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The wiring diagram for plant G signaling

Alejandro C. Colaneri and
The University of North Carolina, Chapel Hill, NC 27599, USA

Alan M. Jones
The University of North Carolina, Chapel Hill, NC 27599, USA

Abstract

Like electronic circuits, the modular arrangement of cell-signaling networks decides how inputs produce outputs. Animal heterotrimeric guanine nucleotide binding proteins (G-proteins) operate as switches in the circuits that signal between extracellular agonists and intracellular effectors. There still is no biochemical evidence for a receptor or its agonist in the plant G-protein pathways. Plant G-proteins deviate in many important ways from the animal paradigm. This review covers important discoveries from the last two years that enlighten these differences and ends describing alternative wiring diagrams for the plant signaling circuits regulated by G-proteins. We propose that plant G-proteins are integrated in the signaling circuits as variable resistor rather than switches, controlling the flux of information in response to the cell's metabolic state.

Introduction

Heterotrimeric, guanine-nucleotide-binding proteins (G-proteins) are conserved elements in, arguably, the most important signal transduction cascades. Genes encoding G-protein components are found in most plants [1,2], but the molecular mechanisms were derived primarily from animal cell studies. Animal and plant heterotrimers are composed of two functional modules, the α subunit and the Gβγ, which are each delimited to the plasma membrane (PM) by lipid tethers [1,2]. The repertoire of genes encoding subunits in plants is small in comparison with animals, making Arabidopsis a good genetic model for G protein studies. Plant G-proteins mutants have altered response to light, glucose, abscisic acid, auxin, jasmonic acid, gibberellins, sphingolipids, and pathogens. In both rice and Arabidopsis, mutations in genes encoding G signaling elements affects hypocotyl length, leaf size and morphology, plant height, silique size, and grain shape and size. Regulatory and signaling pathways for the physiological processes described above are, for the most part well characterized, however a unified molecular mechanism still needs elaboration.

Corresponding authors: Colaneri, Alejandro C. (alejandro.colaneri@outlook.com) and Jones, Alan M (alan_jones@unc.edu).

The G-protein paradigm: animal heterotrimeric G-proteins are molecular timers that control the amplitude and duration of a broad spectrum of instantaneous responses

Animal heterotrimeric G-proteins directly couple the receptor (aka GPCR) with downstream effectors. In the absence of the stimulus, nearly all the cellular G-proteins are at rest at the PM in a heterotrimeric inactive conformation that is bound to GPCRs. In this inactive state, the Gα subunit's guanine-nucleotide-binding site binds GDP. Upon ligand binding, the receptor-agonist complex acts upon the Gα subunit as a guanine nucleotide exchange factors (GEF) and facilitates the diffusion of GDP away from the Gα subunit-binding site, promoting the exchange of GDP for GTP. This event marks the activation of the α-subunit and the Gβγ, which in turn interact with their own set of effector proteins to initiate downstream signaling cascades [3]. The amplitude of these responses depends on the ratio of $\alpha^{GTP}/\alpha^{GDP}$ (activated/deactivated G-proteins) at steady state [4]. Under persistent stimulation, the Gα guanine-binding-site is quickly and constantly replenished with GTP favoring the accumulation of active α^{GTP}. Thus, in animals, G proteins transduce information by rolling the GTPase cycle for the duration of the stimulus. Gα subunits are slow GTPases and the fast deactivation that follows the removal of stimulus depends on GTPase Activating Proteins (GAP) [5]. The GAPs for heterotrimeric G proteins are Regulator of G-protein Signaling' (RGS) protein. GAPs not only ensures timely termination of the response but also potentiate the reaction by speeding up the GTPase cycle during stimulation in a process called dynamic scaffolding [4,5]. In short, animal heterotrimeric G proteins are GTP/GDP-gated molecular timers, which respond instantaneously. Both responsiveness and amplitude of the signal-output are controlled independently by the concerted cooperation of GEFs and GAPs [4,5].

Arabidopsis G-proteins: *the plant paradigm*

Many of the key kinetic principles that control animal G-protein pathways do not apply to plants. Biochemical characterization of the plant G-protein activation-deactivation cycle derives mainly from research on the Arabidopsis heterotrimeric alpha subunit (AtGPA1) and Arabidopsis Regulator of G-protein signaling 1 (AtRGS1), but the principles extend to most other plants [2,6••,7,8•].

Activation

Since the initial discovery of G-proteins in model plants, most researchers adopted the animal paradigm [15] concluding that plant G-proteins operate as switches that set the "on" and "off" states of signaling pathways. Later, we learned that the kinetic properties of plant Gα differ diametrically from animals and therefore, the animal paradigm cannot apply [8•, 12••]. AtGPA1 has an intrinsically high rate of nucleotide exchange. AtGPA1 spontaneously exchanges GDP for GTP thus they do not require a GEF such as a GPCR in animals. Consistent with this property, no receptors with GEF activity are found in plants [48,49]. If plant heterotrimeric G-proteins lie inactive on the PM to be activated by extracellular stimuli, (off-on switch) the existence of plant-specific Guanine nucleotide Dissociation Inhibitors like protein (GDI-like) is needed [10] but, to date, non were found. In the absence

of an extrinsic source of regulation, the plant Gα subunit would exist in a constitutively activated state, (α^{GTP}) freeing most of the Gβγ. Thus, in contrast to animals, the signals that control G-proteins activity would promote the formation of heterotrimer. So far AtRGS1 is the only known G-protein modulator able to perform this function.

A speculative alternative to the two-state model of regulation is that plant G protein components are tunable modulators of signal-output that work not by the absolute number of activated subunits over time but rather by the ratio of active to total G protein component. In this scenario, the modulatory capacity of plant G-proteins is tuned by reaching steady state at different $\alpha^{GTP}/\alpha^{GDP}$ and $^{freed}\beta\gamma/^{het}\beta\gamma$ ratios. This mechanism converts a signal-dependent, two-state output into a continuous dose-dependent output.

Deactivation

As in animals, inactivation of plant Gα subunits depends on hydrolysis of bound GTP [11,12••]. Hydrolysis of GTP is the rate-limiting step for the plant $\alpha^{GTP}/\alpha^{GDP}$ cycle. RGS proteins accelerate the intrinsic GTP hydrolysis rate of Gα subunits *in vitro* [8•] and *in vivo* [13]. Whether the plant RGS GAP activity accelerates signal termination and/or attenuates signaling by lowering fractional activation at the steady state cannot be discerned with *in vitro* assays. The regulation of amplitude and rate of deactivation may be completely different for plant G signaling. The animal Gα repetitively cycles through the duration of the stimulus, and the fraction of each cycle in its active conformation determines the amplitude of the signal [4]. GAPs can shorten the time that Gα s spend activated. But if the deactivation occurs in the vicinity of a receptor, it shortens the time that $G\alpha^{GDP}$ needs to interact with the GEF, speeding the re-entry into the active state. This kinetic scaffolding mechanism allow GAPs to potentiate G-signaling [4,5]. However, given the kinetic properties of plant Gα, this scaffolding mechanism is irrelevant for plant G cycling, prompting us to rethink the role of plant RGS proteins.

Activation and Deactivation During Glucose Stimulation

Plant RGS proteins have a seven-transmembrane domain that topologically resemble animal GPCRs. Although no GEF activity has been demonstrated for this domain, we confirmed a model in which at the idle state Gα operates in a futile cycle in association with the Gβγ and RGS. Glucose stimulation frees the Gβγ, which recruits WITH NO LYSINE (WNK) kinases. There are 11 WNK Ser/Thr kinases in Arabidopsis and 3 of them (WNK8, WNK1 and WNK10) interact and phosphorylate serine residues in the C-terminal region of AtRGS1 [6••]. Phosphorylation is required for AtRGS1 endocytosis in response to glucose however different WNKs operate at different time scales and glucose concentration allowing the cells to perceive both, the intensity and the temporal dimension of the glucose stimulus [14••]. Following RGS internalization and the physical uncoupling from GPA1 the heterotrimer is dissociated, allowing the Gα monomer and the Gβγ dimer to interact with their effectors [14••]. The extension of AtRGS1 endocytosis is a glucose dose dependent process.

Physiological functions and G-protein signaling associated cascades

Studies conducted mostly with Arabidopsis and rice mutants revealed species-specific, tissue-specific and development-specific phenotypes [15]. Both Arabidopsis and rice have the potential to create three to five different heterotrimers (combinations of one alpha, one beta and one of the 3–5 gamma subunits, depending on species) [15]. From the beginning of plant G protein research, the simplicity of the plant G protein repertoire promised to simplify the interpretation of phenotypes, e.g. to establish associations between phenotypes and subunits or to assign specific phenotypes to failures in the activation mechanism. However, systematic analyses of epistatic interactions between genes encoding the different heterotrimeric subunits revealed more complexity than expected [15,16]. If we follow the animal paradigm, expectations of phenotypes for specific mutations in the Gα subunit should constitutively activate Gβγ-dominant pathways. However a quantitative comparison of *gpa1* and *agb1* developmental phenotypes in Arabidopsis growing under standardized non stressed conditions showed that this prediction is not always corroborated [17]. Unique phenotypes for either of the G-protein subunits have also been found in responses to biotic stress. For example, AGB1 mediates cell death downstream of the receptor-like kinase BIR1. Mutations in *BIR1* activate cell death and defense responses but these phenotypes are reverted by a null mutation in *AGB1*. However, this BIR1-AGB1 modulated pathway is insensitive to mutations affecting *GPA1* [18••]. Freedom from control or influence of the other G-subunit is also suggested in PAMP triggered immune responses mediated by FLS2, EFR and CERK1 [18••]. In general, the role of AGB1 and AtGPA1 in immunity varies depending in the plant–pathogen interaction [19,20••,21–25]. In more general terms the data indicates that the mechanism of activation varies with the stimulus and pathway and suggest that the existence of alternative mechanisms that activate or deactivate Gα and the Gβγ without the participation of a heterotrimeric state.

Agonist

Plants carrying mutations affecting G-protein subunits display phenotypes associated with the actions of major phytohormones (e.g. gibberellins, brassinosteroids, abscisic acid, jasmonic acid), agents that triggers oxidative stress (UV, ozone, H_2O_2, NaCl, pathogen associated molecular patterns [PAMPs], tunicamycin), and molecules that signal for energy and growth (glucose, ATP, CLAVATA3 peptide). To date, there is no biochemical evidence for ligand binding to a receptor in the plant G protein pathway. The efficacy of these potential agonists in the plant G-protein pathways needs to be assessed with suitable assays, preferably assays that measure the relationship between agonist concentration and the fractional activation of Gα at steady state but there is no *in vivo* reporter for GTP-bound Gα subunit at this time. The best candidate for an agonist to date is glucose [6••,14••]. Glucose induces the internalization of the 7 transmembrane RGS1 proteins [6••,14••] and induces interaction between AtRGS1 and AtGPA1 [12••]. The activation and relaxation times are in the order of minutes, much slower than the times in animals. The activation kinetics is influenced not only by the concentration of glucose but also by the exposure time. G-proteins are similarly activated by acute glucose doses or prolonged exposure to low glucose concentration. This property of reciprocally sensing dose and duration is another difference between plant and animal G signaling.

Receptors

Given that plant Gα subunits may dispense with GEFs for activation, another family of receptors might better fit the kinetic properties of plant G-proteins. The hypothesis that receptor-like kinases (RLK) discriminate the potentially many extracellular signals that regulate the activation state of G-proteins has gained support over the time. G-protein mutant alleles were isolated from genetic screens for components in diverse RLK signaling pathways [18••,26,27••]. In Arabidopsis, the *agb1*-4 allele rescued a severe growth phenotype associated with the *bir1*-1 allele. BIR1 is a RLK that negatively regulates activation of defense mechanism by the RLK SOBIR1. The data suggest that AGB1 mediates activation of cell death downstream of the SOBIR1. In addition AGB1 mediates immune responses downstream of several pattern-recognition receptors (PRRs). PRRs are RLKs that interact with PAMPs and boost the plant innate immune system [18••]. PAMP-triggered immunity assays showed that *agb1*-4 plants are not able to boost immunity after treatment with flg22, elf18 and chitin [18••]. In maize, the RLK FASCIATED EAR2 (FEA2) is an ortholog of the Arabidopsis leucine-rich repeat receptor CLAVATA 2 (CLV2). CLV2 interacts with the secreted peptide CLAVATA3 (CLV3) and inhibit growth of the shoot apical meristem (SAM). Genetic evidence suggests that a null allele of *COMPACT PLANT2* (*CT2*) an ortholog of *AtGPA1* is epistatic to *FEA2* in a pathway that determines the size of the SAM [27••]. Biochemical data suggest that FEA2 and CT2 interact at the PM. *ct2* embryos have reduced sensitivity to CLV3. Together, the data suggest that FEA2 couples CT2 to transmit signals that control proliferation of stem cells in the maize SAM [27••].

In summary, compelling evidence suggest that signal transduction by G-proteins downstream of RLKs occurs in plants. However, much work still needs to be done to understand the functional relationship between RLKs and G signaling.

Second messengers

Second messengers relay signals both downstream and upstream of the heterotrimer. For example, inhibition of stomatal opening, stimulation of stomatal opening and elicitation of stomatal closure are separate phenomenon controlled by different arrangements of environmental signals, hormones, second messengers and G-proteins. Originally the second messenger sphingosine-1-phosphate (S1P) was found to mediate ABA inhibition of stomatal aperture upstream of G-proteins [28]. Later it was suggested that AtGPA1 is a node of convergence for signals mediated by ABA-S1P and flg22-FLS2 [29]. Downstream of AtGPA1, the second messengers H_2O_2, nitric oxide (NO) and Ca^{2+} regulates the activation of potassium channels that inhibited the stomatal opening [29]. AtGPA1 is also involved in UV-B induced stomatal closure in a linear pathway that involves AtGPA1 dependent production of ROS and NO [30•]. Stomatal closure is also induced by extracellular Ca^{2+} via an extracellular calmodulin that signal through AtGPA1. Production of ROS and NO by NITRIC OXIDE ASSOCIATED1 and RESPIRATORY BURST OXIDASE HOMOLOGS (AtRBOH D and F) are also downstream of AtGPA1 in this signaling cascade [31]. Finally, stomatal opening is mediated by AtGPA1 downstream of extracellular ATP. In turn, AtGPA1 stimulates production of ROS by the NADPH oxidases AtRBOH D and F. The oxidative burst triggers H^+ efflux and Ca^{2+} influx. A rise in cytosolic Ca^{2+} in guard cells is evoked by a variety of processes that regulates stomatal movements [32]. The same signals

under the control of AtGPA1 evokes opposite outcomes in the stomata movement suggesting that key element of these pathways have not yet been identified.

Effectors

Plant G-proteins evolved their own set of molecular surfaces to regulate effectors. The many phenotypes attributed to G-proteins may be explained by multiple coupling to many effectors. An exhaustive screen using yeast complementation assays detected a comprehensive list of putative plant G-protein effectors [33•] revealed many enzymes and transcription factors that are involved in the regulation of the cell wall composition, alongside with altered xylose content in the cell wall of G-protein mutants [33•]. This is consistent with a transcriptomic analysis of *agb1* and *agg1 agg2* mutants infected with a necrotrophic fungi *P. cucumerina* showing misregulation of genes involved in cell wall composition [20••]. Genes that determine the structure and composition of the cell wall were significantly reprogrammed after the infection in both *Col*-0 and G-protein mutants. Some of these genes were found differentially regulated between genotypes (e.g. *Col*-0 vs *agb1* alleles). In contrast to G-proteins mutants, which have a decreased xylose content and increased susceptibility to *P. cucumerina*, the *det3*, *xyl1-2* and *irx6-1* mutants with increased level of xylose or xyloglucan showed decreased susceptibility to the *P. cucumerina* infection [20••]. Mutations of glucuroxylan-synthesis genes enhance tolerance to abiotic stress as well [34]. Mutations affecting acetylation of xylans induce a constitutive stress phenotype [35]. For example, plants lacking TBL29 protein in the Golgi, a member of the TRICHOME BIREFRINGENCE-LIKE (TBL) family are tolerant to drought, salt and cold and have elevated levels of ABA under normal growth conditions [36]. TBL29 contains a DUF231 domain, which is required for O-acetylation of xyloglucans [37]. Interestingly in a trascriptomic study designed to identify glucose-induced, AtRGS1-dependent genes, the *TLB26* gene was the most attenuated in the *rgs1*-2 mutant compared to *Col*-0 [38]. TBL26 also contains a DUF231 domain and it is located on the Golgi membranes (S. Wolfenstetter, unpublished data). In summary, both xylose content and xylan acetylation contribute to the modulation of stress responses. G-proteins regulate these process at different levels: **1)** transcriptional regulation of cell wall biosynthetic genes [20••], **2)** glucose dependent control of xylan acetyl transferase levels in the Golgi [38], **3)** control of sugar transport to the Golgi [39], and **4)** interaction with putative effectors involved in cell wall synthesis or remodeling [33•]. However, it is not clear to what extent the cell wall related enzymes found in the interactome are real effectors. A molecular and kinetic characterization of each of these interactions is needed.

The G-protein interactome is a rich source for identifying novel plant G-protein effectors. To discover new G signaling elements, we performed gene ontology enrichment analysis on these interactors. The resulting functional profiles confirmed many of the previously characterized plant G-protein functions but also suggested new signaling clusters [40]. The most important role of plant G-proteins suggested by this analysis was the response to abiotic stress; similar results were associated with interactors in pea [41]. The response to salinity appeared as the most relevant biological process associated to the interactome and the involvement of plant G-protein in Na^+ stress was corroborated [40]. Na^+-induced senescence is accelerated in *agb1*-2 mutants but delayed in *gpa1*-4 and *rgs1*-2 mutants. The

null *AGB1* allele is epistatic to the null At*RGS1* allele [40]. This suggests that AGB1 is the primary G-protein element mediating salt tolerance or recovery and that AtRGS1's GAP activity on AtGPA1 reduces free Gβγ through heterotrimer formation. This was confirmed using point mutations that disrupt heterotrimer formation [42]. Both AtGPA1 and AGB1 are positive regulators of shoot cell proliferation. Thus, inactivation of plant G-proteins could be a mechanism by which stress attenuates the pace of growth. However, AGB1 also negatively regulates cell death [43•]. Thus, modulation of the unsequestered/heterotrimeric AGB1 ratio during the stress responses may shift the balance between death and growth.

The involvement of G-protein in the balance between growth and senescence during Na^+ stress appears to be a general mechanism in plants. Recently, we showed that *ct2* and *d1*, the KO alleles for the Gα subunit in maize and rice, respectively, are tolerant to Na^+ stress [50].

AtRGS1 regulates the active/inactive G-protein ratio, in part, by its own trafficking. On the PM, AtRGS1 decreases the active pool of AtGPA1 and when endocytosed, the ratio increases [40]. AtRGS1 internalization occurs 10 to 16 h after exposure to Na^+ coinciding with the growth recovery phase [44] and the time that glucose levels increase [45,46]. Thus, glucose may be the molecule that signal RGS internalization during salt stress.

AtRGS1 may respond to changes in the carbon-energy metabolism caused by metabolic stress induced by salt [47]. Once the system reaches homeostasis and growth resumes, the level of internalized RGS will determine a new ratio of active/inactive G-protein that will set the new pace of growth. This relationship between metabolism, G-proteins and salt stress is seen in the G protein inter-actome. Many enzymes in glycolysis and at the entrance into the tricarboxylic acid cycle are potential partners to Arabidopsis G-proteins (Figure 1).

Conclusions and remarks

A major conundrum in plant G-protein research is the stark contrast between the simple composition of the heterotrimers and the complex repertoire of phenotypes in which they were found involved. A major effort was made to identify receptors that discriminate signals upstream of plant G-proteins, but so far the search produced no protein worthy of a "receptor" moniker in plant G signaling [48,49] except AtRGS1 [13] Figure 2 provides an alternative idea. In this model, G-proteins exert a tonic level of modulation in the activities of their effectors. Under favorable conditions, G-proteins promote growth to optimal levels either stimulating growth-promoting-effectors or inhibiting growth-inhibitors. Environmental stresses may uncouple G-proteins from these effectors, and consequently bring growth to its basal level. For example, it was recently suggested that plants lacking the Gα subunit are already growing at basal level and therefore growth is less affected by salt stress in relation to WT [50]. Thus the G-protein-effector complex behave as variable resistors (rheostats) in the signaling circuits that control cell expansion and cell proliferation (Figure 2). In summary, G-proteins modulate the intensity of the signal output (indicated by the symbol ⏦ in Figure 2C) downstream of the master regulators with associated phenotypes related to growth. We speculate that plant G-proteins are active by default, consistent with the kinetic properties of the plant Gα subunits. Modulating proteins such as AtRGS1 shift the fractional pool of active G-proteins. Rather than having bimodal "on" and

"off" states, plant G-proteins reach steady state at different active/inactive ratios. We speculates that under certain stresses (e.g. salt stress) the level of active Gα subunit and Gβγ is controlled by the energy state of the cell, which is sensed by the glucose level, and transduced by AtRGS1. This connects metabolic signaling with other related signaling pathways to produce a coherent and integrated response. We acknowledge that the signaling circuits presented in Figure 2 do not fit all the aspect of plant G-protein signaling. As discussed above there are a number of signaling processes, which appears to be specific for only one of the heterotrimeric subunit, yet so far do not involve an RGS protein. Moreover, the absence of a 7TM RGS protein in the monocot lineage challenges the generalization of current models and creates the need to rethink alternative mechanism or additional components for plant G-protein activation and signaling.

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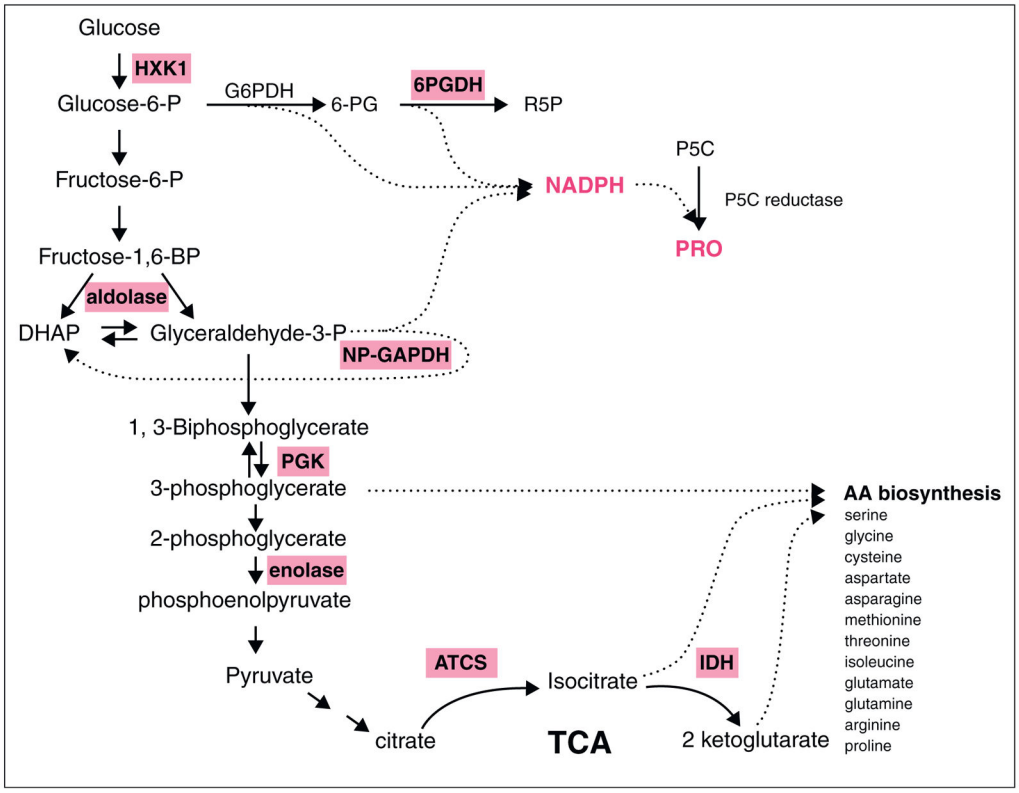

Figure 1. G-proteins interact with Metabolic Enzymes

Enzymes in glycolysis and at the entrance into the tricarboxcylic acid cycle (TCA) that interact with G-proteins are indicated in pink boxes. HXK1, hexokinase, 6PGDH, 6-phosphogluconate dehydrogenase; NP-GAPDH, non-phosphorylating glyceraldehydes 3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; ATCS, *Arabidopsis thaliana* citrate synthase; IDH, isocitrate dyhydrogenase. The subcellular locations of the orthologs for IDH and ATCS are not certain. The other enzymes are cytosolic. Product abbreviation: 6PG, 6-phospho-D-glucono-1,5-lactone; R5P, ribulose 5 phosphate; P5C, pyrroline-5-carboxylate; PRO, proline; DHAP, Dihydroxyacetone phosphate.

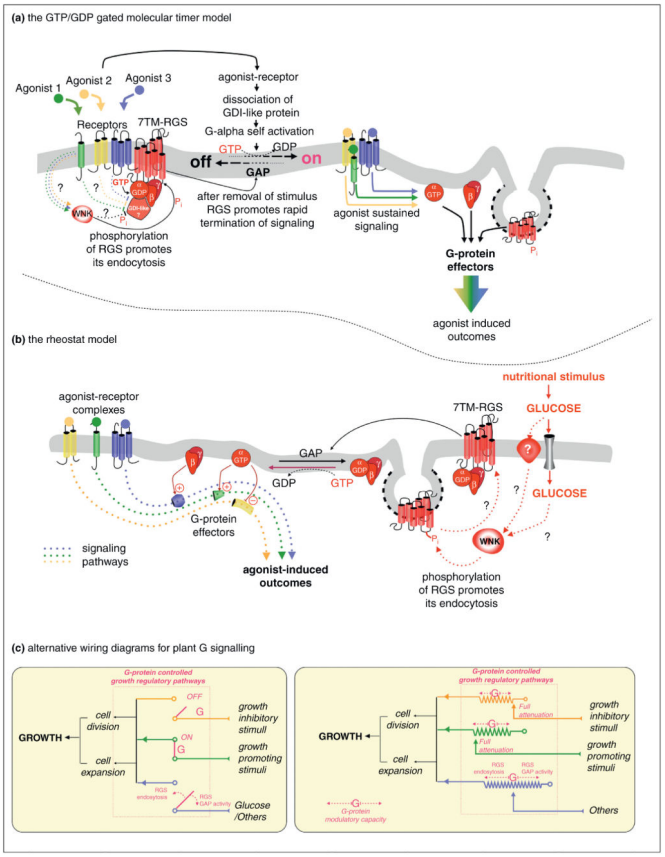

Figure 2. G-protein regulation of plant signaling pathways

(A) In this model the activation of the heterotrimeric G-protein is an obligated step to convey information through the regulated pathway, (*i.e.* activation of G-proteins depend on the transduced signal). G-proteins link receptors and operate as switches. The binding of an agonist to a coupled-receptor releases the heterotrimer from an inhibitory complex (e.g. with a GDI like protein). This promotes Gα self activation, dissociation of the heterotrimer and activation or inhibition of G-protein effectors. G-proteins convey information during receptor stimulation (ON state). Deactivation of receptors lead to deactivation of the G-proteins. GAP activity must accelerate deactivation and stabilize the heterotrimer in its OFF state. How receptors activate plant G-proteins is still unkonw and could include one or more intermediary proteins/steps (dashed arrows with question marks). **(B)** In this model the activation of G-proteins is not controled by the trigger-signal of the singnalling pathways, but rather controlled by the cell's metabolic state. Thus G-proteins integrate metabolic information in the signaling network that controls cell division and cell expansion (i.e. growth). The free G-protein subunits, rather than the heterotrimer, are the ones integrated in the signaling pathways exerting a constitutive modulatory activity. Sustained activation of G-proteins involves the concerted activities of different WNK kinases, which encode intensity and duration of glucose signal to regulate RGS endocytosis. How glucose activates WNKs is still unkonw and could include one or more intermediary proteins/steps (dashed arrows and question marks). Phosphorylation of RGS promotes its endocytosis and physically uncoupling from the G-protein complex, allowing spontaneous nucleotide

exchange in the Gα subunit and release of the Gβγ dimer. **(C)** In analogy with electronic circuits, the signal transduction networks are interconnected modules arranged to convert inputs in outputs. The panel in the left represent a wired diagram of this signaling network where G-proteins operate as switches in the circuits that convey signals triggered by development, stress, hormones, energy, nutrient, and diverse mitogens. Under situations that favor growth, the G-protein coupled pathways that respond to growth-inhibitory-stimuli (yellow) will be switched off at the G-protein module. The opposite (switch on) will occur in the G-protein coupled pathways that respond to growth promoting stimuli (green). In some cases this switch will be controlled by RGS endocytosis/RGS GAP activity, e.g. glucose (blue). The panel in the right represents a wired diagram of the rheostat model in a situation favorable to growth. In this example G-proteins are imposing maximum resistance to transmit information along pathways responding to growth-inhibitory-stimuli (yellow) or not resistance in pathways responding to growth promoting stimuli (green). The G-protein modulatory capacity represented by the variable resistor symbol will depend on the cellular glucose content. In blue we show a pathway that convey information to boost cell division or cell expansion. Under nutrient availability glucose will promote RGS endocytosis shifting the G-protein rheostat to minimum resistance. Opposite, low levels of glucose will promote G-protein inactivation trough RGS GAP activity shifting the G-protein rheostat to maximum resistance. Plants lacking one or the other G-subunit still convey information trought the G-protein associated pathways but with modified intensity.