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Modulation of Root Cell Division by the Heterotrimeric G-proteins in *Arabidopsis*

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ABSTRACT

The root is an ideal system to study cell division *in situ*. The root system originates from a root primordium that is formed during embryogenesis. Stem cells of the root apical meristem (RAM) essentially give rise to all cell types in the primary root. Therefore, the growth of the primary root is in part a consequence of cell cycle maintenance in the RAM. Lateral roots are initiated from the pericycle cells located adjacent to protoxylem poles at some distance from the RAM. The formation of lateral roots requires meristem formation by pericycle founder cells, a process that involves re-entry or activation of the cell cycle. The cell division in the RAM and the formation of lateral roots are regulated by both intrinsic and environmental stimuli. Substantial evidence supports the notion that cell division in the RAM and the activation of pericycle founder cells use distinctive mechanisms. Recent research indicates that the heterotrimeric G-protein subunits have differential roles in the modulation of cell cycle maintenance in the RAM and in the activation of pericycle cells during lateral root formation. Future studies are expected to lead to the determination of the molecular mechanism through which the heterotrimeric G-protein complex executes its modulatory role in root cell division.

Keywords: AGB1, GPA1, lateral root primordium (LRP), pericycle cell, root apical meristem (RAM)
Abbreviations: **AGB1**, Arabidopsis heterotrimeric G-protein β subunit; **CDZ**, cell division zone; **CEZ**, cell elongation zone; **GPA1**, Arabidopsis heterotrimeric G-protein α subunit; **GPCR**, G-protein-coupled receptor; **GUS**, β-glucuronidase; **IAA**, indole-3-acetic acid; **LRP**, lateral root primordium; **NAA**, 1-naphthalene acetic acid; **NPA**, naphthylphthalamic acid; **QC**, quiescent center; **RAM**, root apical meristem; **RGS**, regulator of G-protein signaling; **RHZ**, root hair zone

CONTENTS

INTRODUCTION

In addition to support and fix a plant to soils, the root system is critical for absorbing and transporting water and nutrients from soil to the aerial part of a plant. More importantly, the root system senses biotic (such as pathogens and insects) and abiotic stresses (such as salinity and drought) in soils, therefore is of critical importance for the wellness and fitness of a plant. In model plant Arabidopsis, the root system originates from a few embryonic cells that are formed during embryogenesis. These embryonic cells undergo cell division and cell elongation, and form a root meristem consisting of several different types of root cells, a process that involves pattern formation. When an embryo matures, essentially all cell types have been formed and all cells stay in a relatively inactive status. Upon the presence of suitable environmental factors such as water, oxygen, and suitable temperature, and the absence of inhibitory substances such as abscisic acid, the embryonic roots are ready to penetrate the seed coat during the seed germination process. Subsequently a root system is established through a spatial and temporal cell division and cell elongation. Some root epidermal cells can differentiate into root hairs in a position-dependent manner, and some pericycle cells can be activated to form lateral roots. The root system is essential for the growth, development, and survival of a plant. In this article, we mainly focus on the post-embryogenic root cell division and its regulation in the model plant Arabidopsis. For the cell division of root cells and pattern formation during embryogenesis, readers are referred to two recent excellent review articles and references therein (Scheres *et al*. 2002; Willemsen and Scheres 2004).

CELL DIVISION IN THE ROOT APICAL MERISTEM

In Arabidopsis, once the embryonic root has protruded through the seed coat, the stem cells of the root apical meristem (RAM) generate all cell types through stereotypic divisions followed by cell elongation and differentiation (Scheres *et al*. 2002). Cell division in the primary root is restricted to the root tip region in a zone designated as cell division zone (CDZ). By using a G2-to-M-specific cell cycle marker line *CYCB1;1:GUS* (β-glucuronidase), these cells can be easily recognized (**Fig. 1**). The quiescent center (QC)

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Invited Mini-Review

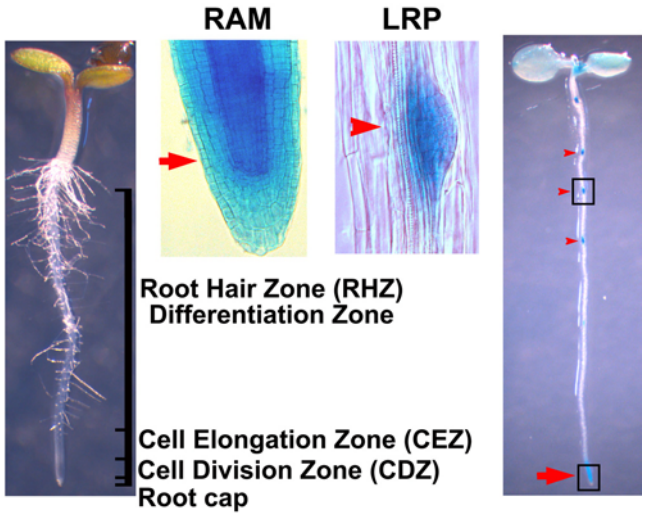

Fig. 1 Root apical meristem (RAM) and lateral root primordium (LRP) in Arabidopsis seedlings. Shown in the left is a 3 d-old, light-grown wild-type (ecotype: Columbia-0) seedling. The regions of root cap, cell division zone (CDZ), cell elongation zone (CEZ), and root hair zone (RHZ) or differentiation zone are indicated. Shown in the right is a 6 d-old, light-grown *CYCB1;1:GUS* (*β-glucuronidase*) seedling (in Columbia-0 ecotypic background) that was stained with the GUS substrate X-Gluc (5-bromo-4-chloro-3-indolyl β-D-glucuronide). Arrow indicates the RAM, and arrowheads indicate LRPs. Shown in the middle are the enlarged images of RAM and LRP that are boxed in the histochemically stained *CYCB1;1:GUS* seedling (right).

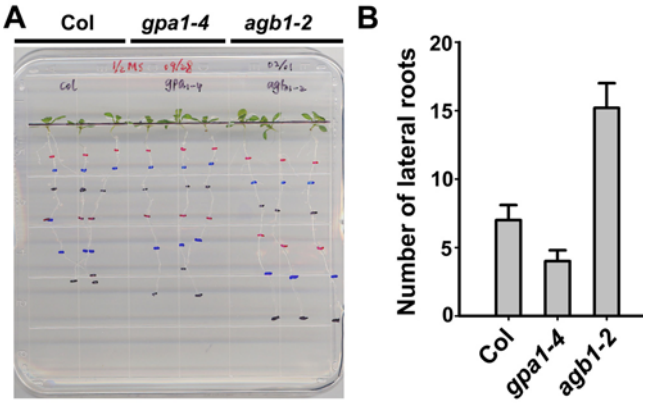

Fig. 2 Root phenotypes of Arabidopsis heterotrimeric G-protein α (*GPA1*) and β subunit (*AGB1*) mutants. (**A**) The growth of primary roots of *gpa1-4* and *agb1-2* mutants. *gpa1-4* is a loss-of-function allele of Arabidopsis heterotrimeric G-protein α subunit (Jones *et al*. 2003). *agb1-2* is a loss-of-function allele of Arabidopsis heterotrimeric G-protein β subunit (Ullah *et al*. 2003). Both *gpa1-4* and *agb1-2* are in Columbia-0 (Col) ecotypic background. Wild-type and mutants were grown vertically on Murashige and Skoog (MS) medium for three days. Then identical seedlings from each genotype were transferred to fresh MS medium. The growth of the primary root was monitored every 24 hours by marking the position of root tip. (**B**) The number of lateral roots in *gpa1-4* and *agb1-2* mutants. The number of lateral roots was counted from 9 d-old light-grown seedlings. Shown are the means of number of lateral roots of 10 seedlings ± S.E.

in the root tip region plays a critical role in maintaining the root meristem identity and the proper cell division in the CDZ. Subsequently, divided cells undergo cell elongation. These elongating cells form a zone, designated as cell elongation zone (CEZ). Therefore, cells in the CEZ are much longer than cells in the CDZ (**Fig. 1**). When cells in the CEZ reach their maximal length, they gain differentiation potential. These elongated cells form a zone designated as the cell differentiation zone or root hair zone (RHZ). The RHZ can be recognized by the appearance of root hair derived from certain root epidermal cells (H-cells) that are located at the junction of two underneath cortex cells (**Fig. 1**). The CDZ, CEZ and RHZ can also be distinguished by the appearance and maturity of the vascular system. For example, no vasculature is formed in the CDZ. The vasculature is developing in the CEZ, and becomes fully developed in the RHZ.

Primary root growth is a combined effect of cell division in the CDZ and cell elongation in the CEZ. It is possible to indirectly calculate both parameters. The growth rate of a primary root can be monitored by measuring the extension of root within a certain time period (**Fig. 2**), and calculated as μm per hour. Because the root cells reach their final length in the RHZ, the length of root cell (i.e. cortex cell) can be measured (as μm) in this region. Therefore, the rate of cell production (number of cells/hr) from the RAM can be expressed as the root growth rate (μm/hr) divided by the average root cortex cell length (μm) in the RHZ. Recently a confocal tracking system has been established to analyze the numbers and orientations of cell divisions in root stem cells (Campilho *et al*. 2006). Using this technique, now it is possible to track cell division timing of root stem cells.

LATERAL ROOT INITIATION BY THE ACTIVATION OR RE-ENTRY OF PERICYCLE FOUNDER CELLS

Lateral root formation involves two major steps: cell cycle activation of the pericycle founder cells and the subsequent establishment of a new meristem (Malamy and Benfey 1997). Lateral roots are initiated from the pericycle cells located adjacent to protoxylem poles at some distance from the root tip (**Fig. 1**), but lateral root founder cells become specified in young root parts before differentiation is accomplished (de Smet *et al*. 2006, 2007). In *Arabidopsis*, the first lateral root is formed in the RHZ at a position that corresponds with the region where pericycle cells progress via the S to G2 phase (Beeckman *et al*. 2001). Then other lateral roots occur in an acropetal pattern (Dubrovsky *et al*. 2006), a process that requires a reentry or activation of cell cycle in the pericycle founder cells. Pericycle founder cells divide transversally and anticlinally, resulting in organized new layers of cells. The lateral root development can be generally categorized into seven stages (Malamy and Benfey 1997). Each stage is characterized by the type and layer of cells in the lateral root primordium (LRP). The LRP can be easily detected in the *CYCB1;1:GUS* line, a well-characterized cell cycle maker for active cell division (**Fig. 1**). Once the LRP has emerged from the root epidermis, it is regarded as a lateral root. The lateral root meristem has an identical cell organization as the RAM. Cell division, elongation, and differentiation in a lateral root follow an almost identical process as that in the primary root. Cell cycle activation in the pericycle founder cells represents a critical point for the regulation of lateral root formation, and has become a focused issue in several excellent review articles (Casimiro *et al*. 2003; Malamy 2005; de Smet *et al*. 2006; Fukaki *et al*. 2007). Because formation of the lateral root results from cell cycle activation or re-entry in the pericycle founder cells, cell division in the pericycle founder cells can be indirectly measured by counting the number of lateral roots, or the number of LRPs and lateral roots.

ROLE OF THE HETEROTRIMERIC G-PROTEINS IN ROOT CELL DIVISION

Cell division in the RAM and cell cycle activation at the pericycle founder cells are regulated by both biological stimuli and environmental factors. For example, all six major plant hormones, auxins, cytokinins, gibberellins, abscisic acid, ethylene, and brassinosteroids, directly or indirectly regulate root cell division. Among these hormones, auxin is the predominant regulator that is required to initiate lateral root formation. Root cell architecture is also regulated by various environmental factors such as nutrition composition and distribution, the availability of water, and the salinity of the

soil (Lopez-Bucio *et al*. 2003; Malamy 2005). Many of these environmental factors may crosstalk with the auxin signaling pathway to regulate root cell division. For a comprehensive list of genes whose expressions are up- or down-regulated by auxin during lateral root initiation, readers are referred to the article by Himanen *et al*. (2004). For a comprehensive list of genes that affect the lateral root initiation and/or development, readers are referred to a recent review article by de Smet *et al*. (2006). Here we mainly focus on the recent progress on the modulation of root cell division by the heterotrimeric G-proteins in Arabidopsis.

The heterotrimeric G-protein complex is a conserved signaling module found in all eukaryotic cells (Gilman 1987). In the classical paradigm, ligand binding to the cell-surface seven-transmembrane (7TM) G-protein-coupled receptor (GPCR) results in a conformational change of the GPCR. The activated GPCR acts as a Guanine Nucleotide Exchange Factor (GEF) to prompt the exchange of GDP for GTP in the G-protein α subunit (Gα), resulting in a conformational change of Gα and subsequent dissociation of Gβγ dimer from the activated Gα (GTP-bound). The GTP-bound Gα and freely-released Gβγ can then interact with downstream effectors, resulting in specific cellular responses and/or activation/suppression of gene expression. Eventually, the intrinsic GTPase activity of the Gα subunit allows the bound GTP to be hydrolyzed into GDP and the G proteins return to an inactive state. The GTPase activity of the Gα can be accelerated by Regulator of G-protein Signaling (RGS) proteins which possess GTPase Accelerating Protein (GAP) activity for Gα (Neubig and Siderovski 2002).

In contrast to its counterpart in mammals, the repertoire of G-protein complex and accessory components in Arabidopsis is much simpler. In particular, the Arabidopsis genome contains genes encoding only one Gα, one Gβ, and two Gγ subunits, one RGS, and several dozens of putative GPCRs (Jones and Assmann 2004; Moriyama *et al*. 2006; Temple and Jones 2007). As in other eukaryotes, the heterotrimeric G proteins play important roles in cell division in plants. The loss-of-function alleles of Arabidopsis heterotrimeric G-protein α (GPA1) and β (AGB1) subunits display defects in cell division throughout development (Perfus-Barbeoch *et al*. 2004). For example, both *gpa1* and *agb1* mutants have reduced cell divisions in the hypocotyls and rosette leaves (Ullah *et al*. 2001, 2003). The small repertoire of the heterotrimeric G-protein complex and accessory components and the availability of loss-of-function alleles of Gα and Gβ subunits in Arabidopsis offer a unique advantage over all multicellular, genetic-model systems for dissecting their role in cell division.

Both the loss-of-function alleles of *GPA1* and *AGB1* also have defects in cell division in the roots. *gpa1* mutants have fewer lateral roots than wild-type plants whereas the cell division in the RAM is normal (Ullah *et al*. 2003; Chen *et al*. 2006). In contrast, *agb1* mutants have more lateral roots than wild-type plants and have longer primary roots that result from increased cell production rate in the RAM (Ullah *et al*. 2003; Chen *et al*. 2006). The typical root phenotypes of *gpa1* and *agb1* mutants are illustrated in **Fig. 2**. In the classical model of G-protein signaling, activation of the Gα releases the sequestration of Gβγ dimer by Gα, therefore an opposite phenotype, such as number of lateral roots of Gα and Gβ loss-of-function alleles, is generally interpreted to mean that the Gβγ subunit is the predominant form of the heterotrimer regulating the cellular process of this given phenotype. In this scenario, AGB1 has more important role than GPA1 in the modulation of the activation of pericycle cells, a prerequisite process for lateral root formation.

On the other hand, the *agb1* mutants have longer primary roots whereas the length of primary roots of the *gpa1* mutants is wild-type (**Fig. 2**) (Chen *et al*. 2006). By analyzing the rate of cell production of the RAM, it was found that the longer primary roots of *agb1* mutants were due to increased cell division (Chen *et al*. 2006). In the classical model of G-protein signaling, as discussed above for lateral root formation, a role for the individual G-protein subunits can be generally determined if an opposite phenotype of Gα and Gβ loss-of-function alleles is observed. If a similar or same phenotype is observed between the loss-of-function alleles of Gα and Gβ, a relative importance of the individual subunits can also be determined. In that case, Gα is generally considered to be the predominant factor of the heterotrimer, because loss of Gβ disrupts Gα coupling with receptor or effector proteins. For example, both *gpa1* and *agb1* mutants have round shape of rosette leaves (Ullah *et al*. 2001, 2003). Therefore GPA1 is considered to be the primary factor of the heterotrimer regulating the cellular process that determines the shape of rosette leaves.

The difference of *gpa1* and *agb1* mutants in cell division in the RAM (i.e. *gpa1*, wild-type; *agb1*, increased cell division) made a direct assignment of individual G-protein subunits in this process ambiguous. In order to circumvent this problem and to dissect the role of heterotrimeric G-protein subunits in the modulation of root cell division, Chen *et al*. (2006) overexpressed *GPA1* and *AGB1* in *agb1* and *gpa1* mutant backgrounds, respectively, examined cell division in the RAM by measuring root cell production rate, and counted the number of lateral roots as an indirect measurement of activation or reentry of cell cycle in the pericycle founder cells. The major advantage of overexpressing Gα (GPA1) in the loss-of-function Gβ (AGB1) mutant background is to allow a direct examination of the role of Gα because Gβ is no longer available for recruitment by Gα to form the heterotrimer. Similarly, overexpression of Gβ in the loss-of-function Gα mutant background allows a direct test of the role of Gβ because the sequestration of Gβ by Gα is eliminated.

By using these transgenic lines as well as the *gpa1* and *agb1* single and double mutants, it was confirmed that AGB1 is the predominant subunit of the heterotrimeric G-protein complex that modulates lateral root formation, and that AGB1 works downstream of GPA1 to exert its modulatory role (Chen *et al*. 2006). In the RAM, interestingly, it was found that the formation of the heterotrimer is required to modulate cell division and that the heterotrimer functions as an attenuator (Chen *et al*. 2006). Furthermore, the activated form of GPA1 (GTP-bound) positively modulates cell division in the RAM.

A MODE FOR THE ACTION OF THE HETEROTRIMERIC G-PROTEINS IN THE MODULATION OF ROOT CELL DIVISION

The analyses of the number of lateral roots (indirect measurement of the activation of cell cycle in the pericycle founder cells) and the rate of cell production in the primary root (indirect measurement of cell division in the RAM) in *gpa1* and *agb1* single and double mutants, as well as in transgenic lines overexpressing *GPA1* and *AGB1* in the *agb1* and *gpa1* mutant backgrounds promote a testable model in which AGB1 acts downstream of GPA1 to negatively modulate pericycle cell activation (**Fig. 3**). In the RAM, the intact heterotrimer attenuates cell division. Because loss-of-function mutations in the sole RGS protein in Arabidopsis, AtRGS1, and overexpression of the constitutively active form of GPA1 (GTPase-dead), resulted in an increased cell division in the RAM (Chen *et al*. 2003), activated GPA1 (GTP-bound) may positively modulate cell division in the RAM (**Fig. 3**).

Himanen *et al*. (2004) established an elegant lateral root induction system to study genes that are involved in the early stage of lateral root initiation. In this system, Arabidopsis seeds were germinated in the presence of 10 μM auxin polar transport inhibitor naphthylphthalamic acid (NPA) for 3 days, then the NPA-pretreated seedlings were transferred to growth medium containing 1-naphthalene acetic acid (NAA), a synthetic auxin, for 0, 2, 4, or 6 h. Total RNAs were isolated from root segments excluding the root apical and adventitious meristems. Therefore, these root

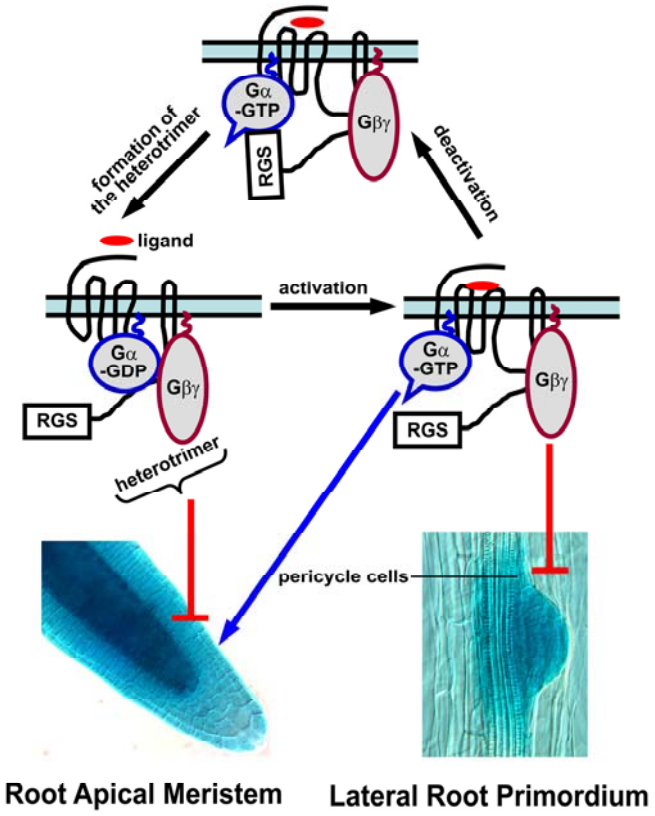

Fig. 3 A model for the action of Arabidopsis heterotrimeric G-proteins in the modulation of root cell division. In Arabidopsis, Gα is encoded by the single gene *GPA1*. Gβ is encoded by the single gene *AGB1*. Gγ is encoded by two genes, *AGG1* and *AGG2*. RGS is encoded by the single gene *AtRGS1*. AtRGS1 is predicted to be a seven transmembrane (7TM) protein and is directly coupled by Gα, but no ligand has been identified for AtRGS1. AtRGS1 contains an RGS-box at its C-terminus, which can accelerate the intrinsic GTPase activity of Gα. Arrows depict acceleration and bar indicates attenuation of cell division. The heterotrimer is a negative modulator of cell division in the RAM whereas activated Gα (GTP-bound) is a positive modulator. Gβγ is the predominant factor of the heterotrimer that negatively modulates the formation of lateral roots.

segments represented the tissue that can specifically respond to auxin to initiate lateral roots. Transcript profiling revealed that *GPA1* is among one cluster of genes (59 genes) whose transcription was strongly induced 2 h after auxin treatment. These results support the notion that GPA1 is involved in the early stage of lateral root initiation.

Because AGB1 negatively modulates lateral root formation, a reduced level of *AGB1* is required for lateral root initiation. Consistent with this scenario, auxin down-regulates the transcription of *AGB1* (Ullah *et al*. 2003). *gpa1 agb1* double mutant phenocopies *agb1* single mutant lateral root phenotype, suggesting that *agb1* is epistatic to *gpa1* (Chen *et al*. 2006). Because overexpression of *GPA1* increases the number of lateral roots in the wild-type, but not in the *agb1* mutant background (Chen *et al*. 2006), these results support that AGB1 acts downstream of GPA1, and suggest that GPA1 may not be required for the function of AGB1 in the modulation of lateral root formation. Because during the initiation of lateral root primordium, the transcript of *GPA1* was elevated whereas that of *AGB1* was down-regulated by auxin (Ullah *et al*. 2003; Himanen *et al*. 2004), it is likely the actual function of GPA1 in the activation of pericycle founder cells is to sequester AGB1, thereby alleviating the attenuation role of AGB1. This notion is consistent with the classical G-protein signaling paradigm in which inactivated form of Gα (GDP-bound) recruits activated Gβγ dimer (freely-released) to form the heterotrimer. Therefore, in the activation of pericycle founder cells, the classical G-protein-coupled signaling system is operating. However, the high or low level of *GPA1* or *AGB1* transcript during the activation of pericycle founder cells may not reflect the actual status of their protein levels. A proteomic approach may lead to more precise information about the interaction between GPA1 and AGB1 proteins in the modulation of lateral root formation.

The role of the heterotrimeric G-proteins in the modulation of lateral root formation is further supported by the analysis of global gene expression profiles in *agb1* mutants (Ullah *et al*. 2003). Wild-type and *agb1* seedlings were treated with 10 μM indole-3-acetic acid (IAA) for 20 min. The gene expression profiles were generated using the Affymetrix Arabidopsis Genome Array Genechips, and compared between wild-type and *agb1* mutants, with or without IAA treatment. Consistent with the attenuator role of AGB1 in root cell division, a set of auxin-regulated genes are deregulated in the *agb1-2* mutants (Ullah *et al*. 2003).

Because neither GPA1 nor AGB1 is required for the cell division in the RAM or the activation of pericycle founder cells, G-proteins are interpreted to have modulatory roles in root cell division. In consistent with this, G-proteins are not directly coupled in the auxin signaling pathways, because both *gpa1* and *agb1* mutants are still responsive to auxin (Ullah *et al*. 2003). Therefore, it remains mysterious how G-proteins exert their modulatory roles on root cell division. Because auxin plays a dominant role in determining the positioning and frequency of lateral root initiation (Himanen *et al*. 2002) and G-proteins are not directly coupled in auxin signaling, it is possible that G-proteins may regulate other aspects of auxin action. For example, the first formative division in the pericycle cells depends on the basipetal transport of auxin whereas the shoot-derived auxin regulates the outgrowth of lateral roots (Casimiro *et al*. 2001; Bhalerao *et al*. 2002), it is possible that G-proteins may modulate root cell division by regulating auxin transport. Both *GPA1* and *AGB1* are expressed in root cells (Huang *et al*. 1994; Chen *et al*. 2006b). It is known that both shoot-derived auxin and root-synthesized auxin regulate lateral root formation (Reed *et al*. 1998; Bhalerao *et al*. 2002; Ljung *et al*. 2005). The shoot-derived auxin is transported acropetally in roots and initiates early lateral root primordial. The root-synthesized auxin is transported basipetally in roots and influence lateral root initiation. Consistent with this, application of auxin polar transport inhibitor NPA to Arabidopsis seedlings inhibits lateral root formation. Inhibition of basipetal auxin transport also completely blocks gravity response in roots while inhibition of acropetal auxin transport only partially reduces it (Rashotte *et al*. 2000), suggesting that the basipetal auxin transport is also required for gravitropism in roots. Therefore, if the heterotrimeric G-proteins indeed modulate lateral root formation through regulating auxin polar transport, one would expect that the loss-of-function G-protein mutants display alternation in gravitropism. Consistent with this scenario, recently it has been found that *agb1* mutants are less responsive to gravistimulation than wild-type in Arabidopsis (Trusov *et al*. 2007). The possible involvement of G-proteins in the modulation of auxin transport deserves further investigation.

What could be the precise nuclear stage in the cell cycle for the modulatory target in the RAM and pericycle by the heterotrimeric G-proteins? Using *cyc1At-CDB:GUS* as a mitotic reporter, Ullah *et al*. (2001) found a reduced expression of the mitotic reporter, suggesting a reduction in cell division in the *gpa1* mutants. Consistent with this, ectopic expression of GPA1 confers increased cell division in Arabidopsis seedlings (Ullah *et al*. 2001). Moreover, overexpression of GPA1 in synchronized tobacco BY-2 cells shortened the G1 phase of the cell cycle and promoted formation of nascent cell plates (Ullah *et al*. 2001). As discussed above, the transcript of *GPA1* is strongly induced 2 h after auxin treatment in the lateral root induction system (Himanen *et al*. 2004). At this stage, pericycle founder cells are at the G1-to-S transition (Himanen *et al*. 2002). These results suggest that GPA1 is involved in the early stage of lateral root initiation, and support the notion that GPA1 may promote cell

division at the G1-to-S transition of the cell cycle.

Because GPA1 is not required for AGB1's function in the attenuation of cell division in the pericycle founder cells, and AGB1 acts downstream of GPA1 (Chen *et al*. 2006), AGB1 may not necessarily target at the same phase of cell cycle (i.e. G1-to-S transition) as that of GPA1. For example, AGB1 may target a later phase of cell cycle (i.e. G2-to-M transition). Further studies are required to determine the exact nuclear stage of the cell cycle at which G-proteins exert their modulatory roles.

CONCLUDING REMARKS

The regulation or modulation of cell division is one of the conserved functions of the heterotrimeric G-proteins in eukaryotes (Knoblich 2001; Bellaiche and Gotta 2005; Wilkie and Kinch 2005; Yu *et al*. 2006). In Arabidopsis, the heterotrimeric G-protein subunits differentially modulate cell division in roots. In addition to Arabidopsis, heterotrimeric G-protein subunits are also present in other plant species. For example, genes encoding heterotrimeric G protein α subunits have been cloned from tomato, alfalfa, lotus, lupin, pea, rice, soybean, spinach, tobaccos, and wild oat (Assmann 2002). However, little is know about the function of the heterotrimeric G-proteins in root cell division in these plant species. Because root system is essential for a plant's wellness and fitness, the manipulation of the heterotrimeric G-protein signaling pathway in plants may lead to potential application in agriculture.

The significance of the finding that intact heterotrimer is functional is twofold: First, this finding provides evidence that the formation of the heterotrimer is required to exert the modulatory role of the G-proteins in the cell division in the RAM. The biochemical and bioinformatic evidence about the assembly of the heterotrimer has been reported in plant cells previously (Ullah *et al*. 2003; Kato *et al*. 2004; Adjobo-Hermans *et al*. 2006), but the significance of such assembly had not been assessed. The assignment of the heterotrimer as a negative modulator for cell division in the RAM provides the first functional evidence for the assembly of the heterotrimer in plant cells. Second, this finding suggests that the heterotrimer itself is signaling. Such a scenario is rare but not unprecedented (Peleg *et al*. 2002). In Arabidopsis, phospholipase D α subunit 1 (PLDα1) was found to bind the inactive form of GPA1 (Zhao and Wang 2004). PLDα1 have both negative and positive roles in G-protein-mediated abscisic acid inhibition of stomatal opening (Mishra *et al*. 2006). These results imply that the inactive form of Gα, presumably in the heterotrimeric complex, could bind effector proteins. This raises the possibility of presence of GPCR-independent signaling pathway in some types of plant cells.

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