Modulation of Cell Proliferation by Heterotrimeric G Protein in Arabidopsis

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The α subunit of a prototypical heterotrimeric GTP-binding protein (G protein), which is encoded by a single gene (GPA1) in Arabidopsis, is a modulator of plant cell proliferation. gpa1 null mutants have reduced cell division in aerial tissues throughout development. Inducible overexpression of GPA1 in Arabidopsis confers inducible ectopic cell division. GPA1 overexpression in synchronized BY-2 cells causes premature advance of the nuclear cycle and the premature appearance of a division wall. Results from loss of function and ectopic expression and activation of GPA1 indicate that this subunit is a positive modulator of cell division in plants.

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Heterotrimeric G proteins regulate cell growth, differentiation, and transformation in animal cells (1). Many growth factors activate receptors that transmit signals to the cytoplasm through heterotrimeric G proteins. Of the 17 Go subunits that have been cloned, 10 couple mitogenic signaling (2, 3). Studies of the interaction between Go subunits and proliferation support the emerging view that the α subunits form a new class of oncocenes (4–6).

16. This was observed for the ORD spectra recorded in a Pockel’s cell instrument as well as for those obtained in a calcite prism instrument.

17. Two important questions are worth remarking on at this point: (i) the UV/vis absorption bands of the homoassociates occur at very different wavelengths than those of the monomeric species (7–13), allowing us to attribute unambiguously the detected chirality to the homoassociate chromophores; (ii) the huge absorptivity of the porphyrin chromophor transitions results in high rotational strengths and leads to high sensitivities in the detection of enantiomeric excesses.


19. True chirality meets the nonexchange condition between enantiomers through the space reversal as well as with the time reversal operators (e.g., a vortex translation). False chirality meets this condition through the space reversal operator (e.g., rotation in a gravitational field) [(18); L. D. Barron, Chem. Soc. Rev. 15, 189 (1986)].

20. In fact, complementary experiments conducted with magnetically stirred solutions where aggregation was supposedly fostered by acidification of a free base porphyrin solution, at near to constant ionic strength, did not show any substantial signature of chiral selection.


of the gpa1-1 mutant (Fig. 2, compare C and D).

The short hypocotyl of gpa1 seedlings was due to a reduced number of elongating cells (Fig. 3), indicating impaired cell division. gpa1 mutants have about 10 hypocotyl cells (Fig. 3A), compared with the typical 20 cells of the WT (Fig. 3B). The number of hypocotyl cells is established during embryogenesis, whereas hypocotyl length after germination is established almost exclusively by cell elongation (15). Maximum cell lengths in gpa1 mutants were normal, and no additional compensating cells were observed in the hook region (Fig. 2C).

Normal leaf morphogenesis is driven by cell division and expansion. Division begins at the apex of the primordium and moves basipetally ahead of a wave of cell expansion to drive the major increase in leaf area. Additional cell divisions within intercalary meristems influence leaf shape. Epidermal leaf cells of 3-week-old gpa1 mutants are significantly larger and fewer at all positions examined in the leaf (Fig. 3E). This increase in cell expansion compensates the reduction in cell division in the gpa1 mutants. The gpa1 mutants exhibit a rotundifolia-like (16) leaf shape when grown in light (Fig. 3C). Rotundifolia encodes cytochrome P450, which might be involved in brassinosteroid synthesis (17). We have found that gpa1 mutants have reduced brassinolide responsiveness (18), consistent with the phenotype of rotundifolia.

To visualize the deduced decrease in cell division, we analyzed a mitotic reporter (19, 20) in the gpa1 background. β-Glucuronidase (GUS) staining of both the apical meristems and basal cells of the first leaf was markedly reduced in gpa1 mutants compared with controls (Fig. 4). Because overall leaf expansion was slightly faster in gpa1 seedlings, comparisons were made to wild-type seedlings that were both developmentally (5-day-old) and chronologically (4-day-old) the same as gpa1 expanding leaves. Although the normal basal pattern of division in the control leaves was apparent as a discrete and intense wave of staining, this pattern was not observed in developing gpa1 leaves. Instead, weak and diffuse GUS staining in aerial tissues was consistently found. The most likely explanation of this result is that Gαs of the nuclear cycle is lengthened in gpa1 cells. As expected, owing to a lack of a root phenotype, GUS staining in gpa1 roots was consistently similar to GUS staining in WS roots, indicating that Gαs does not regulate proliferation in root meristems. Therefore, we view Gαs as an intermediate signal element integrating signals that modulate cell division. Signals modulating division are necessarily different between root and shoot cell types (21).

Inducible, ectopic expression of GPA1 in Arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of arabidopsis conferred inducible, ectopic cell division in multiple organs. Three homozygous lines transformed with GPA1 under the control of a dexamethasone (dex)-inducible promoter showed distinctive phenotypes only after exposure to dex, whereas control plants did not display a dex-dependent phenotype (Fig. 5). The induced phenotypes showed medium to severe reduction in growth (Fig. 5, B and C) that correlated with the level of
GPA1 expression. Each phenotype could be explained by ectopic cell division. This is most evident in the shoot epidermis, where ectopic division planes and decreased cell area in leaves overexpressing GPA1 are abundant (Fig. 5, H to K). Furthermore, overexpression of GPA1 led to excessive cell division in meristematic regions, as well as initiation of adventitious meristems (Fig. 5D).

To determine more precisely how GPA1 modulates cell division, we expressed Arabidopsis GPA1 in synchronized tobacco BY-2 cells (line designated GOX1). The DNA content was measured in synchronized cells 6 hours later, after cells were released from aphidicolin-induced arrest [Web fig. 1, A and B (22)]. The addition of auxin shifts the percentage of control cells in G2 from 15 to 60% during this time; however, synchronized GOX1 cells advance to the maximum G2 percentage in the absence of auxin. Furthermore, whereas synchronized control cells had not synthesized a cell plate 24 hours after release from aphidicolin inhibition, 50% of GOX1 cells showed a nascent cell plate during this time [Web fig. 1, C and D (22)]. The auxin-induced advance in nuclear cycle was also demonstrated by increased [3H]thymidine incorporation in control cells [Web fig. 1E (22)]. The results indicate that overexpression of GPA1 leads to increased cell division by shortening G1, consistent with the lengthened G1 phase predicted by the behavior of the loss-of-function mutants. Additional support for a role for GPA1 in modulating cell division is shown with the use of Mas7, an activator of Ga. The addition of Mas7, but not the inactive analog Mas17, markedly increased DNA synthesis in control cells, consistent with the Arabidopsis and BY-2 GPA1 overexpression data [Web fig. 1 (22)].

In mammals, the βγ subunit of heterotrimeric G proteins also triggers cell proliferation, but indirectly, by way of the mitogen-activated protein kinase (MAPK) pathway (1, 2, 23–25). Because Gβγ does not change conformation upon binding to Ga (26, 27), its downstream actions are solely dependent on Ga activation and subsequent dissociation of the heterotrimeric complex (24). One interpretation of the current results is that a plant Gβ modulates cell division because activation of Ga releases sequestration of Gβγ subunits in the cell. Therefore, a possible consequence of Ga overexpression could manifest its phenotype on a MAPK pathway regulated by the Gβγ subunits. Signal transduction by auxin, a prominent modulator of plant cell division and elongation, appears to use a MAPK pathway. Activation of the MAPK cascade suppresses auxin signal transduction (28), and therefore the partial inhibition of cell division in gpa1 plants might result from Gβγ suppression of a
MAPK pathway. Additionally, it is plausible that Gβγ release regulates a potassium channel, as shown for brain cell GIRK2 (29).

Cell division and elongation are fundamental cellular processes in the life cycle of plants. Stimuli from multiple signaling pathways become integrated at some point to modulate proliferation. Because gpa1 mutants are compromised in multiple signal transduction, GPA1 represents this point of integration for many signals. For example, ABA regulation of ion channels in guard cells is completely eliminated (30). In addition to the indirect evidence that auxin signal transduction uses GPA1, we find that gpa1 mutants are less sensitive to gibberellic acid, brassinolide, and ACC and are hypersensitive to sugars. Intuitively, multiple signaling inputs are expected to modulate a single (or few) critical pathway(s) involved in cell division and elongation in plants. Now that a critical player in the cell proliferation pathways has been identified, further studies should clarify the mechanism through which it acts and how it integrates different signaling pathways leading toward cell proliferation.

References and Notes

10. T-DNA-tagged Arabidopsis mutants (60,480) (31) were generated at the University of Wisconsin Knockout Arabidopsis facility (www.biotech.wisc.edu/ Arabidopsis/) and screened for insertions. Primers specific for the T-DNA left-border (5'-CATTTATATAAAACGGTGGCGACATCTAC-3') and the T-DNA right-border (5'-TGCGAAAACCTGCCTCTTAGGACACTTGAT-3') were used in tandem with GUS-specific primers (forward: 5'-GACCCCTTTGCGCATATTTGCTGATTTTG-3'; and reverse: 5'-GCTAGTCGAGACACAT TAGA-3').
18. W5, gpa1-1, and gpa1-2 seedlings were grown for 2 days in the dark on various concentrations of brassinolide (BR, 0 to 10 μM). W5 and gpa1 hypocalytic growth was inhibited by BR to 45% and 73% of the control lengths, respectively.
19. cyc1At-CDB-GUS contains Arabidopsis cyc1At promoter and 5′ portion of the cyclin coding region fused in-frame to the reporter β-glucuronidase (GUS) gene. The fusion contains sequences encoding the cyclin destruction box (CDB). cyc1At-CDB-GUS plants were crossed into gpa1 mutant plants. gpa1 mutants were selected from a F1 population grown for 2 days in dark. The selected plants were allowed to grow for an additional 48 to 72 hours in light before staining. The seedlings were stained for GUS activity.
22. Supplementary data are available on Science Online at www.sciencemag.org/cgi/content/full/292/5524/2066/DC1.
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