Letunic, I., Goodstadt, L., Dickens, N. J., Doerks, T., Schultz, J., Mott, R., Ciccarelli, F., Copley, R. R., Ponting, C. P., and Bork, P. (2002). Recent improvements to the SMART domain-based sequence annotation resource. *Nucleic Acids Res.* **30,** 242–244.

McEwen, D. P., Gee, K. R., Kang, H. C., and Neubig, R. R. (2002). Fluorescence approaches to study G protein mechanisms. *Methods Enzymol.* **344,** 403–420.

McGuffin, L. J., Bryson, K., and Jones, D. T. (2000). The PSIPRED protein structure prediction server. *Bioinformatics* **16,** 404–405.

Morton, T. A., and Myszka, D. G. (1998). Kinetic analysis of macromolecular interactions using surface plasmon resonance biosensors. *Methods Enzymol.* **295,** 268–294.

O'Shannessy, D. J., Brigham-Burke, M., Soneson, K. K., Hensley, P., and Brooks, I. (1994). Determination of rate and equilibrium binding constants for macromolecular interactions by surface plasmon resonance. *Methods Enzymol.* **240,** 323–349.

Popov, S., Yu, K., Kozasa, T., and Wilkie, T. M. (1997). The regulators of G protein signaling (RGS) domains of RGS4, RGS10, and GAIP retain GTPase activating protein activity *in vitro*. *Proc. Natl. Acad. Sci. USA* **94,** 7216–7220.

Remmers, A. E., Posner, R., and Neubig, R. R. (1994). Fluorescent guanine nucleotide analogs and G protein activation. *J. Biol. Chem.* **269,** 13771–13778.

Ross, E. M. (2002). Quantitative assays for GTPase-activating proteins. *Methods Enzymol.* **344,** 601–617.

Snow, B. E., Brothers, G. M., and Siderovski, D. P. (2002). Molecular cloning of regulators of G-protein signaling family members and characterization of binding specificity of RGS12 PDZ domain. *Methods Enzymol.* **344,** 740–761.

Sunyer, T., Codina, J., and Birnbaumer, L. (1984). GTP hydrolysis by pure Ni, the inhibitory regulatory component of adenylyl cyclases. *J. Biol. Chem.* **259,** 15447–15451.

Tesmer, J. J., Berman, D. M., Gilman, A. G., and Sprang, S. R. (1997). Structure of RGS4 bound to AlF_4^- activated G(i alpha 1): Stabilization of the transition state for GTP hydrolysis. *Cell* **89,** 251–261.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25,** 4876–4882.

Wieland, T., Chen, C. K., and Simon, M. I. (1997). The retinal specific protein RGS-r competes with the gamma subunit of cGMP phosphodiesterase for the alpha subunit of transducin and facilitates signal termination. *J. Biol. Chem.* **272,** 8853–8856.

Zhang, X. F., Marshall, M. S., and Avruch, J. (1995). Ras-Raf complexes *in vitro*. *Methods Enzymol.* **255,** 323–331.

[20] AtRGS1 Function in *Arabidopsis thaliana*

By JIN-GUI CHEN and ALAN M. JONES

Abstract

Arabidopsis thaliana RGS1 is a novel ''regulator of G-protein signaling'' (AtRGS1) protein that consists of an N-terminal seven transmembrane domain characteristic of G-protein-coupled receptors and a C-terminal RGS box. AtRGS1 modulates plant cell proliferation. *Atrgs1*

0076-6879/04 $35.00

mutants are insensitive to glucose and less sensitive to fructose and sucrose, suggesting that sugar signaling in Arabidopsis involves AtRGS1. In addition, sugar metabolism and phosphorylation by hexokinase (HXK) are not required for AtRGS1-mediated sugar signaling, suggesting that AtRGS1 functions in a HXK-independent glucose signaling pathway.

Introduction

Glucose is not only a nutrient for eukaryotic cells, but also serves as a potent signal controlling growth and development (Rolland *et al*., 2001, 2002). In the yeast *Saccharomyces cerevisiae*, glucose-induced filamentous growth preceded by an increase in the intracellular cAMP level requires Gpr1p, a G-protein-coupled receptor (GPCR) and the Gα subunit Gpa2p (Colombo *et al*., 1998; Forsberg and Ljungdahl, 2001; Kraakman *et al*., 1999; Rolland *et al*., 2000, 2001; Versele *et al*., 2001).

Plants, like other eukaryotes, use heterotrimeric G-protein signaling in the regulation of growth and development (Assmann, 2002; Jones, 2002; Ullah *et al*., 2001, 2002, 2003); however, compared to metazoans, plants utilize a far simpler and atypical system. The *Arabidopsis* genome contains only one gene encoding a canonical G-protein α subunit (*GPA1*), one gene encoding a G-protein β subunit (*AGB1*), and two genes encoding G-protein γ subunits (*AGG1* and *AGG2*) (Assmann, 2002; Jones, 2002). In contrast, there are an estimated 20 Gα, 6 Gβ, and 12 Gγ genes in mammals (Vanderbeld and Kelly, 2000). To date, a plant GPCR with its cognate ligand has not been identified.

Regulators of G-protein signaling (RGS) proteins accelerate the deactivation of G-protein α subunits to reduce GPCR signaling (Neubig and Siderovski, 2002). A novel RGS protein (AtRGS1) has been identified in Arabidopsis (Chen *et al*., 2003). The Arabidopsis AtRGS1 protein has an unusual modular construction: a predicted N-terminal seven transmembrane (7TM) domain characteristic of GPCRs and a C-terminal RGS box; therefore, AtRGS1 appears to be a structural hybrid of a GPCR and an RGS protein. The RGS box of AtRGS1 binds to the *Arabidopsis* GPA1 protein in a nucleotide-dependent manner, accelerates its intrinsic GTPase activity, and complements the pheromone supersensitivity phenotype of the yeast RGS mutant, *sst2*Δ, indicating that the RGS box of AtRGS1 is functional. AtRGS1 is a critical modulator of plant cell proliferation, and some evidence suggests that it may mediate sugar signaling (Chen *et al*., 2003). *Atrgs1* mutants have altered sensitivity to high concentrations of glucose, raising the possibility that AtRGS1 is a cell surface sugar receptor coupled by GPA1.

In addition to the pathway involving the GPCR Gpr1, *S. cerevisiae* contains three other glucose-sensing pathways: one involving hexokinase

(HXK), one utilizing glucose carrier-like proteins and one using glycolytic intermediates as possible metabolic messengers (Rolland *et al*., 2001). The GPCR pathway is desensitized by one of the four yeasts RGS proteins, RGS2 (Versele *et al*., 1999). While less defined as in yeast, plants may also have at least three distinct glucose signal transduction pathways: a hexokinase (HXK)-dependent pathway (Jang *et al*., 1997; Moore *et al*., 2003; Xiao *et al*., 2000), a HXK-independent pathway (Ciereszko *et al*., 2001; Martin *et al*., 1997; Mita *et al*., 1997), and a glycolysis-dependent pathway that depend on the catalytic activity of HXK (Xiao *et al*., 2000). These pathways can be dissected using sugar analogs that are differently transported and metabolized.

Materials and Methods

Plant Materials

The genotypes of T-DNA insertion mutants *Atrgs1-1* and *Atrgs1-2* are described in Chen *et al*. (2003). All mutants used here are in the ecotype Columbia (Col-0) *Atrgs1-1* is from the Torrey Mesa Research Institute Arabidopsis T-DNA ''SAIL'' (formerly GARLIC) Collection (www.tmri.org). SAIL is an insertion collection that has been generated from approximately 100,000 individual T-DNA mutagenized Arabidopsis plants (Columbia ecotype). It should be noted that effective as of January 1, 2004, Syngenta Biotechnology, Inc. (SBI) will no longer be distributing seeds from the SAIL collection to academic researchers (http://www.tmri.org/pages/collaborations/garlic files/GarlicDescription.html). However, of the approximately 100,000 lines in the SAIL collection, about 90% are being donated to the Arabidopsis Biological Resource Center (ABRC) in Columbus, Ohio, so researchers will be able to access these publicly accessible lines directly through the ABRC (http://www.arabidopsis.org/abrc/). Sequence information for the entire SAIL collection will be deposited into GenBank and is also being provided to Joe Ecker at the Salk Institute to enter into his public site for finding Arabidopsis mutants (http://signal.salk.edu/tabout.html). *Atrgs1-2* was obtained from another publicly accessible strain repository, the Salk Institute sequence-indexed T-DNA insertion mutant collection (http://signal.salk. edu/cgi-bin/tdnaexpress), which contains 141,486 T-DNA sequences, and the seeds were ordered from ABRC (http://www.arabidopsis.org/abrc/). Plants homozygous for *Atrgs1-1* and *Atrgs1-2* are isolated, and the insertion is confirmed by sequencing at UNC-Chapel Hill, using T-DNA left border primers (for *Atrgs1-1*: 5′-TAGCATCTGAATTTCATAACCAATCTCGATACAC-3′, and for *Atrgs1-2*: 5′-GGCAATCAGCTGTTGCCCGTCTCACTGGTG-3′). Loss of detectable *AtRGS1*

transcripts in *Atrgs1-1* and *Atrgs1-2* mutants is verified by reverse transcriptase polymerase chain reaction (PCR) (Chen *et al*., 2003)

AtRGS1 Cloning and Expression

The entire open reading frame of *AtRGS1* (At3g26090) is amplified by PCR from a cDNA library made from seedlings grown in light for 10 days and cloned into the pENTR/D-TOPO vector (Invitrogen, Carlsbad, CA) and then subcloned into Gateway plant transformation destination vectors pB2GW7 and pK7FWG2, respectively (Karimi *et al*., 2002) by LR recombination reaction. In both constructs, expressions of *AtRGS1* (in vector pB2GW7) or *AtRGS1-GFP* (in vector pK7FWG2) are driven by the 35S promoter of cauliflower mosaic virus. Both constructs are transformed into *A. thaliana* (Columbia–0 ecotype) by *Agrobacterium*-mediated transformation (Bechtold and Pelletier, 1998). At least three independent transgenic lines are analyzed for each construct.

Chemical Reagents

All sugars and sugar analogs are purchased from Sigma-Aldrich (St. Louis, MO). Murashige & Skoog (M&S) basal medium with Gamborg's vitamins is purchased from ICN Biomedicals Inc. (Aurora, OH). Phytoagar is purchased from Research Products International Corp. (Mt. Prospect, Ill).

Sugar Sensitivity Assay

Wild-type (Col-0) and *Atrgs1-1* and *Atrgs1-2* mutant seeds are sown, chilled, and light treated, and the plants are grown under identical condition until maturation. When the plants are dried and seeds turn brown, seeds are collected from each genotype separately by breaking the siliques and are cleaned by removing debris. The seeds are put into a small envelope (2.5×4.0 in.) and stored at 23° with low humidity (about 20%). For sugar sensitivity assays, seeds are sterilized with 80% ethanol for 2 min, followed by 30% bleach with 0.1% Tween 20 for 10 min, and then washed with sterile deionized water six times under sterile condition. Sterilized seeds are stratified at 4° by placing them in the dark for 48 h, sown on plates containing 1/2 M&S salts with Gamborg's vitamins (pH adjusted to 5.7 with 1 *N* KOH), 0.5% phytoagar, and different concentrations or types of sugars or sugar analogs, and germinated and grown horizontally at 23° with constant fluorescent light (75 μmol/m^2/s). Ten days later, the growth of seedlings is photographed by a digital camera linked with a dissection

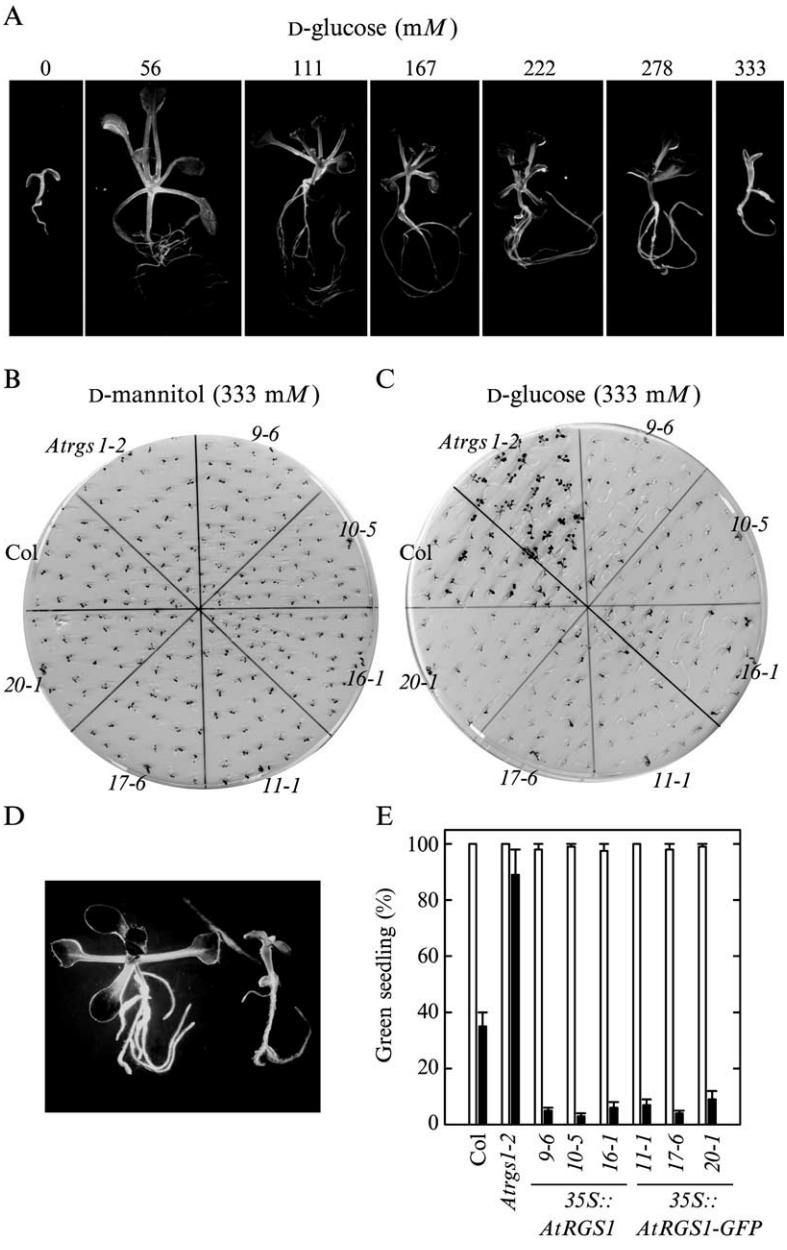


FIG. 1. AtRGS1 mediates glucose responses in *Arabidopsis*. (A) Glucose dose-dependent developmental responses of wild-type (Col-0) *Arabidopsis* seedlings. Seedlings were photographed after 14 days grown at 23°, constant light. (B) *Atrgs1* mutants have a wild-type

microscope, and the lengths of hypocotyls and primary root are also measured under a dissection microscope.

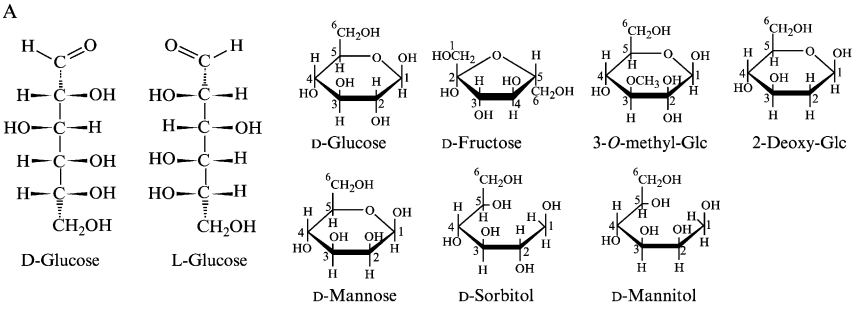
The timing for maximal differences between the genotypes may vary depending on the degree of seed dormancy. This variable is a function of the time from harvest and the temperature and relative humidity during seed storage. Germination is a complex trait. Full germination capacity is not realized until after several weeks of an after-ripening process for which temperature and humidity are important influences. Light and chilling (stratification) are typically required for germination and, as such, must also be controlled in comparative studies on germination. Finally, germination potential is lost over time and is influenced by storage conditions. Therefore, it is important to use seeds lots that are produced and harvested identically for germination or sugar sensitivity assay because sugar also affects seed germination. The percentage of green seedling is defined here as the number of green seedlings divided by the total number of seeds. Each experiment should be repeated at least twice. About 50 seeds are scored for each treatment of each genotype.

Experimental Results

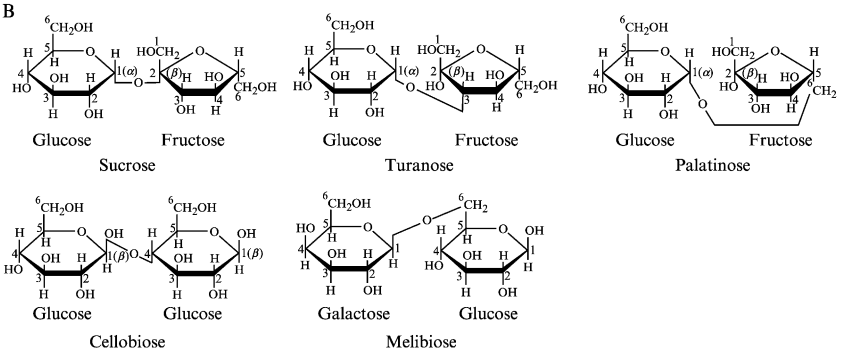
Sugars affect both seed germination and early seedling development. At low concentrations, sucrose and D-glucose promote seedling development (Fig. 1A), but are not prerequisites for seed germination. At high concentrations, sugar inhibits both seed germination (Price *et al*., 2003; Ullah *et al*., 2002) and early seedling development. Arabidopsis seedling growth in the presence of 333 m*M* D-glucose (6%) is arrested, as evidenced by the absence of postembryonic leaves, inhibition of root elongation, accumulation of anthocyanin (''reddish'' coloring), and lack of chlorophyll (Fig. 1A and D). This developmental arrest has been used for the genetic

response to D-mannitol. T-DNA insertion mutant *Atrgs1-2* and transgenic lines overexpressing *AtRGS1* (lines *9-6*, *10-5*, and *16-1*) or *AtRGS1-GFP* (lines *11-1*, *17-6*, and *20-1*) were sown on 1/2 MS plates containing 333 m*M* (6%) D-mannitol. In transgenic lines, expression of *AtRGS1* or *AtRGS1-GFP* was driven by the 35S promoter of cauliflower mosaic virus. (C) *Atrgs1* mutants are insensitive to high concentration of D-glucose. Seeds in B were sown on 1/2 MS plates containing 333 m*M* (6%) D-glucose. Seedlings in B and C were photographed after 10 days grown at 23°, constant light. (D) Green seedling *(left)* and development arrested seedling (*right*) on 333 m*M* (6%) D-glucose. Seedlings were photographed after 10 days grown at 23°, constant light. (E) Quantitative analyses of sugar sensitivities in *Atrgs1-2* mutants and transgenic lines overexpressing *AtRGS1* or *AtRGS1-GFP*. Open bar: 333 m*M* D-mannitol. Closed bar: 333 m*M* D-glucose. The percentages of green seedlings were scored after 10 days grown at 23°, constant light. Shown are means ± SE of three replicates. (See color insert.)

A



B



C

Compound	Structure	Transportable	Metabolizable	Phosphorylatable by HXK
Monosaccharide				
D-glucose		Yes	Yes	Yes
L-glucose		No	No	No
D-fructose		Yes	Yes	Yes
3-O-methyl-glucose		Yes	No	No
2-Deoxy-glucose		Yes	No	Yes
D-mannose		Yes	No	Yes
D-sorbitol		Yes	No	Yes
D-mannitol		Yes	No	Yes
Disaccharide				
Sucrose	Glc[1–2]Fru	Yes	Yes	Yes
Turanose	Glc[1–3]Fru	No	No	Yes
Palatinose	Glc[1–6]Fru	No	No	Yes
Cellobiose	Glc[1–4]Glc	Yes	Yes	Yes
Melibiose	Gal[1–6]Glc	Yes	No	Yes

FIG. 2. Sugar and sugar analogs used in *Atrgs1* sugar sensitivity assay. (A) Monosaccharides. (B) Disaccharides. (C) Some biological characteristics of these sugars and sugar analogs.

selection of mutants with an altered response to sugars (Rolland *et al*., 2002; Smeekens, 2000).

Seedling development of *Atrgs1* mutants is less sensitive to high concentrations of sugar (Chen *et al*., 2003), suggesting that sugar signaling in plants may involve the heterotrimeric G-protein complex. Consistent with this, overexpression of *AtRGS1* conferred hypersensitivity to glucose (Fig. 1C and E). The insensitivity of *Atrgs1* mutants to glucose is not due to osmotic stress because *Atrgs1* mutants and *AtRGS1* overexpressors have wild-type responses to 333 m*M* (6%) D-mannitol (Fig. 1B and E).

Sensitivities of *Atrgs1* mutants to different monosaccharide and disaccharide types (Fig. 2) were tested to dissect the three potential signaling pathways in plants described earlier. In all these experiments, the particular sugar/disaccharide or analogs were the sole sugar source. Each of these sugar analogs has altered characteristics in transport, metabolism, or phosphorylation compared to glucose and sucrose (Fig. 2C). L-Glucose is an unnatural enantiomer of D-glucose and is transported poorly (Gogarten and Bentrup, 1989; Oliveira *et al*., 2002), metabolized poorly (Chevalier *et al*., 1996; Koch *et al*., 2000), and phosphorylated poorly (Tiessen *et al*., 2003). L-Glucose at 333 m*M* completely inhibited seed germination both in wild-type and in *Atrgs1* mutants (Fig. 3). D-Fructose and D-glucose are constitutional isomers. *Atrgs1* mutants are less sensitive to 333 m*M* D-Fructose than wild type, evident by longer roots and hypocotyls, and the formation of true leaves, compared with stunted wild-type seedlings having only the cotyledons (the embryonic leaf pair). However, the resistance of *Atrgs1* mutants to D-fructose was less compared to the same concentration of D-glucose (Fig. 3). 3-*O*-Methyl-D-glucose is a nonmetabolizable D-glucose analog and is not a substrate for HXK. At 50 m*M*, 3-*O*-methyl-D-glucose inhibits seedling growth but not seed germination both in wild-type and in *Atrgs1* mutants. The phenotypes of seedlings grown in 3-*O*-methyl-D-glucose mimic the phenotypes of seedlings grown in the absence of sugar. This implies that sugar metabolism and phosphorylation by HXK are required for early seedling growth and development.

2-Deoxy-D-glucose and D-mannose are also nonmetabolizable D-glucose analogs, but they are substrates for HXK. Both 2-deoxy-D-glucose and D-mannose cause developmental arrest at relatively low concentrations. At 5 m*M*, 2-deoxy-D-glucose and D-mannose completely block seed germination, whereas it requires approximately 333 m*M* D-glucose to block seed germination. At 1 m*M* 2-deoxy-D-glucose or D-mannose, both wild-type and *Atrgs1* mutants can germinate, although at a low rate, and form green seedlings (Fig. 3). However, root production was blocked completely. This inhibition can be restored by metabolizable sugars (Pego *et al*., 1999), suggesting that sugar metabolism is required for seedling development.

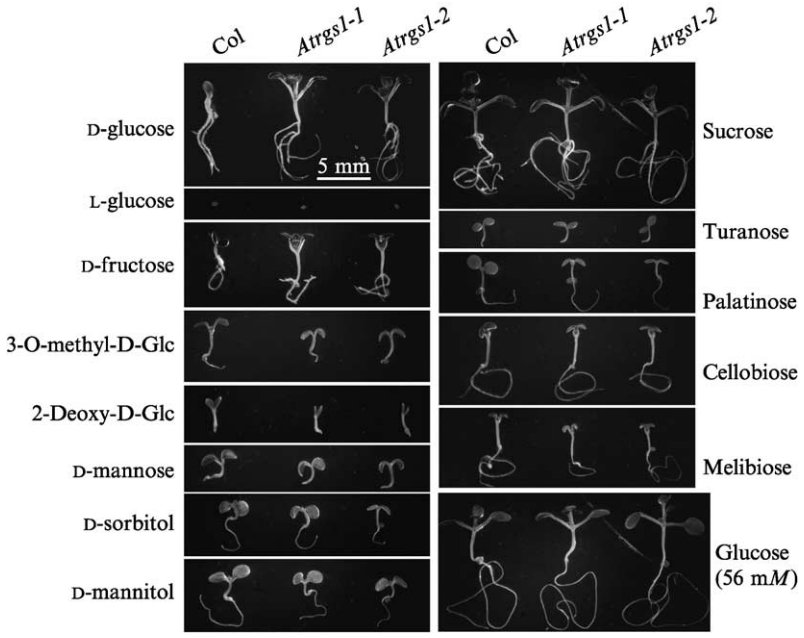


FIG. 3. Sugar specificities of *Atrgs1* mutants. D-Glucose, L-glucose, D-fructose, D-sorbitol, and D-mannitol were used at 333 m*M*. 3-*O*-Methyl-D-glucose (3-O-methyl-D-Glc) was used at 50 m*M*. 2-Deoxy-D-glucose (2-Deoxy-D-Glc) and D-mannose were used at 1 m*M*. All disaccharides (sucrose, turanose, palatinose, cellobiose, and melibiose) were used at 200 m*M*. Wild-type (Col) and *Atrgs1* mutants grown at 56 m*M* D-glucose were used as controls. In all these experiments, the particular sugar/disaccharide or analog was the sole sugar source. Seedlings were photographed after 10 days grown at 23°, constant light. Seeds on 333 m*M* L-glucose did not germinate. (See color insert.)

Because no significant difference in growth phenotype was observed between wild-type and *Atrgs1* mutants in either 3-*O*-methyl-D-glucose (transportable, nonmetabolizable, not a substrate for HXK) or 2-deoxy-D-glucose or D-mannose (both transportable, nonmetabolizable, substrates for HXK), sugar metabolism and phosphorylation by HXK are not responsible for the glucose insensitivity observed in *Atrgs1* mutants. AtRGS1 probably functions in an HXK-independent glucose-signaling pathway.

Both D-sorbitol and D-mannitol are reduced forms of D-glucose and are used as comparable osmotic solutes here. No difference between wild-type and *Atrgs1* mutants was observed when they were grown at 333 m*M* (6%) D-sorbitol or D-mannitol (Fig. 3).

Plants sense a wide variety of sugars, but among soluble carbohydrates, disaccharide sucrose is the predominant form. Sucrose may also act as a

signaling molecule in plants, although no sucrose sensor has been identified so far. Because glucose and sucrose are interconverted in the plant cell metabolically, the effect of sucrose on seedling development could be attributed to the constituent hexoses (glucose and fructose), sucrose, or both. At 200 m*M* (6.85%) sucrose, the accumulation of chlorophyll (dark-green leaves) was evident in wild-type Arabidopsis seedlings. At this concentration, the phenotypic differences between wild-type and *Atrgs1* mutants were subtle. However, the sensitivity of wild-type and *Atrgs1* mutants on 300 m*M* (10.27%) sucrose was comparable to that on 333 m*M* glucose (data not shown).

Turanose and palatinose are structural isomers of sucrose composed of glucose and fructose with different glycosidic linkages (Fig. 2). They are not synthesized in higher plants and cannot be cleaved or transported by plant enzymes. Neither turanose nor palatinose competes for sucrose transport (Sinha *et al*., 2002). However, in tomato suspension culture cells, both turanose and palatinose can specifically activate MAPK activity, whereas glucose and sucrose elicit only weak MAPK activation probably due to an osmotic effect because the same concentration of mannitol has a similar effect (Sinha *et al*., 2002). These findings indicate that nonmetabolizable sucrose isomers such as turanose and palatinose can activate distinctly different signal transduction pathways from metabolizable sugars. Here we show that both turanose and palatinose at 200 m*M* inhibit seedling development dramatically (Fig. 3) and that turanose blocks root formation completely. No significant difference was observed between wild-type and *Atrgs1* mutants. These results further support the idea that sugar metabolism is required for early seedling development, but is not required for AtRGS1-mediated sugar signaling.

Cellobiose, a disaccharide obtained by the partial hydrolysis of cellulose, consists of two D-glucopyranoses joined by a 1,4′,-β-glycoside bond (Fig. 2). Cellobiose cannot be digested by humans and cannot be fermented by yeast. However, it is metabolizable in plants (Loreti *et al*., 2000). Cellobiose at 200 m*M* also has an inhibitory effect on seedling growth and development (Fig. 3) and has an equal effect on wild-type and *Atrgs1* mutants. Melibiose is composed of galactose and glucose (Fig. 2). In barley embryos, melibiose is a nonmetabolizable disaccharide (Loreti *et al*., 2000). Melibiose at 200 m*M* inhibits Arabidopsis seedling growth and development to a similar extent in wild-type and *Atrgs1* mutants (Fig. 3).

This structure–function analysis of disaccharides indicates that a fructose moiety is needed for disaccharide sensing. Alteration of the fructosyl moiety, such as in turanose and palatinose, or replacing the fructose moiety with glucose or galactose, such as in cellobiose and melibiose, results in

significant inhibition of seedling growth and development and masks the sugar insensitivity or hyposensitivity observed in *Atrgs1* mutants.

Taken together, *Atrgs1* mutants are insensitive to D-glucose and less sensitive to fructose and sucrose. Because sugar metabolism and phosphorylation by HXK are not required for AtRGS1-mediated signal signaling, AtRGS1 most likely functions in an HXK-independent glucose signaling pathway.

Acknowledgments

Work in A.M.J.'s laboratory on the Arabidopsis G protein is supported by the NIH (GM65989-01) and NSF (MCB-0209711).

References

Assmann, S. M. (2002). Heterotrimeric and unconventional GTP binding proteins in plant cell signaling. *Plant Cell.* **14**(Suppl.), S355–S373.

Bechtold, N., and Pelletier, G. (1998). In planta Agrobacterium-mediated transformation of adult *Arabidopsis thaliana* plants by vacuum infiltration. *Methods Mol. Biol.* **82,** 259–266.

Chen, J. G., Willard, F. S., Huang, J., Liang, J., Chasse, S. A., Jones, A. M., and Siderovski, D. P. (2003). A seven-transmembrane RGS protein that modulates plant cell proliferation. *Science* **301,** 1728–1731.

Chevalier, C., LeQuerrec, F., and Raymond, P. (1996). Sugar levels regulate the expression of ribosomal protein genes encoding protein S28 and ubiquitin-fused protein S27a in maize primary root tips. *Plant Sci.* **117,** 95–105.

Ciereszko, I., Johansson, H., and Kleczkowski, L. A. (2001). Sucrose and light regulation of a cold-inducible UDP-glucose pyrophosphorylase gene via a hexokinase-independent and abscisic acid-insensitive pathway in Arabidopsis. *Biochem. J.* **354,** 67–72.

Colombo, S., Ma, P., Cauwenberg, L., Winderickx, J., Crauwels, M., Teunissen, A., Nauwelaers, D., de Winde, J. H., Gorwa, M. F., Colavizza, D., and Thevelein, J. M. (1998). Involvement of distinct G-proteins, Gpa2 and Ras, in glucose- and intracellular acidification-induced cAMP signalling in the yeast *Saccharomyces cerevisiae*. *EMBO J.* **17,** 3326–3341.

Forsberg, H., and Ljungdahl, P. O. (2001). Sensors of extracellular nutrients in *Saccharomyces cerevisiae*. *Curr. Genet.* **40,** 91–109.

Gogarten, J. P., and Bentrup, F. W. (1989). Substrate specificity of the hexose carrier in the plasma membrane of Chenopodium suspension cells probes by transmembrane exchange diffusion. *Planta* **178,** 52–60.

Jang, J. C., Leon, P., Zhou, L., and Sheen, J. (1997). Hexokinase as a sugar sensor in higher plants. *Plant Cell* **9,** 5–19.

Jones, A. M. (2002). G-protein-coupled signaling in Arabidopsis. *Curr. Opin. Plant Biol.* **5,** 402–407.

Karimi, M., Inze, D., and Depicker, A. (2002). GATEWAY vectors for Agrobacterium-mediated plant transformation. *Trends Plant Sci.* **7,** 193–195.

Koch, K. E., Ying, Z., Wu, Y., and Avigne, W. T. (2000). Multiple paths of sugar-sensing and a sugar/oxygen overlap for genes of sucrose and ethanol metabolism. *J. Exp. Bot.* **51**(GMP Special issue), 417–427.

Kraakman, L., Lemaire, K., Ma, P., Teunissen, A. W., Donaton, M. C., Van Dijck, P., Winderickx, J., de Winde, J. H., and Thevelein, J. M. (1999). A *Saccharomyces cerevisiae* G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. *Mol. Microbiol.* **32,** 1002–1012.

Loreti, E., Alpi, A., and Perata, P. (2000). Glucose and disaccharide-sensing mechanisms modulate the expression of α-amylase in barley embryos. *Plant Physiol.* **123,** 939–948.

Martin, T., Hellman, H., Schmidt, R., Willmitzer, L., and Frommer, W. B. (1997). Identification of mutants in metabolically regulated gene expression. *Plant J.* **11,** 53–62.

Mita, S., Murano, N., Akaike, M., and Nakamura, K. (1997). Mutants of *Arabidopsis thaliana* with pleiotropic effects on the expression of the gene for β-amylase and on the accumulation of anthocyanin that are inducible by sugars. *Plant J.* **11,** 841–851.

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

Neubig, R. R., and Siderovski, D. P. (2002). Regulators of G-protein signalling as new central nervous system drug targets. *Nature Rev. Drug Discov.* **1,** 187–197.

Oliveira, J., Tavares, R. M., and Geros, H. (2002). Utilization and transport of glucose in *Olea europaea* cell suspensions. *Plant Cell Physiol.* **43,** 1510–1517.

Pego, J. V., Weisbeek, P. J., and Smeekens, S. C. M. (1999). Mannose inhibits Arabidopsis germination via a hexokinase-mediated step. *Plant Physiol.* **119,** 1017–1023.

Price, J., Li, T. C., Kang, S. G., Na, J. K., and Jang, J. C. (2003). Mechanisms of glucose signaling during germination of Arabidopsis. *Plant Physiol.* **132,** 1424–1438.

Rolland, F., De Winde, J. H., Lemaire, K., Boles, E., Thevelein, J. M., and Winderickx, J. (2000). Glucose-induced cAMP signalling in yeast requires both a G-protein coupled receptor system for extracellular glucose detection and a separable hexose kinase-dependent sensing process. *Mol. Microbiol.* **38,** 348–358.

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

Rolland, F., Winderickx, J., and Thevelein, J. M. (2001). Glucose-sensing mechanisms in eukaryotic cells. *Trends Biochem. Sci.* **26,** 310–317.

Sinha, A. K., Hofmann, M. G., Romer, U., Kockenberger, W., Elling, L., and Roitsch, T. (2002). Metabolizable and non-metabolizable sugars activate different signal transduction pathways in tomato. *Plant Physiol.* **128,** 1480–1489.

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

Tiessen, A., Prescha, K., Branscheid, A., Palacios, N., McKibbin, R., Halford, N. G., and Geigenberger, P. (2003). Evidence that SNF1-related kinase and hexokinase are involved in separate sugar-signalling pathways modulating post-translational redox activation of ADP-glucose pyrophosphorylase in potato tubers. *Plant J.* **35,** 490–500.

Ullah, H., Chen, J. G., Temple, B., Boyes, D. C., Alonso, J. M., Davis, K. R., Ecker, J. R., and Jones, A. M. (2003). The β-subunit of the Arabidopsis G protein negatively regulates auxin-induced cell division and affects multiple developmental processes. *Plant Cell* **15,** 393–409.

Ullah, H., Chen, J. G., Wang, S., and Jones, A. M. (2002). Role of a heterotrimeric G protein in regulation of Arabidopsis seed germination. *Plant Physiol.* **129,** 897–907.

Ullah, H., Chen, J. G., Young, J. C., Im, K. H., Sussman, M. R., and Jones, A. M. (2001). Modulation of cell proliferation by heterotrimeric G protein in *Arabidopsis*. *Science* **292,** 2066–2069.

Vanderbeld, B., and Kelly, G. M. (2000). New thoughts on the role of beta-gamma subunit in G-protein signal transduction. *Biochem. Cell Biol.* **78,** 537–550.
Versele, M., Lemaire, K., and Thevelein, J. M. (2001). Sex and sugar in yeast: Two distinct GPCR systems. *EMBO Report* **21,** 574–579.
Versele, M., Winde, J., and Thevelein, J. M. (1999). A novel regulator of G protein signalling in yeast, Rgs2, downregulates glucose-activation of the cAMP pathway through direct inhibition of Gpa2. *EMBO J.* **18,** 5577–5591.
Xiao, W., Sheen, J., and Jang, J. C. (2000). The role of hexokinase in plant sugar signal transduction and growth and development. *Plant Mol. Biol.* **44,** 451–461.

[21] Identification and Functional Analysis of the *Drosophila* Gene *loco*

By SEBASTIAN GRANDERATH and CHRISTIAN KLÄMBT

Abstract

In contrast to vertebrates, the fruit fly *Drosophila melanogaster* contains only a small number of regulator of G-protein signaling (RGS) domain genes. This article reviews current knowledge on these genes. Although the fruit fly is particularly amenable to genetic analysis and manipulation, not much is known about the functions and mechanisms of action. The best-studied RGS gene in *Drosophila* is *loco*, a member of the D/R12 subfamily. The four different protein isoforms all contain RGS, GoLoco, and RBD domains. This article describes the identification and functional analyses of *loco* in the *Drosophila* system and discusses some mechanistic models that may underlie *loco* function.

Introduction

One of the many pathways cells used to couple cell surface-bound receptors to intracellular signaling systems are heterotrimeric G proteins. These membrane-associated complexes comprise a GTP-hydrolyzing subunit called Gα and a Gβ/Gγ heterodimer. In its inactive form, Gα is bound to GDP and forms a complex with the Gβ/Gγ subunit. Following activation, GTP displaces GDP and GαGTP dissociates from the trimer. Both the free Gα subunit and the G$\beta\gamma$ complex are capable of activating specific downstream signaling components. Intrinsic GTPase of Gα activity hydrolyzes GTP to GDP and inorganic phosphate; the subsequent reassociation of Gα and G$\beta\gamma$ subunits terminates signal transduction.

0076-6879/04 $35.00