J. Plant Biol. (2011) 54:57–64
DOI 10.1007/s12374-011-9145-z

ORIGINAL RESEARCH

Molecular Identification and Physiological Characterization of a Putative Novel Plasma Membrane Protein from *Arabidopsis* Involved in Glucose Response

Moon-Soo Chung · Ping Huang · Chan Man Ha · Ji Hyung Jun · Sung-Ju Ahn · Fu-chun Zhang · Hyun-Jong Bae · Baik Ho Cho · Cheol Soo Kim

Received: 29 September 2010 / Revised: 29 December 2010 / Accepted: 2 January 2011 / Published online: 19 January 2011

Abstract We carried out functional analysis of a putative novel *Arabidopsis* plasma membrane glucose-responsive regulator, designated AtPGR, which contains seven predicted transmembrane domains. Several evidences showed that AtPGR is a glucose-related protein, but its biological functions have yet to be reported in any plant. Analyses of the *AtPGR* promoter-β-glucuronidase (GUS) construct and RNA in situ hybridization revealed substantial gene expression in the vasculature of various tissues, especially in the phloem region. Glucose treatment induced the highest levels of GUS activity, reaching a peak at 3 h and declining thereafter, consistent with glucose-mediated regulation of the *AtPGR* promoter. We generated an *atpgr* RNAi knockdown mutant and found that this plant grew and developed normally. Ectopic expression of *AtPGR* gene modulated the induction of glucose and 2-deoxyglucose insensitivity under stress conditions. By way of contrast, cotyledon greening of *atpgr* RNAi knockdown mutant seeds enhanced sensitivity to glucose and 2-deoxyglucose. Taken together, these results suggest that AtPGR functions as a potential glucose-responsive regulator in carbohydrate metabolism.

Keywords At5g19930 · Glucose · Glucose insensitivity · Glucose regulator · Phloem · Plasma membrane

Abbreviations

2-DG	2-Deoxyglucose
Frc	Fructose
Glc	Glucose
Glc-6-P	Glucose-6-phosphate
GUS	β-Glucuronidase
GFP	Green fluorescent protein
HXK	Hexokinase
Man	Mannose
3-OMG	3-*O*-Methylglucose
MS	Murashige and Skoog

Electronic supplementary material The online version of this article (doi:10.1007/s12374-011-9145-z) contains supplementary material, which is available to authorized users.

M.-S. Chung · P. Huang · B. H. Cho (✉) · C. S. Kim (✉)
Department of Plant Biotechnology,
Chonnam National University,
Gwangju 500-757, South Korea
e-mail: chobh@chonnam.ac.kr

C. S. Kim
e-mail: cskim626@chonnam.ac.kr

C. M. Ha · J. H. Jun
Department of Plant and Microbial Biology,
University of California,
Berkeley, CA 94720, USA

S.-J. Ahn · H.-J. Bae
Department of Bioenergy Science and Technology,
Chonnam National University,
Gwangju 500-757, South Korea

F.-c. Zhang
College of Life Science and Technology, Xinjiang University,
Urumqi 830046, China

PM	Plasma membrane
qPCR	Quantitative real-time PCR
RT-PCR	Reverse transcription-PCR
RNAi	RNA interference
WT	Wild type

Introduction

Sugar signal transduction cascades are important components of regulatory networks in cells. Compared to the situation in bacteria, yeast, and animals, participants in the sugar sensing and signaling pathways in plants are poorly understood. In plants, sugar sensing and signaling play pivotal roles in controlling many aspects of growth, metabolism, and development throughout the whole plant life cycle (Rolland et al. 2006). Furthermore, sugars can be conserved as polymers giving rise to storage components such as starch and fructans, or structural components like cellulose. Both after de novo synthesis of sucrose by CO_2 assimilation and after glucose (Glc) production by starch degradation, these soluble carbohydrates have to be transported from the respective "source" tissues to the different carbohydrate consuming "sink" tissues. The coordinated modulation of gene expression in source and sink organs is to a large extent choreographed by the sugar status in the cells. In general, low sugar levels promote photosynthesis and mobilization of energy reserves, whereas high sugar levels stimulate growth and storage of starch and other carbohydrates. There are numerous examples of genes whose expression is regulated by sugars, and carbon metabolite-mediated regulation of gene expression appears to be a central and fundamental mechanism common to all higher plants (Koch 1996).

Three Glc signal transduction pathways in plants have been suggested (Xiao et al. 2000). These are *Arabidopsis thaliana* hexokinase 1 (AtHXK1)-dependent pathway in which gene expression was correlated with the AtHXK1-mediated signaling function. Second was glycolysis-dependent pathway that was influenced by catalytic activity of both AtHXK1 and heterologous yeast HXK2. Third is the HXK-independent pathway in which gene expression is independent of AtHXK1. However, by using two independent knockout mutations for HXK1 isoform, it was concluded that glucose-6-phosphate metabolism is uncoupled from HXK1-dependent signaling (Moore et al. 2003). A mechanism for glucose sensing by HXK1, wherein a change in conformation by substrate binding initiates a signaling cascade, has been proposed (Harrington and Bush 2003). It still remains unclear if HXK senses Glc in a linear concentration-dependent manner or it is flux sensor. Hexose sensing and signaling functions are, however, dependent on subcellular localization, translocation, and interactions with downstream effectors of HXK (Rolland et al. 2002). Multiple Glc signal transduction pathways that control diverse genes and processes are intimately linked to developmental stages and environmental conditions (Xiao et al. 2000).

In the present study, we sought to identify and physiologically characterize a putative plasma membrane (PM)-localized glucose-responsive regulator protein designated *Arabidopsis* PM Glc-responsive regulator (AtPGR). This protein seems to be single gene in *Arabidopsis*, and AtPGR (At5g19930) is primarily expressed in the phloem tissues. Based on subcellular localization studies and Glc sensitivity assays in wild-type (WT) or transgenic plants during Glc treatment, we provide several lines of evidence showing that AtPGR is involved in Glc response in *Arabidopsis*.

Materials and Methods

Plant Materials, Growth Conditions, and Glc Induction

Arabidopsis plants were grown in growth chambers under intense light at 22°C, 60% relative humidity, and a 16-h day length. The plants were challenged with Glc via the submersion of 10-day-old *Arabidopsis* seedlings in a solution containing 5% Glc. Samples were obtained at 0, 1, 3, 6, 9, and 12 h of Glc stress, frozen in liquid nitrogen, and stored at −80°C.

In Situ Hybridization

Plant fixation and in situ hybridization were performed as described previously (Jackson 1992). For the probes, the full-length cDNA sequence of *AtPGR* was amplified and cloned in the pGEM T-easy vector as described below. Probes for in situ hybridization were transcribed using the digoxigenin labeling mix (Roche, Mannheim, Germany).

Overexpression Construct of AtPGR

Total RNA was isolated from *Arabidopsis* leaves using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RT-PCR was utilized to obtain full-length *AtPGR* cDNA (At5g19930). The generated product was then cloned into the pGEM T-easy vector (Promega, Madison, WI, USA) for DNA sequence analysis. RT-PCR primers were forward primer 5′-GCTCTAGAGCATGGAAACGTCGCCGCAA-3′ (*Xba*I site is shown in italics) and reverse primer 5′-GGGGTACCCCT CAGAAAATGTACACAGA-3′ (*Kpn*I site is shown in italics) on the basis of the sequence information in a cDNA

database (NCBI, http://www.ncbi.nlm.nih.gov). Amplification proceeded for 35 cycles as follows: 94°C, 30 s; 57°C, 30 s; and 72°C, 1 min. The PCR-amplified products were double-digested with *Xba*I and *Kpn*I and directionally cloned into the plant expression vector pBI121. The resulting construct was introduced into *Agrobacterium tumefaciens* strain GV3101 by *in planta* vacuum infiltration. Homozygous lines (T_3 generation) from 11 independent transformants were obtained, and two lines (OX7-6 and OX15-1) evidencing high levels of transgene expression were selected for phenotypic characterization. Kanamycin resistance of the T_2 generation from these two selected lines was segregated as a single locus.

RNAi Construct of AtPGR

A fragment of approximately 250 bp including the first exon and first intron of *AtPGR* was amplified from *Arabidopsis* genomic DNA by PCR with the following primers: sense 1 primer 5′-TCACTGCAGGGTACCATG GAAACGTCGCCGCAA-3′ (*Pst*I and *Kpn*I site are shown in italics) and antisense 1 primer 5′-CGAGGATC CACCTTTCAGATTCAGCAG-3′ (*Bam*HI site is shown in italics). The resulting PCR product was digested with *Kpn*I and *Bam*HI and ligated to the *Kpn*I-*Bam*HI-cleaved vector pMD18-T vector (Takara, Shiga, Japan). The cloned fragments were sequenced to ensure that the correct DNA fragment was amplified and cloned. This plasmid served as a template to generate a second PCR fragment to complete the inverted first exon repeat construct. The second PCR primers were sense 1 primer and antisense 2 primer 5′-AGCGGATCCACCT GAAACCGGCGGTGAAG-3′ (*Bam*HI site is shown in italics). This second PCR fragment was cleaved with *Bam*HI and *Pst*I and inserted into the *Bam*HI-*Pst*I sites of the template plasmid. Then, the double-strand RNA constructs were double-digested with *Kpn*I and *Pst*I and directionally cloned into the plant expression modified vector pCAMBIA1301. *Arabidopsis* plants were transformed with the construct, and the resultant T_3 homozygous transgenic lines (*ri5-1* and *ri11-1*) were evaluated for Glc and Glc analog sensitivity.

Results

Identification and Amino Acid Sequence Analysis of the AtPGR (At5g19930) Gene

AtPGR was identified as a glucose-related gene in the Affymetrix ATH1 GeneChip assay (Price et al. 2004). Present analysis determined that AtPGR was comprised of 867 bp and harbored one single open reading frame encoding a protein of 288 amino acids with a calculated molecular weight of 30 kDa harboring seven predicted transmembrane domains (Fig. 1) by the SOSUI program (http://bp.nuap.nagoya-u.ac.jp/sosui). A BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed only a single copy of the gene in the *Arabidopsis* genome. An amino acid sequence alignment between AtPGR and rice, maize, insect, and human orthologs is shown in Supplementary Fig. S1. Overall homology values of 38–68% identity and 58–82% similarity were observed between AtPGR and the bilaterian proteins. On the basis of amino acid sequence alignments, two highly conserved regions in the center and C-termini were identified in the homologous proteins (Supplementary Fig. S1), although no function was assigned to these regions.

Subcellular Localization of AtPGR Protein

To determine the subcellular localization of AtPGR, a green fluorescent protein (GFP) reporter gene was fused in-frame to the AtPGR coding region in order to generate a GFP-AtPGR fusion protein in transgenic *Arabidopsis* plants. As shown in Fig. 2, the fluorescence signal of the GFP-AtPGR construct was detected in the putative PM of the transgenic *Arabidopsis*; however, the majority of the GFP protein was located in the cytoplasm of the transgenic *Arabidopsis* root cells, and the GFP fluorescence signal was weak (Fig. 2). Furthermore, in an effort to verify the expected plasma membrane localization, the root cells were plasmolyzed by salt treatment to exclude the possibility of cell wall association of AtPGR. As is shown in Fig. 2, the GFP fluorescence in the plasmolyzed root cells was detached slightly from the cell wall. This result shows that AtPGR is localized in the PM or attached at the PM.

Expression Analysis of AtPGR Gene in *Arabidopsis*

In order to know spatial pattern of *AtPGR*, we performed RNA in situ hybridization experiment. In transverse sections of 10-day-old seedling plants, *AtPGR* is not expressed in the shoot apical meristem region. However, it is strongly expressed in vasculature of developing leaf primodia, especially in the phloem region (Supplementary Fig. S2a). In contrast, sense probe did not give any signal in our experiment (Supplementary Fig. S2b).

To gain more information on the promoter activity of *AtPGR* gene, we generated *AtPGR* promoter-β-glucuronidase (GUS) plants. The results indicated that *AtPGR* was mainly in the vasculature of various tissues (Fig. 3a–f), and no staining was visible in root tip (Fig. 3b, c). In the root, cross-sectional analysis revealed that GUS activity was localized primarily in the phloem tissues (Fig. 3g).

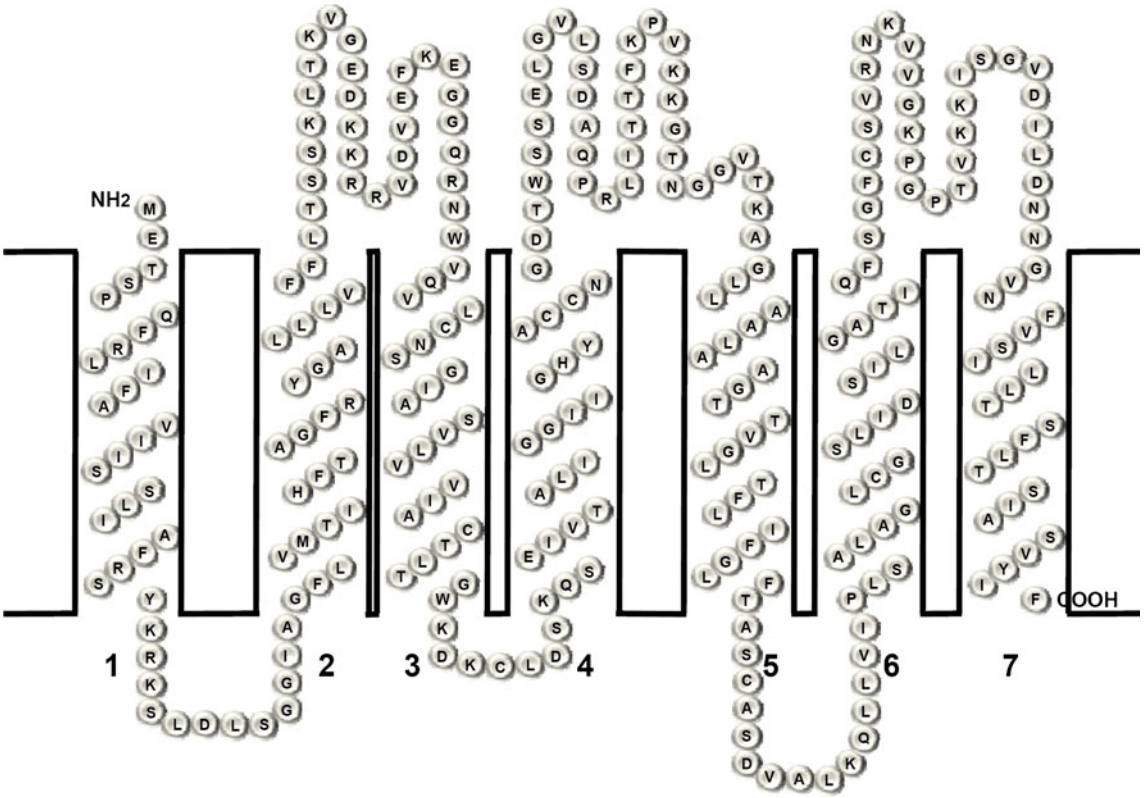

Fig. 1 Diagramatic representation of AtPGR structure. The diagram was drawn based on the prediction of hydrophobicity profile of AtPGR. The seven putative transmembrane helices are *numbered 1–7*. Invariant residues are noted using the *single-letter abbreviations* for the amino acids

AtPGR Gene Is Regulated by Glucose

In a previous report, Glc-inducible expression of *AtPGR* was confirmed by quantitative real-time PCR (qPCR; Price et al. 2004). As shown in Fig. 4a, *AtPGR* transcript reached its maximum level at about 3 h, rapidly declining thereafter, to be nearly abolished 12 h after Glc treatment. The sugar-repressible *CAB1* (Krapp et al. 1993) gene was used as a control for the Glc treatment (Fig. 4b). To quantitatively analyze *AtPGR* promoter strength during Glc treatment, GUS activity was determined by a fluorimetric assay. Transgenic plants 10 days after germination were treated with 5% Glc or untreated to measure GUS activity. As shown in Fig. 4c, Glc treatment induced the highest levels of GUS activity, reaching a peak at 3 h, and declining thereafter. These results strongly suggest that in transgenic *Arabidopsis* the *AtPGR* promoter is regulated by Glc.

AtPGR Overexpression or Knockdown Reduces or Increases Glc Sensitivity in Seed Germination, Respectively

To investigate the in vivo function of AtPGR, we induced *AtPGR* overexpression in *Arabidopsis* under the control of the 35S promoter. Eleven homozygous lines (T_3 generation) were obtained, and two lines (OX7-6 and OX15-1) exhibiting high levels of transgene expression (Fig. 5a) were selected for phenotypic characterization. The comparison of *AtPGR*-overexpressing lines with WT plants demonstrated no morphological alterations or retardation of growth. In an effort to further evaluate the functional consequence of the loss of *AtPGR*, we generated *atpgr* RNA interference (RNAi) lines using first exon cDNA sequences. *AtPGR* expression was assessed by reverse transcription-PCR (RT-PCR) in two randomly selected two independent *atpgr* RNAi lines (*ri5-1* and *ri11-1*). The results demonstrated that *AtPGR* expression was knocked down in the RNAi lines (Fig. 5a).

In an effort to evaluate the effects of *AtPGR* expression on germination with Glc treatment, the seeds of WT, *atpgr*, and *AtPGR*-overexpressing plants were germinated on Murashige and Skoog (MS) medium (Murashige and Skoog 1962). Macroscopically, there is scant difference in terms of seed germination among the WT, *atpgr*, and *AtPGR*-overexpressing plants (Fig. 5b). The germination ratio among WT, *atpgr*, and *AtPGR*-overexpressing plants were similar and adequate on MS medium (Fig. 5b, c). As the seeds of the WT, *atpgr*, and *AtPGR*-overexpressing plants were germinated on MS medium containing 5% Glc, the cotyledon greening rate of WT was only slightly above 30% 10 days after germination. Less than 16% of the *atpgr*

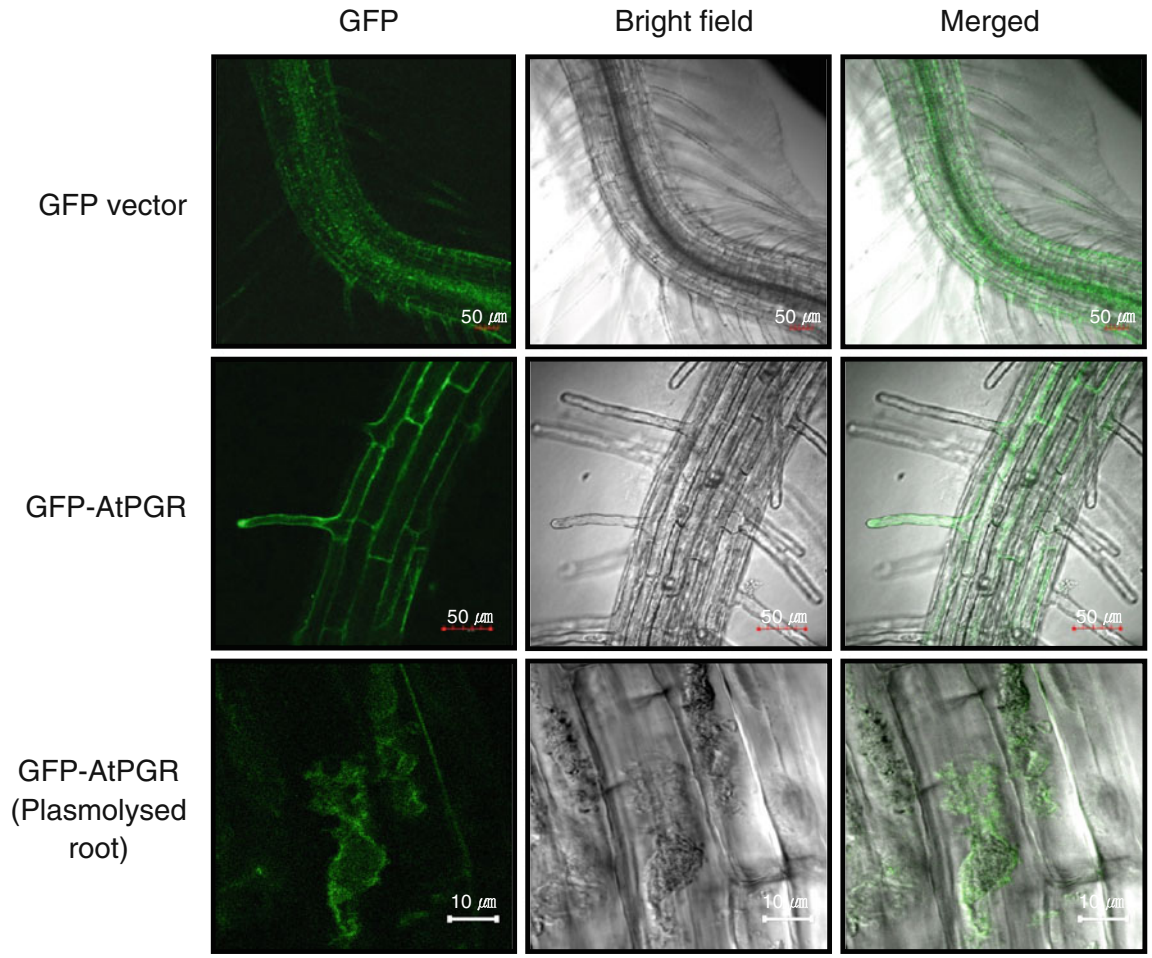

Fig. 2 Plasma membrane localization of AtPGR. *Arabidopsis* root cells overexpressing GFP showed a diffused localization pattern in the cytosol. *Arabidopsis* root cells overexpressing GFP-AtPGR exhibited putative PM localization. *Bars*, 50 μm. In order to induce plasmolysis of the root cell, GFP-AtPGR transgenic seedlings were incubated at 4.5 M NaCl solution for 5–10 min prior to GFP analysis. Ten-day-old transgenic seedlings grown on MS agar plates were analyzed for GFP expression using confocal microscopy. Confocal and bright-field images of GFP fluorescence were simultaneously acquired and superimposed. *Scale bar*, 10 μm

cotyledons (lines *ri5-1* and *ri11-1*) expanded and turned green, as compared to 66–77% in OX-7 and OX-15 (Fig. 5c). In order to further characterize the *atpgr* RNAi lines, WT, *atpgr*, and *AtPGR*-overexpressing seeds were germinated in MS media supplemented with Glc and allowed to grow vertically for 8 days. In the presence of 5% Glc, primary root elongation of *atpgr* reached only 75% of that of WT, whereas primary root elongation of the *AtPGR*-overexpressing plants reached to 200% relative to WT (data not shown). These results demonstrated that the *atpgr* lines were more likely to be sensitive to Glc than was the WT. However, the *AtPGR*-overexpressing plants were demonstrated to be more insensitivity to exogenous Glc than were the WT and *atpgr* RNAi plants. To rule out the possibility that the difference in Glc response between the transgenic and the WT plants was due to an osmotic effect, we used mannitol, 3-*O*-methylglucose (3-OMG) and 2-deoxyglucose (2-DG) in control experiments. No apparent difference was observed among the WT, *atpgr* RNAi lines, and *AtPGR*-overexpressing plants when they were germinated on 350 mM mannitol (data not shown) or 20–150 mM 3-OMG (Supplementary Fig. S3), a Glc analog that is not phosphorylated by HXK. However, the cotyledon greening rate of *atpgr* lines was much more affected than those of WT and *AtPGR*-overexpressing plants by treatment with low concentrations of 2-DG (Supplementary Fig. S4), a glucose analog that is phosphorylated by HXK. As in the glucose assay, the cotyledon greening percentage of *AtPGR*-overexpressing plants was higher than that of WT (Supplementary Fig. S4). To obtain information on how fructose (Frc) or mannose (Man) treatments act on transgenic cotyledon greening, the seeds of the WT, *atpgr*, and *AtPGR*-overexpressing plants were germinated in MS media supplemented with 6% Frc or

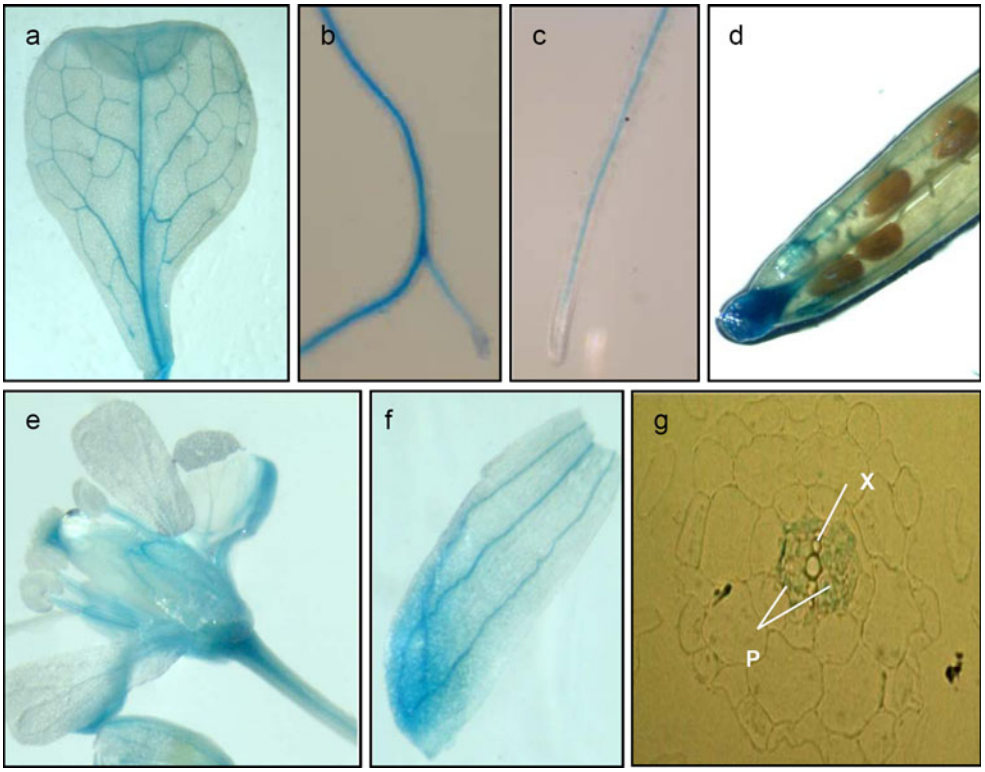

Fig. 3 AtPGR promoter-GUS expression pattern in transgenic *Arabidopsis* plants. **a** GUS staining in a mature leaf. Note the preferential GUS expression in the leaf vasculature. **b** GUS expression in a young root vasculature. **c** GUS expression pattern in a root, showing that *AtPGR* is not expressed at the root tip. **d** Vascular staining in mature silique. **e** GUS staining in a flower. Note the GUS expression in the filament vasculature, style tip, and inflorescence stem vasculature. **f** GUS expression in a sepal vasculature. **g** Cross section of a root, showing GUS expression in the phloem. *X* xylem, *P* phloem

0.1% Man, then permitted to grow for 10 days. As shown in Supplementary Fig. S5, Frc or Man treatments resulted in no significant differences in the cotyledon greening rate after 10 days among WT, *atpgr*, and *AtPGR*-overexpressing plants. These results are consistent with the suggestion that AtPGR is a necessary component for the Glc-triggered developmental leaf growth process.

Discussion

This work demonstrates that AtPGR can function as a Glc-responsive regulator *in planta*. The transgenic plants over-expressing AtPGR show sugar hyposensitivity and the RNAi plants show sugar hypersensitivity in a variety of assays, suggesting that sugar response in *Arabidopsis* involves AtPGR.

AtPGR protein is the localization of constitutively expressed GFP-AtPGR fusion proteins to the putative PM of transgenic root cells (Fig. 2) and amino acid sequence analyses revealing that AtPGR consists of seven predicted transmembrane domains (Fig. 1). To date, genetic and biochemical evidence indicates that one *Arabidopsis* protein, the regulator of G-protein signaling 1 (RGS1), has independent roles in glucose sensing and phytohormone responses (Rolland et al. 2006). AtRGS1 combines a receptor-like seven transmembrane domain with an RGS domain, interacts with the *Arabidopsis* G-protein α subunit (AtGPA1) in a glucose-regulated manner, and stimulates AtGPA1 GTPase activity (Chen and Jones 2004). Furthermore, genetic studies indicate that AtRGS1 interacts with additional components to serve as a plasma membrane sensor for glucose (Grigston et al. 2008). In yeast, there are two hexose-transporter-like PM proteins, which act as Glc sensors rather than transporters (Ozcan et al. 1998). Therefore, AtPGR proteins should be considered as potential candidates for dual sugar sensor and signaling proteins.

Presently, staining of *AtPGR* promoter-GUS was always observed in the vasculature of leaves, roots, inflorescences, siliques, anther filaments, and sepals; also, root and shoot apical meristem transverse sections revealed *AtPGR* expression in the phloem (Fig. 3 and Fig. S2). According to quantitative analysis of *AtPGR* promoter strength during glucose treatment, *AtPGR* promoter is regulated by Glc (Fig. 4c). Vascular tissues have been implicated in multiple plant systemic responses, such as auxin polar transport (Galweiler et al. 1998), blue light perception (Sakamoto and Briggs 2002), drought stress perception (Endo et al. 2008), glucose response (Karve and Moore 2009), and the transport of macromolecules, minerals, and nutrients (Okumoto et al. 2002; Kataoka et al. 2004; Lough and Lucas 2006). Elucidation of the mechanisms that regulate glucose response in this tissue will be the next challenge for a full understanding of the systemic glucose signaling machinery of plants.

Glc is most likely the predominant hexose signal in gene regulation in plants and other organisms. Just like sucrose,

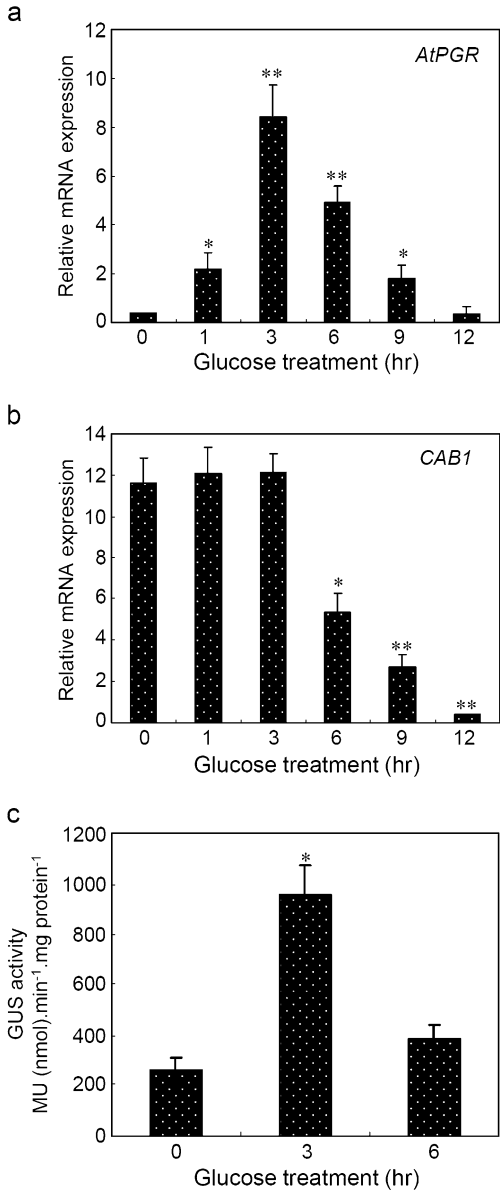

Fig. 4 Expression of the *AtPGR* gene in *A. thaliana*. **a**, **b** qPCR analysis of the expression of *AtPGR* (**a**) and *CAB1* (**b**) involved in glucose response. All quantifications were made in three independent isolated RNA samples obtained from plants treated with 5% Glc at the indicated times. *Error bars* indicate standard deviations of three independent biological samples. Differences between the expression of *AtPGR* or *CAB1* in 10-day-old *Arabidopsis* seedlings untreated and treated with Glc are significant at the $0.01<P<0.05$ (*) or $P<0.01$ (**) levels. **c** GUS activity of *Arabidopsis* plants with Glc. Plants exposed to 0% to 5% Glc at the indicated times. *Error bars* represent standard deviations. Differences between the GUS activity in 10-day-old *Arabidopsis* seedlings untreated and treated with Glc are significant at the $0.01<P<0.05$ (*) levels

Glc represses photosynthesis and germinative and post-germinative developmental programs in different plant systems (Smeekens 2000; Rolland et al. 2002). Glc is phosphorylated in plant cells by both unspecific HXKs and glucose-specific HXKs (glucokinases). The glucose analog 2-DG and Man are transported into the plant cells and phosphorylated by HXK to 2-DG-6-phosphate and Man-6-phosphate, respectively (Loreti et al. 2001). Two other Glc analogs, 6-deoxyglucose (6-DG) and 3-OMG, are transported into the cells but not phosphorylated by HXK

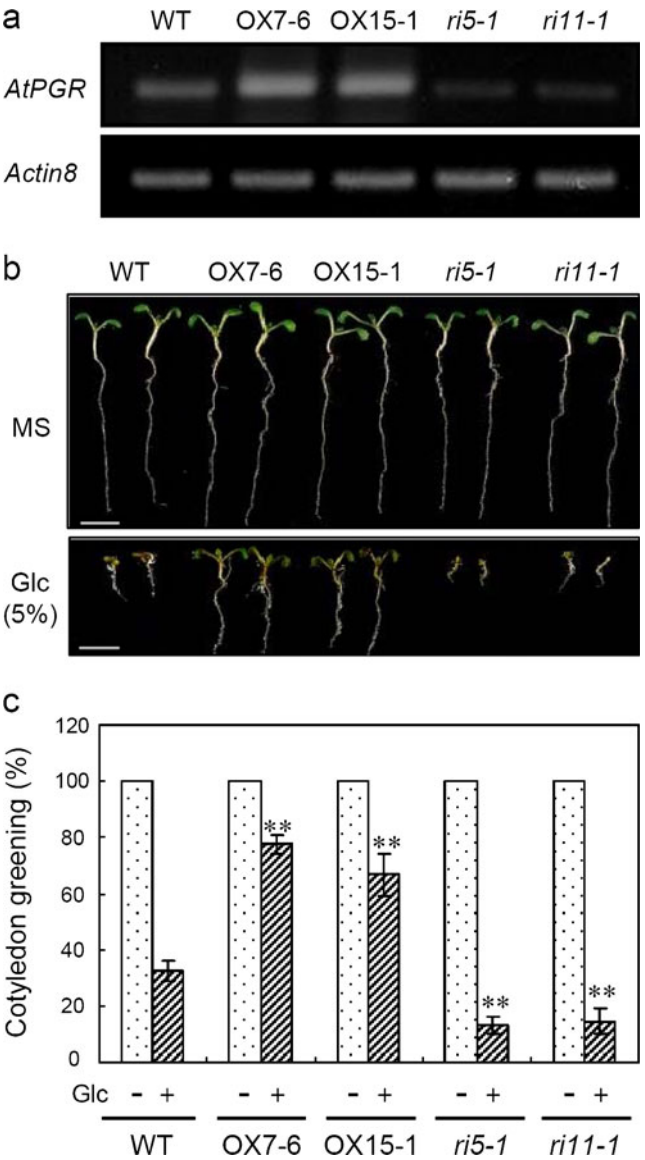

Fig. 5 Glc sensitivity of *AtPGR*-overexpressing and *atpgr* RNAi transgenic plants. **a** Expression levels of *AtPGR* in WT, two independent *AtPGR*-overexpressing (OX7-6, OX15-1) and *atpgr* RNAi (*ri5-1*, *ri11-1*) transgenic lines determined by RT-PCR by using total RNA isolated from 10-day-old seedlings. *Actin* was used in RT-PCR as an internal control. **b** Effect of Glc treatment on seedling growth. *Bars*, 5 mm. **c** Effect of Glc treatment on cotyledon greening. Seeds were sown on MS agar plates supplemented without (−) or with (+) 5% Glc and permitted to grow for 10 days, and seedlings with green cotyledons were counted (triplicates, $n=50$ each). Cotyledon greening was defined as complete cotyledons expanded and turned green. *Error bars* represent standard deviations. Differences between WT and transgenic plants grown in the same conditions are significant at the $P<0.01$ (**) levels

(Smeekens 2000; Loreti et al. 2001; Rolland et al. 2002). Generally, Glc-induced repression of photosynthesis and seed germination can be mimicked by 2-DG and Man, but not by 6-DG or 3-OMG, demonstrating that hexose transport as such does not suffice for gene repression, but that the sensor is intracellular and that hexose phosphorylation is essential. Frc is also phosphorylated by HXK, and it is likely that this mediates signals via the same pathway as Glc. *AtPGR*-overexpressing transgenic plants showed enhanced insensitivity to Glc in comparison to the WT, whereas the *atpgr* RNAi lines displayed enhanced sensitivity to Glc in cotyledon greening (Fig. 5), which implies that AtPGR is a component in the regulation of Glc or Glc-mediated stress response pathways in *Arabidopsis*. As in the glucose assay, RNAi transgenic plants were hypersensitive to low concentration of 2-DG, as shown by the inhibition of cotyledon greening (Supplementary Fig. S4). The *AtPGR*-overexpressing transgenic plants were 2-DG hyposensitive and appeared green (Supplementary Fig. S4). By testing other Glc analogs, 3-OMG, Frc, or Man treatments resulted in no significant differences in the cotyledon greening rate among WT, *atpgr*, and *AtPGR*-overexpressing plants. These observations suggest that AtPGR is a necessary component for the Glc-triggered developmental leaf growth process.

Further studies targeted at the identification of the direct targets of AtPGR would help us to elucidate the molecular mechanisms underlying AtPGR activity in glucose response.

Acknowledgments This work was supported in part by grants from the Basic Research Promotion Program (KRF-2008-314-F00005) funded by the South Korea government and the Basic Science Research Program funded by the Ministry of Education, Science and Technology of Korea (NRF-2010-0022026).

References

Chen J-G, Jones AM (2004) AtRGS1 function in *Arabidopsis thaliana*. Meth Enzymol 389:338–350

Endo A, Sawada Y, Takahashi H, Okamoto M, Ikegami K, Koiwai H, Seo M, Toyomasu T, Mitsuhashi W, Shinozaki K, Nakazono M, Kamiya Y, Koshiba T, Nambara E (2008) Drought induction of *Arabidopsis* 9-cis-epoxycarotenoid dioxygenase occurs in vascular parenchyma cells. Plant Physiol 147:1984–1993

Galweiler L, Guan C, Muller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. Science 282:2226–2230

Grigston JC, Osuna D, Scheible W-R, Liu C, Stitt M, Jones AM (2008) D-Glucose sensing by a plasma membrane regulator of G signaling protein, AtRGS1. FEBS Lett 582:3577–3584

Harrington N, Bush DR (2003) The bifunctional role of hexokinase in metabolism and glucose signaling. Plant Cell 15:2493–2496

Jackson AC (1992) Detection of rabies virus mRNA in mouse brain by using in situ hybridization with digoxigenin-labelled RNA probes. Mol Cell Probes 6:131–136

Karve A, Moore B (2009) Function of *Arabidopsis* hexokinase-like1 as a negative regulator of plant growth. J Exp Bot 60:4137–4149

Kataoka T, Hayashi N, Yamaya T, Takahashi H (2004) Root-to-shoot transport of sulfate in *Arabidopsis*: evidence for the role of SULTR3;5 as a component of low-affinity sulfate transport system in the root vasculature. Plant Physiol 136:4198–4204

Koch KE (1996) Carbohydrate modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol 47:509–540

Krapp A, Hofmann B, Schafer C (1993) Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis? Plant J 3:817–828

Loreti E, de Bellis L, Alpi A, Perata P (2001) Why and how do plants sense sugars? Ann Bot 88:803–812

Lough TJ, Lucas WJ (2006) Integrative plant biology: role of phloem long-distance macromolecular trafficking. Annu Rev Plant Biol 57:203–232

Moore B, Zhou L, Rolland F, Hall Q, Cheng W-H, Liu Y-X, Hwang I, Jones T, Sheen J (2003) Role of *Arabidopsis* glucose sensor HXK1 in nutrient, light and hormonal signaling. Science 300:332–336

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497

Okumoto S, Schmidt R, Tegeder M, Fischer WN, Rentsch D, Frommer WB, Koch W (2002) High affinity amino acid transporters specifically expressed in xylem parenchyma and developing seeds of *Arabidopsis*. J Biol Chem 277:45338–45346

Ozcan S, Dover J, Johnston M (1998) Glucose sensing and signaling by two glucose receptors in the yeast *Saccharomyces cerevisiae*. EMBO J 17:2566–2573

Price J, Laxmi A, Martin SK, Jang J-C (2004) Global transcription profiling reveals multiple sugar signal transduction mechanisms in *Arabidopsis*. Plant Cell 16:2128–2150

Rolland F, Moore B, Sheen J (2002) Sugar sensing and signaling in higher plants. Plant Cell 14:S185–S205

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol 57:675–709

Sakamoto K, Briggs WR (2002) Cellular and subcellular localization of phototropin 1. Plant Cell 14:1723–1735

Smeekens S (2000) Sugar-induced signal transduction in plants. Annu Rev Plant Physiol Plant Mol Biol 51:49–81

Xiao W, Sheen J, Jang J-C (2000) The role of hexokinase in plant sugar signal transduction and growth and development. Plant Mol Biol 44:451–461