GCR1, the putative *Arabidopsis* G protein-coupled receptor gene is cell cycle-regulated, and its overexpression abolishes seed dormancy and shortens time to flowering

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Although signaling through heterotrimeric G proteins has been extensively studied in eukaryotes, there is little information about this important signaling pathway in plants. We observed that expression of *GCR1*, the gene encoding the only known (but still putative) G protein-coupled receptor of *Arabidopsis thaliana*, is modulated during the cell cycle and during plant development. Overexpression of *GCR1* in tobacco (*Nicotiana tabacum*) BY-2 cells caused an increase in thymidine incorporation and in the mitotic index of aphidicolin synchronized cells. Overexpression of *GCR1* in *Arabidopsis* caused two remarkable phenotypes: seed dormancy was abolished and time to flowering was reduced. Molecular markers of these two developmental processes (phosphatase *PP2A* and *MYB65* in germination; *LFY* during flowering) were up-regulated in *GCR1* overexpressors. These data are consistent with the hypothesis that *GCR1* may be a regulator of the cell cycle and that this regulation underlies the developmental changes observed in the *GCR1* transformants.

S ignaling through heterotrimeric G proteins is a highly conserved signal transduction mechanism responsible for transmitting extracellular signals to the cytoplasm in diverse eukaryotes (1). In animal cells these proteins are key regulators of cell growth and differentiation (2, 3). G protein-coupled receptors (GPCRs) are the initial components of this pathway. Activation of GPCRs results from ligand binding and triggers the initiation of a cascade within the cell, resulting in changes in cellular functions including the activation of many genes. Transduction of the signal is mediated by the α subunit or the βγ subunit complex of the heterotrimeric G protein when these components interact with their downstream targets (e.g., ion channels and adenylate cyclase). Normally, upon GPCR activation, GTP binds to Gα, resulting in a conformational change of the heterotrimeric G protein complex and subsequent synthesis or activation of second messengers through specific effectors. Gα has intrinsic GTPase activity and bound GTP is hydrolyzed to GDP.

The human genome contains >800 GPCRs, and these receptors are involved in hundreds of signal transduction pathways. On the other hand, only a single gene *GCR1* (4) encoding a putative GPCR has been identified in *Arabidopsis thaliana*. Whether other genes encoding proteins with seven membrane-spanning helices encode GPCRs is not clear (5, 6). The *A. thaliana* genome has only one canonical gene (*GPA1*) encoding Gα (7) although at least one other Gα-like gene has been identified (8). In addition there is one Gβ (9) and two Gγ (10, 11).

It appears therefore that in the case of plant evolution this signaling pathway did not diversify to the extent as it did in animals.

Animals respond to environmental change by physiological adjustments, but plants respond primarily by changes in development. In plants, growth responses to environmental signals and to internal developmental signals involve meristems, spe-

cialized regions where cells actively divide (12). Whether and how the rate of cell division determines the overall plant development rate remains a topic of active research and discussion. Signals such as auxin, cytokinin, brassinosteroids, light, sucrose, and stress all modulate cell proliferation and development of plants (13–17). Recent evidence indicates that overexpression in tobacco BY-2 cells of the *Arabidopsis GPA1* gene, which encodes the Gα-subunit of a heterotrimeric G protein, leads to a premature advance of the nuclear division cycle, whereas a *gpa1 Arabidopsis* null mutant has reduced cell division in its aerial parts (18). These results raise the possibility that GPCRs are regulators of the cell cycle. A different line of experimentation links the modulation of cell division to the overall growth rate of plants. Overexpression of *Arabidopsis* cyclin D2 led to a reduction of the G_1 phase of the cell cycle and an increase in the overall growth rate of transgenic tobacco plants (19). D-type cyclins have been suggested to control both the commitment to cell division and the responses of plant cells to extracellular signals during G_1 (16, 20). To investigate the possible role of *GCR1* in the cell cycle and during plant development, we examined the developmental expression pattern of this gene and the effect of its overexpression on plant development. Here we report that *GCR1* has a cell cycle associated expression pattern (like cyclin D2) in *Arabidopsis* and that its overexpression in BY-2 cells increases the incorporation of thymidine into DNA and elevates the mitotic index of the cells. Overexpression of *GCR1* in *Arabidopsis* creates transgenic plants that have an early flowering phenotype and produce seeds that lack dormancy.

Materials and Methods

Plants. *A. thaliana* ecotype Columbia was used. The *gpa1–2* mutant, a knockout of Gα, was obtained from the Ohio State University *Arabidopsis* Stock Center. *Nicotiana tabacum* BY-2 cells (Bright Yellow 2) were provided by N. Raikhel (Michigan State University, East Lansing, MI).

Vector Construction, *Arabidopsis*, and BY-2 Cells Transformation. The *GCR1* ORF was PCR-amplified from the full-length cDNA clone, supplied by R. Hooley (University of Bristol, Bristol, U.K.) by using the following primers: 5′-TCTAGACCCGG-GATGTCGGCGGTTCTCACA-3′ and 5′-ATGACTCGAGT-TGCTGGTCCTTCGGTCTTG-3′. Primers were designed to generate a *Sma*I restriction site at the 5′ end and a *Xho*I site at

the 3′ end of the *GCR1* ORF. The amplified fragment was sequenced to assure fidelity and cloned into the binary vector pMON530. Competent *Agrobacterium tumefaciens* C58 (21) were transformed with the pMON-*GCR1* plasmid and used to produce transgenic *Arabidopsis* and BY-2 cell lines. *Arabidopsis* plants were transformed as described by Bechtold and Pelletier (22). T1 transformants were selected by germination on medium supplemented with kanamycin sulfate (25 μg·ml^{-1}) and cephotaxime (100 μg·ml^{-1}). Green seedlings were transferred to soil after 2 wk of selection and grown at 21°C under a long-day regime.
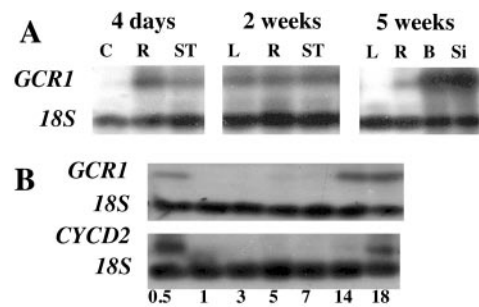
BY-2 cells were transformed as described (23). The selected transgenic calli were grown in the dark on selective medium at 24°C and transferred to fresh medium every 4 wk. For all of the experiments, liquid cultures were obtained for the different transgenic lines and synchronization was achieved by a 24-h subculture in 5 mg·liter^{-1} aphidicolin as described by Combettes *et al.* (24).

Thymidine Incorporation and Mitotic Index. DNA synthesis was measured by incubating 1-ml samples of the synchronized cell suspension with 1 μCi of [^{3}H]thymidine for 20 min at 27°C in Eppendorf microtubes with gentle shaking. Cells were collected by centrifugation for 5 min at 1,800 × *g*, and the pellet was frozen in dry ice. DNA was extracted with Plant DNAzol Reagent (GIBCO/BRL), resuspended in water, and the [^{3}H]radioactivity was determined with a scintillation counter.

Mitotic indices were determined at various times by counting mitotic figures in at least 400 cells stained with 0.25 mg·liter^{-1} Hoechst dye and 0.2% Triton X-100 by using a UV light microscope.

Northern Blot and Reverse Transcription–PCR (RT-PCR). Total RNA was prepared from plants at different developmental stages and BY-2 cell pellets by using the RNeasy Plant minikit (Qiagen, Chatsworth, CA). To remove contaminating genomic DNA, the samples were treated with DNAase I. Two micrograms of total RNA was annealed to oligo(dT)$_{12–18}$ and reverse-transcribed by using Maloney-murine-leukemia virus (M-MLV) RT (GIBCO) to obtain cDNA. The *GCR1* ORF, a 300-bp fragment from the *LFY*-coding region, and 800 bp of the protein phosphatase *PP2A* gene were PCR-amplified from total cDNA, gel-purified, and sequenced to assure accuracy, before being labeled with [^{32}P]dCTP (Rediprime II, Amersham Pharmacia) and used as probes for Northern and Southern blot experiments. Northern analysis was performed as described (25). Relative quantitative RT-PCR was performed by using as internal standard the 18S ribosomal RNA primers/competimers (Ambion) in a ratio of 3:7. All RT-PCRs were verified through Southern blot experiments by using specific probes. The sequences of the gene-specific primers used for RT-PCR are the following: *GCR1*(A), 5′-ATGTCGGCGGTTCTCACAGC-3′; *GCR1*(Z), 5′-GTAACGACCAGGAGCCAGAATTC-3′; *LFY*(A), 5′-GACGCCGTCATTTGCTACTCTC-3′; *LFY*(Z), 5′-CGTCGT-CATCCTCACCTTCGTT-3′; 5*PP2A*(A), 5′-ATGAGCA-GATCTCGCAG-3′; *PP2A*(Z), 5′-TCCTGACCAAATGT-ATATCC-3′; *AtMY65*(A), 5′-CACGGCGAGGGTAACTG-GAA-3′; *AtMY65*(Z), 5′-TGAAGGGAGCTCCAGCTTCA-3′; *AtCYCD2*(A), 5′-ATGGCTGAGAATCTTGCTTGT-3′; and *AtCYCD2*(Z), 5′-TCATTGTTTTCTCCTCCTCTT-3′.

Membrane Preparation and GTPγ ^{35}S Assay. Leaves detached from 4-wk-old plants or BY-2 cell pellets were homogenized in extraction buffer (50 mM Tris·HCl/1% β-mercaptoethanol/protease inhibitors, pH 7.4) containing 12% (wt/vol) sucrose. The membrane fraction was obtained by centrifugation at 100,000 *g* for 60 min. Membranes were suspended in GTPγS-binding assay buffer (20 mM Hepes/100 mM NaCl/10 mM

MgCl$_2$, pH 7.4), and protein concentration was determined by the Bradford assay (26).

Fifty microliters of membrane suspension (protein concentration of 200 μg·ml^{-1}) was incubated in triplicate with GTPγ^{35}S (final concentration 30 pM) in a reaction volume of 100 μl. The reaction was carried on for 1 h at room temperature (27). The membrane preparations were then collected by centrifugation at 4,000 × *g* for 15 min, and the supernatant was removed by aspiration. The amount of bound radioactivity was determined by using a Wallac Microbeta Trilux (Perkin–Elmer) spectrometer and the radioactivity bound to a control well (no microsomes) was subtracted.

Results

Expression of GCR1 Is Developmentally Regulated. In their first study on the expression of *GCR1*, Plakidou-Dymock *et al.* (4) noted that this gene is expressed at such low levels that the signal cannot be detected by Northern hybridization. Using RT-PCR with primers that distinguish between mRNA and DNA, we found expression in various plant organs, with the highest expression in flowering buds and small siliques of 5-wk-old plants. In 4-d-old seedlings, we observed high expression in the small roots, lower expression in the hypocotyl, and no expression in the cotyledons (Fig. 1*A*). By 2 wk there was similar expression in the various organs (leaves, roots, and shoot tip), and by 5 wk the expression in the leaves had disappeared, the expression in the roots was still present, and there was high expression in the flower buds and young siliques (Fig. 1*A*).

High expression in the meristematic regions and indications in the literature that G proteins may play a role in the cell cycle (18) prompted us to test whether *GCR1* is expressed at specific times during the cell cycle. Using aphidicolin synchronized *Arabidopsis* root tips, we compared the expression of *GCR1* to that of cyclin D2 (*CYCD2*), a cell cycle associated regulator of protein kinase activity that is expressed in early G$_1$. *Arabidopsis* seedlings that had just germinated, as determined by the emergence of the root tip, were transferred for 24 h to aphidicolin, which blocks the cell division cycle in the early S phase. The cell cycle blocker was then removed and the seedlings were allowed to grow in normal medium. *GCR1* expression was found to be high immediately after the removal of the block, disappeared at 1 h, to reappear 14 h later (Fig. 1*B*). The expression of *CYCD2* showed a similar pattern of expression, except that it did not reappear until 18 h after removal of the aphidicolin. Mitosis normally occurs 6–8 h after removing the aphidicolin block (28).

Fig. 1. (*A*) Relative quantitative PCR of *GCR1* expression in *Arabidopsis* at different developmental stages. RNA was prepared from cotyledons (C), roots (R), shoot tips (ST), leaves (L), buds (B), and 3-mm-long siliques (Si). (*B Upper*) Time course of *GCR1* expression in roots of 2-d-old aphidilcolin synchronized *Arabidopsis* seedlings. (*Lower*) *CYCD2* expression in the same seedlings. This expression pattern of *CYCD2* confirms that mitosis has been synchronized. 18s rRNA used as relative RT-PCR internal standard.
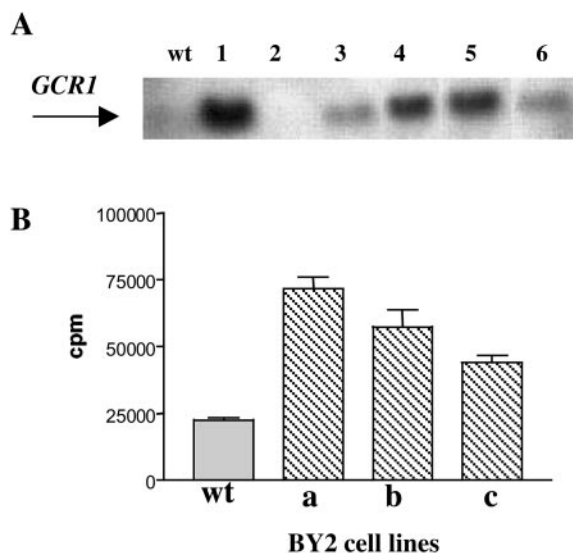
Fig. 2. (A) Northern blot analysis of *GCR1* mRNA abundance. Each lane contains 10 μg of RNA from six different BY-2-transformed lines (1–6). *GCR1*-coding sequence was used as probe. (B) GTPγ^{35}S-binding assay. Receptor activity was measured in membrane preparations by determining the binding of the nonhydrolyzable GTP analog GTPγ^{35}S. Binding of GTPγ^{35}S in *Arabidopsis* T2 plants overexpressing *GCR1* (hatched bar) was significantly higher compared to the wt *Arabidopsis* plant (open bar). Error bars indicate SD.

Overexpression of GCR1 Increases Mitotic Index of BY-2 Cells. The modulation of *GCR1* during the cell cycle indicated to us that it might be instructive to determine the effect of overexpression of *GCR1* on the cell cycle and the expression of cell cycle-associated genes. For this line of experiments, we first used a tobacco (*Nicotiana tabacum*) cell line (BY-2) that is easily synchronizable and has been used extensively for cell cycle studies (29, 24). Transformation of BY-2 cells with the *Arabidopsis GCR1* gene driven by the CaMV35S promoter yielded cell lines that had different levels of GCR1 mRNA as determined by Northern blot (Fig. 2*A*).

To determine whether the transformed cell lines contained higher levels of active receptor, we assayed the binding of GTPγS by microsomes by using the three lines that had the highest level of *GCR1* expression. G protein coupling causes GPCR activation and the level of activation can be measured by using GTPγ^{35}S binding because this compound cannot be hydrolyzed to GDP by the intrinsic GTPase activity of Gα. The bound radioactivity is a measure of the abundance of Gα bound to active GPCR (30, 27). The amount of GTPγS bound to microsomes from the transformed BY-2 lines was 2–3 times higher than that bound to microsomes from wild-type (wt) BY-2 cells (Fig. 2*B*). The results are a strong indication that the transformed cells contained more active GCR1 than wt cells and that this GCR1 was coupled to Gα.

To determine the effect of this greater abundance of active *GCR1* on the cell cycle, we synchronized wt and GCR1 overexpressing BY-2 cells with aphidicolin for 24 h, and after removal of the cell cycle blocker, we measured thymidine incorporation (Fig. 3*A*) and counted mitotic indices (Fig. 3*B*). In wt BY-2 cells, thymidine incorporation showed two clear peaks: one at 1 h after removal of the aphidicolin and a second one at 15 h. This result is consistent with a block by aphidicolin in early S phase so that DNA synthesis starts immediately after the removal of the block. The cell cycle under these conditions is about 13–14 h. In the *GCR1*-overexpressing cells, there was a considerable increase in thymidine incorporation, not only during the times of peak incorporation, but at other times as well, suggesting that the rate of DNA synthesis may be enhanced in these cells.
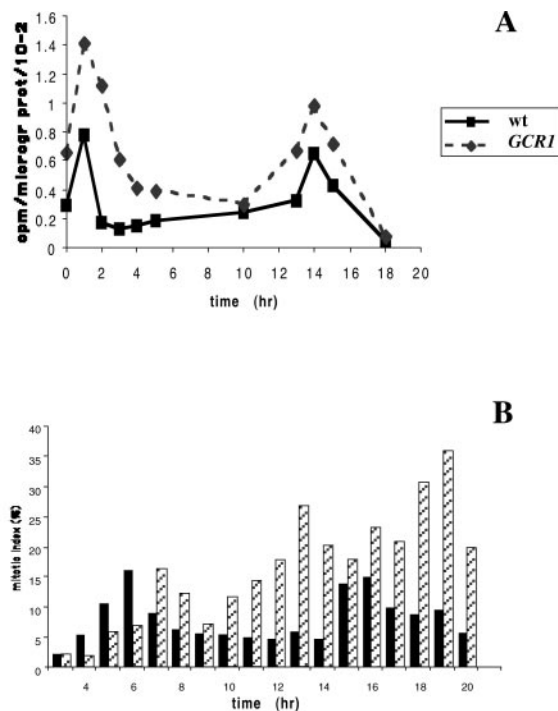

Fig. 3. (A) Thymidine incorporation by aphidilcolin-synchronized wt and *GCR1* overexpressing BY-2 cells. The experiment was carried out with duplicate cell samples and repeated three times. (B) Mitotic indices of the same cultured cells; wt = solid bars; hatched bars = *GCR1*-overexpressing cells The experiment was carried out with duplicate cell samples and repeated three times. Error bars indicate SD.

Examination of the mitotic index in the wt cells showed peaks of mitoses at 6 h and 19 h (Fig. 3*B*), in each case ≈5 h after the S phase. Mitotic indices were higher in the *GCR1*-transformed cell lines, with peaks at 5–6 h and 19 h. There was little difference in the mitotic index associated with the first peak, but at later times, the mitotic index was consistently higher in the *GCR1*-transformed cells.

Overexpression of GCR1 in *A. thaliana* Abolishes Seed Dormancy. Having obtained this interesting result, we overexpressed *GCR1* under the control of the CaMV35S promoter in *Arabidopsis* plants and checked the expression of *GCR1* by using Northern blot hybridization. The results showed, as expected, different levels of expression in the transformed lines (Fig. 4*A*). We prepared microsomes from wt and *GCR1*-transformed plants and determined GTPγ^{35}S binding (Fig. 4*B*). We included microsomes from the *gpa1–2* mutant of *Arabidopsis*, a Gα knockout that has no Gα protein (18). The results extend those shown in Fig. 2. Overexpression of *GCR1* resulted in more GTPγ^{35}S binding, suggesting more active GCR1 coupled to Gα at the plasma membrane. Binding of GTPγ^{35}S to the membranes of the *gpa1–2* mutant was less than in wt, suggesting that total binding in wt membranes reflects binding to GPA1 (a Gα protein), but possibly also to other Gα proteins (8) or to small G proteins (31). Overexpression of *GCR1* in *gpa1* could be used to demonstrate whether GPA1 is coupled to GCR1.

Arabidopsis seeds require either stratification (a cold period) or after-ripening for prompt germination but the seeds of the overexpressing lines appeared to lack dormancy. When freshly harvested wt seeds were sown on agar plates, few (1–2%) germinated within the first 7 d; even after 14 d only 35% had germinated in the experiment shown in Fig. 5*A*. However, freshly harvested seeds from the *GCR1*-transformed plants behaved
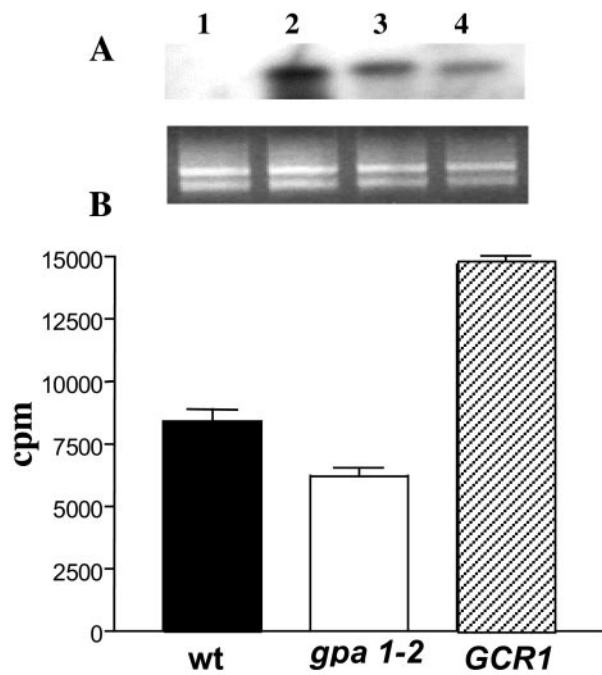
Fig. 4. (*A Upper*) Northern blot analysis of *GCR1* mRNA abundance. Each lane contains 10 mg of RNA from 4-wk-old wt plants (lane 1) or from three different *Arabidopsis* T2 plants (lanes 2–4) grown at 21°C under a long-day regime. *GCR1* ORF was used as probe. (*Lower*) Ethidium bromide-stained gel. (*B*) GTPγ35S-binding assay. Receptor activity was measured in membrane preparations by determining the binding of the nonhydrolyzable GTP analog GTPγ35S. *Arabidopsis* wt plant (solid bar), *GCR1* overexpressing line (hatched bar), and *gpa1–2* line, a Gα knockout (open bar).

differently: 40–60% germinated in the first week and 80–100% within the second week (Fig. 5*A*). Seeds from both wt and *GCR1* transformants germinated promptly after stratification (not shown). If the seeds were allowed to after-ripen for 3 wk, differences in percentage of germination were less marked but still present (data not shown). Fig. 5*B* shows the appearance of 1-wk-old seedlings grown from freshly harvested seeds of wt and three transformed lines. The GCR1 overexpressors had already grown into robust seedlings, but the wt seeds had not yet germinated. The expression of *GCR1* was monitored by relative quantitative RT-PCR. We detected no expression of *GCR1* in wt at 2, 5, or 7 d but strong expression in the transformed seedlings (data not shown).

Overexpression of *GCR1* Enhances the Expression of Germination-Associated Genes. Seed dormancy and germination are regulated by gibberellins (GAs) and abscisic acid, and release from dormancy is accompanied by changes in expression of many genes (32, 33). In a number of species including *Arabidopsis*, seed dormancy can be overcome by GA. In barley (*Hordeum vulgare*), where the control of germination by GA and GA-induced gene expression have been studied for many years, the expression of such genes depends on the transcription factor GAMYB. The expression of *GAMYB* is itself GA-dependent (34, 35). To find the closest *Arabidopsis* homolog of *GAMYB*, we performed a BLAST search of the *Arabidopsis* database and identified *MYB9*, which is identical to *AtMYB65* (partial clone). Using RT-PCR we isolated a full-length *AtMYB65* cDNA and sequenced it to verify its identity to *MYB9* (data not shown). When the expression of *AtMYB65* measured by RT-PCR in wt was compared to that in *GCR1*-transformed plants after 2 and 7 d of germination, we found high expression of *AtMYB65* in the transformed lines but not in wt plants (Fig. 5*C*).

A preliminary report, made by Gonzales-Paz *et al.* at the Plant
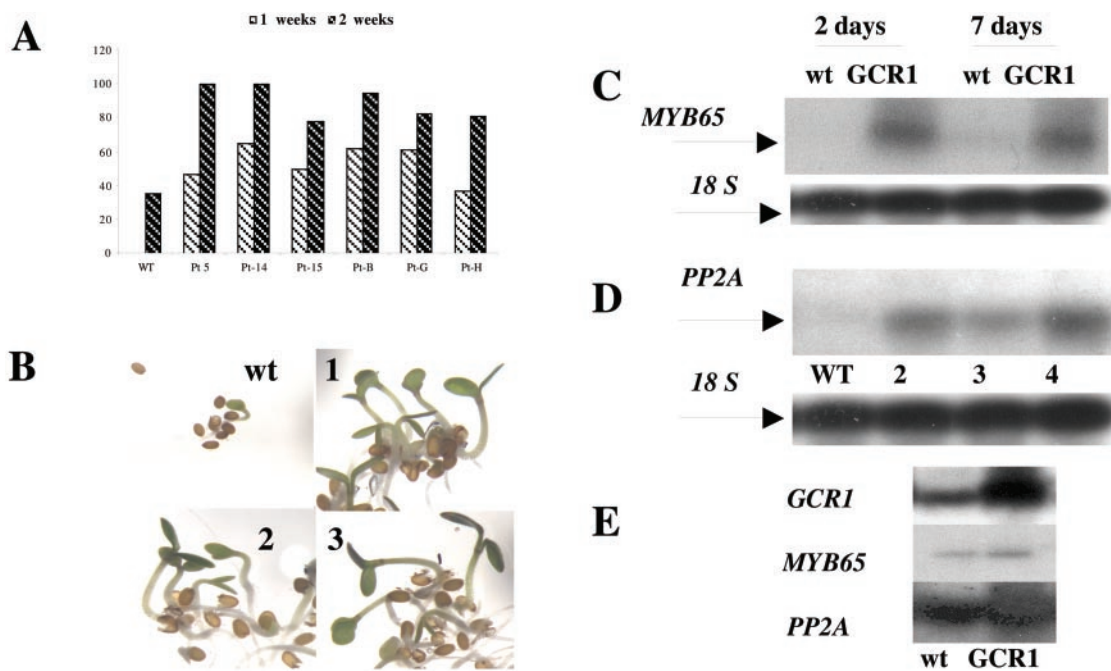
Fig. 5. (*A*) Percentage germination after 1 and 2 wk of seeds sown immediately after collection from dry pods of wt *Arabidopsis* and six different *GCR1*-overexpressing lines. Note that after 1 wk no wt seeds had germinated. (*B*) *Arabidopsis* seeds and seedlings of wt and three different T3 lines overexpressing *GCR1* (1, 2, and 3); seeds were collected at maturity and immediately sown on germination medium and kept at 21°C under a long-day regime. After 7 d the seedlings were photographed. (*C*) Relative competitive RT-PCR of *MYB65* expression in 2- and 7-d-old wt plants and a T3 line of overexpressing *GCR1* plants; 18s rRNA used as relative RT-PCR internal standard. (*D*) Relative competitive RT-PCR of the regulatory subunit of protein phosphatase 2A (*PP2A*) expression in wt (lane 1) and in three different T3 lines overexpressing *GCR1*. RNA was prepared from 7-d-old plants. 18s rRNA used as relative RT-PCR internal standard. (*E*) Expression of *GCR1*, *MYB65*, and *PP2A* in 2-wk-old plants measured by RT-PCR in wt and a *GCR1*-overexpressing line.
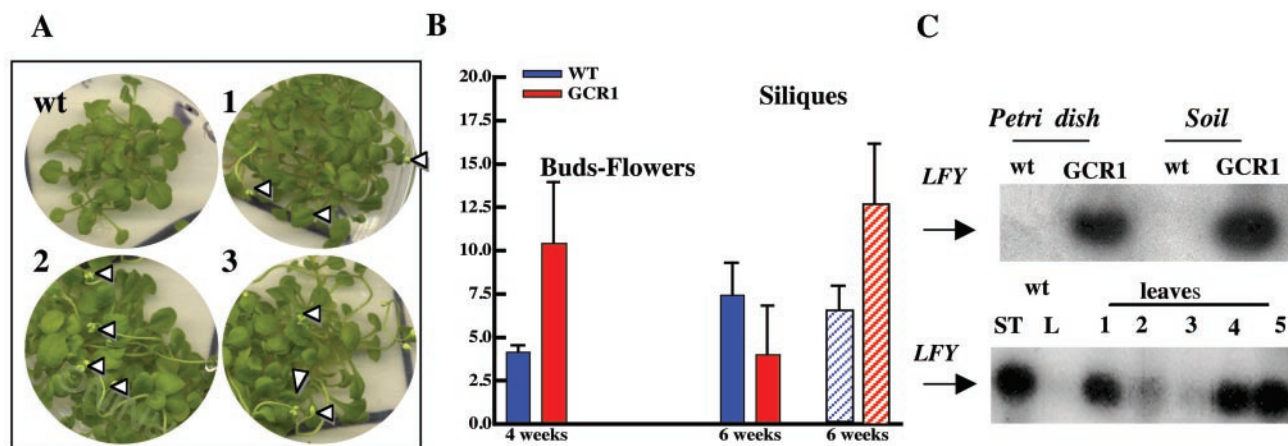
Fig. 6. (A) wt *Arabidopsis* plants and plants of three different T3 lines overexpressing *GCR1* (1, 2, and 3) were sown on germination medium in Petri dishes, stratified, and kept for 4 wk at 21°C under a long-day regime. White arrowheads indicate flowers. (B) Number of buds (solid bars) and siliques (hatched bars) counted after 4 and 6 wk in 60 wt plants and 60 *GCR1*-overexpressing plants (six different lines) grown at 21°C under a long-day regime. Error bars indicate SD. (C Upper) RT-PCR of *LEAFY* (*LFY*) expression in 1-wk-old seedlings of *Arabidopsis* wt grown in a Petri dish (lane 1) or in soil (lane 3) and in a *GCR1*-overexpressing *Arabidopsis* line grown in a Petri dish (lane 2) or in soil (lane 4). (Lower) RT-PCR of *LFY* expression in wt and in five different T3 lines overexpressing *GCR1*. RNA was prepared from shoot tips (ST) or leaves (L) collected from 4-wk-old wt and from leaves of different overexpressing lines.

Growth Regulator Conference in Brno, Czech Republic, in the summer of 2001,[§] indicated that release from dormancy in beechnut is accompanied by the activation of the gene encoding the catalytic subunit of the serine/threonine phosphatase PP2A. PP2A also has been implicated in GA responses during germination (36). Using RT-PCR, we found no detectable expression of the gene for the catalytic subunit of PP2A in the wt seeds 7 d after sowing, but expression was easily detected in the *GCR1*-transformed lines at this time (Fig. 5D). However, 2 wk after sowing, when a substantial number of wt seeds had formed plantlets, we found expression of *GCR1*, *AtMYB65*, and the *PPA2* catalytic subunit gene in wt and *GCR1*-transformed lines (Fig. 5E). These results are consistent with the interpretation that GCR1 initiates a cascade that leads to germination in seeds that would otherwise be dormant.

Overexpression of *GCR1* Accelerates Flowering and the Expression of Genes that Control Flowering. *Arabidopsis* is a long-day plant that bolts and flowers under the proper daylength conditions. Under short days, flowering is delayed. For the experiments described here all seeds were first exposed to a cold period so that they would germinate synchronously. After 4 wk of growth in Petri dishes and under long-day conditions, we observed many fully formed flowers in the *GCR1*-transformed plants, but wt plants had only a few flowers at this time (Fig. 6A). A quantitation of this phenomenon with plants grown in soil (Fig. 6B) shows that at 4 wk there were twice as many flowers per plant in the *GCR1* overexpressing lines as in wt. By 6 wk the *GCR1*-transformed plants had fewer flowers but more than twice as many siliques as wt plants. Thus the entire reproductive process occurred earlier in those plants.

Flowering in *Arabidopsis* is under the control of *LEAFY* (*LFY*), a meristem identity gene that is activated by GA (37–39). *LFY* is expressed in the meristem, and expression increases normally after 2 wk of growth (40). We found high levels of *LFY* expression in 7-d-old seedlings of *GCR1*-transformed lines, whether grown from stratified seeds in a Petri dish or in soil (Fig. 6C Upper), whereas expression was still undetectable in wt plants. After 4 wk (Fig. 6C Lower) *LFY* was expressed in the buds

of wt plants but not in the leaves (as expected). In the transformed lines *LFY* was expressed in the shoot tips (not shown) and also ectopically in the leaves. Variation in the expression of *LFY* in the leaves may reflect the level of expression of *GCR1*, including possible silencing, or the amount of active GCR1 protein at the plasma membrane.

Discussion

Based on indirect approaches, GPCR signaling has been implicated in a number of processes in plants, but there is as yet little direct information about the function of this pathway in plants. So far only one GPCR gene, *GCR1*, has been found in the *Arabidopsis* genome (5). Here we show that *GCR1* is expressed in a developmentally specific manner and is regulated during the cell cycle. We demonstrate that overexpression of the *GCR1* receptor results in greater binding of GTPγS to isolated membranes, providing evidence that this putative receptor is coupled to a GTP-binding protein (presumably Gα). We also show that overexpression in BY-2 tobacco cells increases DNA synthesis and the mitotic index of these cultured cells, whereas overexpression in *Arabidopsis* plants produces two interesting developmental phenotypes: loss of seed dormancy and shortening of time to flower and fruit set.

It may be too early to draw these observations together into one overarching hypothesis, but it is tempting to suggest that *GCR1* plays a role in the regulation of the cell cycle and that the developmental consequences of its overexpression are the result of a modulation of the cell cycle. The results with the aphidicolin synchronized *Arabidopsis* seedlings show that *GCR1* is expressed immediately after this blocker is removed and again 13 h later. These are the times when DNA synthesis peaks in these synchronized cells. Expression of *GCR1* precedes the expression of *CYCD2*, a cell cycle-regulated gene that is expressed in early G_1 (16). In BY-2 cells, overexpression increases incorporation of thymidine into DNA, suggesting higher levels of DNA synthesis, which then support higher levels of mitotic activity observed between 6 and 19 h after removal of the block. Sucrose has been shown to trigger the cell cycle in plant (16) and we have found that sucrose also triggers transcription of *GCR1* (data not shown). Because GCR1 is still a putative receptor whose ligand and effector(s) remains to be identified, we cannot yet test whether this receptor is a cell cycle regulator.

The results described here for the phenotypes of the *GCR1* overexpressing *Arabidopsis* plants are consistent with the interpretation that G protein-coupled signaling may be limiting for certain developmental stages. Furthermore, the *GCR1* transformants behaved as if they were more sensitive to their endogenous GAs and/or less sensitive to their endogenous abscisic acid. GA is involved in the breaking of dormancy and the induction of flowering and both processes were accelerated in the *GCR1* transformants. A number of other studies also support the interpretation that GA signaling is linked to GPCR signaling. For example, the dwarf rice mutant *d1*, which is a Gα null, has short internodes and is GA insensitive (41). The aleurone cells of *d1* produce very little α-amylase when exposed to GA, and the induction of *GAMYB* in aleurone cells is greatly attenuated in the mutant. We observed that *GCR1* overexpression turns on *MYB65*, the *Arabidopsis* homolog of *GAMYB*. We also have found that germination of the seeds from *GCR1* overexpressing lines is less sensitive to exogenous abscisic acid than seeds from control plants (data not shown). This lower sensitivity could simply be caused by an increased sensitivity to GA. It is curious in this respect that the stomatal response of *gpa1* is less sensitive to abscisic acid (42).

The interpretation of an increase in mitotic index is never straightforward. It doesn't necessary mean that the cell cycle is shorter. Rather, the increase may be an indicator that M is longer and that one or more of the other phases (G_1, S, or G_2) are shorter. However, such compensation was not observed when CycD2 was expressed in tobacco plants (19). Overexpression of *GPA1* from a regulated promoter was shown to lead to excessive cell division in meristem and the initiation of additional meristems (18). At which point the GCR1-signaling pathway interacts with GA signaling might be identified in the future by the isolation of mutants in those pathways and/or by biochemical and pharmacological approaches. Further insights into the mechanism of GCR1 action will come from crossing these overexpressing plants with various hormone insensitive mutants. We suggest on the basis of our data that it will be fruitful to identify chemicals (agonists and inverse agonists) that modulate GCR1 activity in plants with the goal of modifying growth and development in specific ways.

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