, TFs), and consequently regulates the expression of downstream genes.

G protein signaling and its potential application have been extensively investigated in the medical fields, while a growing number of experiments in plants over the last decade have identified several striking differences in the G protein complex and the associated regulatory systems between plants and humans. The most notable difference is that the plant Gα proteins are able to self-activate, and this self-activation removes the requirement for GPCRs (Urano et al., 2012a). Thus far, more and more GPCR-like candidates and other receptor-like kinases (RLKs) have been shown to be involved in plant G protein signaling. Except for the classical GPCRs, plants conserve the core G protein components but show less diversity in gene copy numbers and have higher sequence-structural diversity compared to those of humans. In this review, these similarities and differences from the established norm and the possible roles of G protein signaling in the regulation of plant growth, development, and adaptation to the environment are discussed. In particular, the practical improvements of yield potential in crops by modulating the non-canonical Gγ subunits are also examined.

2. GPCR is not an indispensable sensor for plant G proteins to identify signals

In animals, the Gα subunit is composed of a helical domain and a Ras domain. The Gα protein binds to GDP and forms a stable heterotrimer with the Gβγ dimer in steady-state condition. In the presence of GPCRs, the helical domain changes conformation. This structural change causes GDP to dissociate from the Ras domain, resulting in subsequent GTP binding and activation (Rasmussen et al., 2011). The plant Gα subunits, such as *Arabidopsis* GPA1 and rice RGA1, have highly conserved protein structures compared to vertebrate Gα proteins, while the most striking difference is that the plant Gα subunit is able to self-activate in the absence of GPCRs. The helical domain of the plant Gα protein can spontaneously dissociate from GDP and bind GTP, suggesting that this spontaneous fluctuation removes the requirement for GPCR or other guanine nucleotide exchange factors (GEFs) (Urano et al., 2012a).

Biologists have investigated whether GPCRs are present in plants for a long time. Based on sequence comparison, fold recognition and transmembrane helix prediction analyses, several GPCR-like candidates were predicted to be a GPCR in *Arabidopsis*, including G-coupled receptor 1 (GCR1), GCR2, mildew resistance locus O (MLO) proteins, GPCR-type G protein 1 (GTG1) and GTG2 (Taddese et al., 2014). The GCR1 protein shares sequence similarity to *Dictyostelium* cAMP receptors and was reported to interact physically with GPA1 (Pandey and Assmann, 2004). Genetic studies indicated that both the *GPA1* and *GCR1* genes are required for blue light-mediated photomorphogenic growth and abscisic acid (ABA)-mediated stress responses (Pandey and Assmann, 2004; Warpeha et al., 2006, 2007), and further transcriptome analyses also showed that both *GCR1* and *GPA1* share a substantial number of downstream target genes (Chakraborty et al., 2015). However, no direct GEF activity of GCR1 on GPA1 has been demonstrated, and phenotypic comparisons among the *gpa1*, *gcr1*, and *gpa1 gcr1* mutants suggested that *GCR1* acts independently of G protein complex in at least some aspects of brassinosteroid (BR)- and gibberellin (GA)-regulated seed germination (Chen et al., 2004). The GTG1 and GTG2 proteins, which have high sequence homology to human GPR89a (G protein-coupled receptor 89A), have been proposed to be plant GPCRs due to their interaction with the GPA1 protein and their GTP-binding and GTPase activities (Pandey et al., 2009). However, a recent study showed that the GTG1-GFP fusion protein