Re-evaluation of the cytokinin receptor role of the Arabidopsis gene GCR1

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Summary

GCR1 has been tentatively identified in Arabidopsis thaliana as the first plant G-protein coupled receptor (GPCR) (Josefsson and Rask 1997) implicated in the cytokinin sensory pathway (Plakidou-Dymock et al. 1998). A protein fusion of GCR1 and green fluorescent protein has been expressed in Arabidopsis and shown GCR1 to be located on the plasma membrane. Studies of plants with altered GCR1 expression have led us to question GCR1’s involvement in cytokinin signaling. Transgenic Arabidopsis plants containing sense and antisense constructs for GCR1 have been produced and over- and under-expression confirmed. The analysis of 12 antisense and 17 sense lines has failed to reveal the previously reported «Dainty» phenotype or altered cytokinin sensitivity. We have used the «Gauntlet» approach to test the plants’ response to various plant hormones although this has not yet identified a mutant phenotype. The yeast-two hybrid system has been used and so far there is no evidence to suggest GCR1 interacts with heterotrimeric G proteins. Before GCR1 can be identified as genuine G-protein coupled receptor, the identification of a ligand and a proof of association with heterotrimeric G-proteins should be obtained.

Key words: Arabidopsis thaliana – antisense – cytokinin – gauntlet – GCR1 – G-protein – receptor – signal transduction

Abbreviations: BA benzyladenine. – CLSM confocal laser-scanning microscopy. – G-protein GTP-binding protein. – GFP green fluorescent protein. – GPCR G-protein coupled receptor. – QRT-PCR quantitative reverse transcription polymerase chain reaction

Introduction

The receptor occupies the starting position in any signaling pathway and as such, the search for plant receptors has been the focus of many researchers hoping to gain an understanding of plant signaling. For many, the most obvious start-
This means that genes are now being identified and cloned based on varying degrees of homology to other genes from plants or other organisms and now we are presented with the novel problem of assigning functions to them. GCR1 is one such gene that has tentatively been identified, yet attempts to ascertain its function remain unsuccessful.

One of the largest protein families and the largest class of receptors are the G-Protein coupled receptors (GPCRs). This diverse family shares only a small degree of primary sequence homology although there have been a few attempts to establish relationships based on sequence comparisons (Probst et al. 1992, Josefsson and Rask 1999). GPCRs are characterized by a functional coupling to heterotrimeric G-proteins as well as a conserved seven transmembrane domain structure. They have been found in a wide variety of organisms. Even though heterotrimeric G-proteins have been identified in plants, GCR1 is so far the most promising candidate for a plant GPCR (Josefsson and Rask 1997, Plakidou-Dymock et al. 1998).

Despite the limited homology between groups of GPCRs, GCR1 was identified simultaneously by three groups including ourselves using homology based methods (Humphrey and Botella 1996, Josefsson and Rask 1997, Plakidou-Dymock et al. 1998) EST analysis files from the cDNA Sequencing Analysis Project at the University of Minnesota’s Plant Molecular Informatics Center revealed an Arabidopsis EST with significant homology to a family of GPCRs from Dictyostelium discoideum. with significant homology to a family of GPCRs from Dictyostelium discoideum. Arabidopsis Molecular Informatics Center revealed an Arabidopsis EST with significant homology to a family of GPCRs from Dictyostelium discoideum. After obtaining the cDNA clone (Genbank # T04329) from the Arabidopsis Biological Resource Center at Ohio State University, the clone was sequenced and hydrophobicity analysis of the amino acid sequence revealed seven hydrophobic transmembrane domains characteristic of all GPCRs (Fig. 1). The sequence of this gene was later published by Josefsson and Rask (1997) and then by Plakidou-Dymock et al. (1998) who named the gene GCR1. Interestingly, the work published by Plakidou-Dymock et al. (1998) suggests a role for GCR1 in cytokinin signaling. The production of antisense GCR1 plants led to the identification of the «Dainty» phenotype in 6 out of 24 lines. This phenotype was characterized by reduced leaf expansion and the production of a single flowering stem at the 5–8 leaf stage. In addition to these morphological characteristics, the antisense plants were found to exhibit a reduced sensitivity to the cytokinin benzyladenine (BA). Although not directly implying that GCR1 is a cytokinin receptor, these results do suggest it may play a functional role in cytokinin signaling. This result also provided the first suggestion of heterotrimeric G protein involvement in cytokinin response.

Heterotrimeric G proteins have been implicated in a number of plant signaling processes including auxin and gibberellic response, photochrome signaling, stomatal aperture control and pathogen defence response (reviewed in Millner and Causier 1996, Hooley 1998). All of these processes would therefore be expected to involve a functional GPCR, yet so far none have been identified.

Materials and Methods

Northern and Southern blot analysis

Total RNA was extracted from roots, stems, cauline leaves, flowers and two-week-old seedlings of Arabidopsis thaliana (ecotype Landsberg erecta). Genomic DNA was purified from an intermediate step in the RNA extraction and Northern and Southern blot analysis was carried out essentially as described by Etheridge et al. (1999). DNA probes were labeled using the Gigaprime® labeling kit (Bresatec, Australia). As a control for sample loading, blots were stripped and hybridized to a ribosomal DNA probe (Gerlach and Bedbrook 1979). Northern blots were exposed to film and densitometry analysis was carried out using GelWorks 1D software.

GFP localization

A GCR1/green fluorescent protein (GFP) fusion construct was made by cloning the coding region of GCR1 in frame with the mGFP5 coding region (Haseloff et al. 1997) lacking the ER targeting sequence. This fusion was placed under the control of the CaMV 35S promoter in the pSOV binary vector (Mylne and Botella 1998) and transferred to Agrobacterium tumefaciens strain LBA4404 by triparental mating (Svab et al. 1995). Flowering Arabidopsis thaliana (Columbia) plants were transformed by vacuum infiltration (Bechtold et al. 1993) and selection of T1 seeds carried out in soil by spraying seedlings with 0.4 % BASTA® ( Hoecht, Germany). Transgenic Arabidopsis seedlings were grown on plates containing germination medium (GM: 1 × MS Salts, 1 × MS Vitamins, 1 % sucrose, 1 % agar) (Murashige and Skoog 1962) for approximately 10 days, then mounted in water under glass coverslips for microscopy. Seedlings were examined for GFP expression using a Bio-Rad MRC-600 confocal laser-scanning microscope equipped with a krypton-argon laser, the Bio-Rad K1-K2 filter set and a Zeiss 63 × Planapo 1.4 NA oil immersion objective. Images were composed using Confocal Assistant and Adobe Photoshop software.

Protein–protein interactions

The yeast two hybrid system was used to test the interaction between various fragments of GCR1 with the Arabidopsis G-protein alpha and beta subunits (GPA1, GBA1) (Ma et al. 1990, Weiss et al. 1994). Three fragments of the GCR1 protein were separately cloned in frame into the pAS1-CYH2 vector (Clontech, USA). These were the 3rd intracellular loop region consisting of the following amino acid sequence (GONCM…KVLNR), the C-terminal tail (GFNSS…TEDOQ) and a region starting at the 3rd intracellular loop and ending at the C-terminus (GVRM…TEDOQ) (see Fig. 1). GPA1 and GBA1 were amplified by RT-PCR using primers designed against the published sequences. After amplification the products were sequenced and cloned in frame into the pACT2 vector (Clontech, USA). The two plasmids were co-expressed in the yeast strain Y190 on selective media and assayed for interaction by the β-galactosidase filter lift assay described in Bartel et al. (1993).

Sense and antisense transgenics

GCR1 was cloned into the pBI121 binary vector (Jefferson et al. 1987) by removing the uidA gene and inserting the full length cDNA in ei-
Re-evaluating the receptor role of GCR1

Figure 1. Predicted model of GCR1 illustrating the seven transmembrane domains and extracellular N-terminus. Analysis of the amino acid sequence of GCR1 was carried out using the PHDtopology program (Rost et al. 1996) to predict hydrophobic membrane spanning regions. Conserved cysteine residues thought to be involved in disulfide bond formation are shown as filled in circles. Arrows indicate start of the third intracellular loop and C-terminal tail as used for protein interaction studies.

ther sense or antisense orientations with respect to the CaMV 35S promoter. Arabidopsis plants were transformed as described above. Seeds from separate transformations were kept separate to ensure independent transformants were obtained. Seeds were germinated on GM containing 50 $\mu$g/mL kanamycin for selection. The selection process was repeated until homozygous T3 lines were obtained.

Antisense genomic PCR

Genomic DNA was extracted from each of the antisense lines and a pBI121 control as described above and used as template for PCR. Primers corresponding to the 3' end of the 35S promoter and the 5' end of the GCR1 gene were used to detect the presence of the GCR1 antisense construct. As an internal PCR control, two primers designed to amplify a native Arabidopsis gene were also used. PCR reactions (25 $\mu$L) were prepared as follows: 1× PCR Buffer II (Perkin Elmer, USA), 0.2 $\mu$mol/L each primer, 1.5 mmol/L MgCl$_2$, 200 $\mu$mol/L each dNTP and 1 unit Taq polymerase. Thermocycling conditions consisted of an initial denaturing step (94 °C, 3 min) followed by 40 cycles of 94 °C (30 s), 60 °C (30 s), 72 °C (1 min 30 s).

Real time quantitative RT-PCR

Total RNA was extracted from 25-d-old plants as described by Etheridge et al. (1999). RNA was obtained for 12 independent homzygous T3 antisense lines as well as for plants transformed with the unmodified pBI121 vector. RNA was quantified spectrophotometrically and 1$\mu$g used for reverse transcription with the GeneAmp Gold RNA PCR Core kit (Roche Molecular Systems, USA) according to the manufacturer's instructions. Two primers were specifically designed to amplify a 680 bp fragment of the native GCR1 cDNA, the sequences of which were 5'-AAG ATA CCA CGC GAC GGA AA-3' (forward) and 5'-CAC TCG AGC CAT CTG TTC ACC TTT AA-3' (reverse). A different set of specific primers were designed for the Arabidopsis β-tubulin1 cDNA (Oppenheimer et al. 1988) to amplify a 900 bp fragment for use as an RNA loading control, these were 5'-TTG CCT TCA AGG GTT TCA AGT-3' (forward) and 5'-GTC TTC TTC ATC AGC GGT AGC-3' (reverse). PCR conditions were optimized on a conventional PCR machine and products analyzed by gel electrophoresis to ensure the production of a single clear product. PCR reactions were then carried out in quadruplicate with controls in duplicate on the ABI Prism Sequence Detector 7700 (Perkin Elmer, USA) using SYBR Green I as the fluorescent probe with fluorescence detection during the primer annealing and extension phases. PCR reactions (25 $\mu$L) were prepared as follows: 1× SYBR Green PCR buffer (Perkin Elmer, USA), 0.2 $\mu$mol/L each primer, 2 mmol/L MgCl$_2$, 200 $\mu$mol/L each dNTP, 1.25 units AmpliTaq Gold$^\text{™}$ (Roche Molecular Systems, USA) and 1$\mu$L of RT reaction products. Thermocycling conditions consisted of an initial denaturing step (95 °C, 12 min) followed by 45 cycles of 94 °C (30 s), 58 °C (30 s), 72 °C (1 min). Data was processed using Microsoft Excel.

Transgenic phenotype analysis

Surface-sterilized Arabidopsis seed were germinated on plates of GM supplemented with various plant hormones at the concentrations indicated in Table 1. The BA response test was carried out on all transgenic lines but for simplicity, a single antisense (a11) and sense (s2) line was used for the other hormone tests. After 3 days incubation at 4 °C, plates were placed vertically in a growth chamber and maintained for 7 days at 22 °C under 50 $\mu$E/m$^2$/s light provided by white fluorescent tubes with a 16 hour light photoperiod except for the
Figure 2. GFP tagged GCR1 reveals a plasma membrane location. GFP was fused to the C-terminal end of GCR1 and expressed in *Arabidopsis* under control of the CaMV 35S promoter. (A–D) Confocal laser-scanning microscope (CLSM) images of leaf epidermal cells expressing the GCR1/GFP fusion. (E) CLSM image of wild type *Arabidopsis* leaf epidermis showing level of background fluorescence. Images (D) and (E) were photographed using identical microscope settings. Scale bar represents 25 µm. (F) *Arabidopsis* leaf epidermis expressing the mGFP5-ER gene.

Results

**GFP tagging reveals plasma membrane location**

GFP was fused to the C-terminal end of GCR1 and expressed in *Arabidopsis* under control of the CaMV 35S promoter. Confocal laser-scanning microscopy of leaf epidermal cells as shown in Figure 2 reveals GFP fluorescence localized to the outer edge of the cell in discrete clusters. It is possible that this represents localization to the tonoplast which has been pressed up against the cell wall. For comparison, the ER-targeted GFP (Fig. 2F) shows the amount and distribution of the cytoplasm in epidermal cells. Therefore the proximity of the GCR1-GFP fluorescence to the cell wall suggests that GCR1 is most likely located on the plasma membrane. No *Agrobacterium* growth was observed when GFP expressing seedlings were grown on nutrient media and observation of an *Agrobacterium* culture under CLSM revealed no significant GFP fluorescence. Thus, the possibility of persistent agrobacteria causing the apparent GFP expression pattern was ruled out.

**No evidence for G-protein interaction**

The use of GCR1 in the yeast two hybrid system was limited due to its hydrophobic membrane spanning structure. The multiple membrane spanning domains in the protein suggested that folding and transport to the yeast nucleus may not occur efficiently. Evidence from studies with other GPCRs suggested the importance of the intracellular loops for interaction with heterotrimeric G-proteins (Strader et al. 1994). For these reasons, we chose to test only cytoplasmic parts of seedlings grown on ACC which were kept in the dark. For the ABA response test seedlings were germinated on plain GM and after 3 days transferred to media supplemented with ABA and grown for a further 7 days. Seedlings were photographed with a digital camera and root and/or hypocotyl lengths measured with the aid of Scion Image software (Scion Co., USA). In addition to the plate assays described above, measurements and observations were made on transgenic plants grown on soil under normal growth conditions.
GCR1 for interactions with the *Arabidopsis* Gα (*GPA1*) and Gβ (*GBA1*). The third intracellular loop, the region from the third intracellular loop through to the C-terminus and the C-terminal tail (see Fig. 1) of GCR1 were tested for interaction with Gα and the C-terminal tail was also tested for interaction with Gβ. All of the GCR1 protein fragments tested failed to give a positive interaction with either Gα or Gβ.

**Production and identification of GCR1 underexpressing lines**

*Arabidopsis* was transformed with the GCR1 antisense construct and grown through to the third (T₃) generation. Twelve independent homozygous antisense lines as well as a pBI121 control were used for further analysis. PCR was performed on genomic DNA extracted from each line to confirm transformation with the antisense construct (see Fig. 3).

Identification of down-regulation in GCR1 expression is extremely difficult as wild type GCR1 expression is at the lower limit of detection for most techniques. Conventional Reverse Transcription-PCR techniques (RT-PCR), although sensitive, can be inaccurate when end-point quantification is used (Livak 1996). Therefore, for quantifying GCR1 expression in transgenic *Arabidopsis* lines we adopted the Real Time Quantitative RT-PCR (QRT-PCR) method which monitors PCR product formation during each cycle (Freeman et al. 1999).

Identical reverse transcription reactions and subsequent PCRs were performed using total RNA extracted from all transgenic lines including the pBI121 control. Results of the Real Time QRT-PCR are shown in Figure 4. The cycle number at which the product first appeared indicated the relative abundance of initial template. The results for the β-tubulin control PCRs were identical for all lines indicating equal loading of initial RNA template. All of the antisense lines gave results indistinguishable from the pBI121 control except for no. 11. For simplicity only lines 1–3 and number 11 have been shown. The GCR1 product in the pBI121 control and most of the antisense lines first appeared at cycle 32 compared to antisense line 11 which appeared much later in cycle 35. Based on our own experiments performing amplification of...
template dilutions, this difference in cycle number corresponded approximately to a ten-fold reduction in amount of initial template indicating antisense line 11 has a ten-fold reduction in GCR1 expression.

Southern blot analysis was performed on antisense line 11 and hybridised with a probe for the CaMV 35S promoter (Fig. 5). The resulting banding pattern indicates a single T-DNA insertion consistent with the observed 3 : 1 segregation ratio. This result was confirmed by hybridisation with a GCR1 probe (data not shown).

Production and identification of GCR1 over-expressing lines

A construct containing the GCR1 cDNA in sense orientation under the control of the CaMV 35S promoter was transformed into Arabidopsis with the aim of over-expressing the gene. The large amounts of transcript produced by the 35S promoter allowed the use of conventional Northern analysis for identification of over-expressing lines. Northern analysis of 17 independent homozygous T3 lines is shown in Figure 6. All sense lines exhibited very high GCR1 RNA levels compared to the undetectable expression in the wild type and pBI121 controls.

Southern blot analysis was carried out on the representative line s2 (Fig. 7) and indicates the presence of multiple copies of the transgene.

Transgenic phenotype analysis

After identifying GCR1 under- and over-expressing lines, our first aim was to investigate the supposed involvement of GCR1 in cytokinin signaling as reported by Plakidou-Dymock et al. (1998). These authors analyzed an antisense GCR1 transgenic line finding that roots were over twice as long as those of wild type controls when grown on 6 µmol/L BA and therefore suggesting a reduced sensitivity to cytokinins. We aimed to replicate their experiment with our antisense lines and use our over-expressing lines to investigate the possibility of the converse phenotype – a cytokinin hypersensitivity.

All 12 antisense and 17 over-expressing lines were assayed for cytokinin response as described by Plakidou-Dymock et al. (1998). All lines were also grown on media without cytokinin to assess the amount of natural variation in normal root growth (Fig. 8). All transgenic antisense lines, including line a11 which has been proven to be strongly down-regulated, showed the same degree of root growth inhibition as the wild type and pBI121 controls. In addition, analysis of all 17 transgenic over-expressing lines failed to detect an increase in sensitivity to cytokinins.

Further analysis of the antisense and sense lines were carried out and are summarized in Table 1. Phenotype analysis of both under- and over-expressing plants has shown no al-
Figure 8. Cytokinin assays of GCR1 antisense (A) and sense lines (B) including wild type (WT) and pBI121 controls (pBI). Seedlings were germinated on media with or without 5 µmol/L BA, grown for 7 days and root lengths measured. Each bar represents the average root length of 20 seedlings ± standard error.

Table 1. Phenotype Analysis of GCR1 antisense and sense lines as compared to WT and pBI121 controls. All observations and tests were carried out on multiple independent lines except for those indicated with an asterisk which were performed with single sense (s2) and antisense (a11) lines.

<table>
<thead>
<tr>
<th>Observation/Test</th>
<th>Significant difference from WT?</th>
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<tbody>
<tr>
<td>No. rosette leaves at bolting</td>
<td>Antisense No</td>
</tr>
<tr>
<td>Rosette diameter</td>
<td>No</td>
</tr>
<tr>
<td>Days to bolting</td>
<td>No</td>
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<tr>
<td>Days to flowering</td>
<td>No</td>
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<tr>
<td>Height of primary bolt</td>
<td>No</td>
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<tr>
<td>Days to onset of senescence</td>
<td>No</td>
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<tr>
<td>Root length</td>
<td>No</td>
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<tr>
<td>Root growth on 5 µmol/L BA</td>
<td>No</td>
</tr>
<tr>
<td>Root growth on 10 µmol/L IAA*</td>
<td>No</td>
</tr>
<tr>
<td>Root growth on 10 µmol/L ACC in dark*</td>
<td>No</td>
</tr>
<tr>
<td>Root growth on 1 µmol/L abscisic acid*</td>
<td>No</td>
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<tr>
<td>Root growth on 1 µmol/L epibrassinolide*</td>
<td>No</td>
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<tr>
<td>Hypocotyl growth on 10 µmol/L gibberellin*</td>
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terated response to any of the plant hormones auxin, cytokinin, abscisic acid, gibberellin, ethylene and brassinosteroid. Under our growth conditions and under a variety of test conditions, intense observation has failed to reveal a distinct GCR1 mutant phenotype.

Discussion

GCR1 is located on the plasma membrane

The GCR1-GFP fluorescence is localized in discrete clusters rather than spread evenly across the membrane. This could suggest an association with particular membrane structures such as plasmodesmata or caveolae. It has previously been shown that receptors, G-proteins and effectors all occur in a greater abundance within caveolae suggesting a discrete localization of the G-protein signaling process (Anderson 1998).

GPCR targeting experiments with other GPCRs have shown that all of them are predominantly localized to the plasma membrane (e.g., Barak et al. 1997, Xiao et al. 1997). Interestingly, many of these studies show that even with the GFP molecule attached, the receptors are fully functional and exhibit all the same characteristics as GPCRs in their native state. This has enabled detailed studies of the processes involved in receptor desensitization. These studies show the receptor is evenly distributed along the whole membrane until repeated stimulation by its ligand which causes the receptors to aggregate and display a punctate distribution (Kallal et al. 1998, Tarasova et al. 1997). There has also been a report of a GPCR-GFP fusion displaying this punctate distribution in the absence of ligand (Chun et al. 1994) suggesting that different GPCRs may have different membrane distributions. The distribution seen for GCR1 is therefore in accordance with the idea of it being a GPCR.
Elucidating the function of GCR1

There is still much work to be done before we can confidently classify GCR1 as a member of the G-protein coupled receptor family. Evidence so far is limited to a small degree of homology to GPCRs from other organisms (Josefsson 1999), a predicted seven transmembrane domain structure and a plasma membrane location. Two crucial pieces of evidence are missing – a proof of association with heterotrimeric G-proteins and the identification of a ligand.

Proof of a functional association with heterotrimeric G-proteins is important for classification of GCR1 as a true GPCR. The yeast two hybrid system has previously been used to demonstrate interactions between GPCR fragments and Gox’s (Klein et al. 1997, Sun et al. 1997). Our results do not suggest any interaction between GCR1 and GPA1. However, these results do not rule out the possibility of an in vivo interaction as it is possible that interactions require the full, correctly folded GCR1 protein. Also, as there have been two Gox’s cloned from soybean (Gotor et al. 1996), we can’t yet rule out the possibility of there being a second Gox in Arabidopsis.

Another possibility is suggested by studies with other GPCRs indicating the importance of tertiary structure. In this model the receptor undergoes a conformational change upon ligand binding which then allows the interaction with Gox to occur (Strader et al. 1994, Wess 1998). This means that in the absence of the receptor’s ligand, there would be no interaction with Gox. Until a GCR1 ligand is found, there will be some restraints on the types of studies that can be used to investigate G-protein interaction. One approach which has been successfully used with other GPCRs (Wess 1998) would be to dissect the cytoplasmic parts of the receptor into smaller fragments and examine their effect on G-protein activation.

So far the absence of a distinct mutant phenotype has revealed no clues as to the identity of GCR1’s possible ligand. The absence of a phenotype is hardly surprising considering the body of evidence accumulating from T-DNA insertional mutagenesis, (Bouchez et al. 1999) which suggest fewer than 10% of genes, when knocked out, result in a visible phenotype when plants are grown in ‘optimal’ conditions. Subsequently a number of laboratories have adopted the ‘Gauntlet’ approach of screening mutants against a variety of plant hormones, growth inhibitors and stress conditions looking for altered responses (Meissner et al. 1999). This approach has so far been unsuccessful in revealing a phenotype for the GCR1 mutants.

The absence of decreased cytokinin sensitivity observed in our antisense lines is in clear contrast with the results reported by Plakidou-Dymock et al. (1998). Although we have attempted to replicate the experimental conditions used by these authors, we can not discard the possibility that minor variations in chemical suppliers or slight differences in growth conditions could account for the discrepancy of the results. However, the results presented by Plakidou-Dymock et al. (1998) were based on the analysis of a single antisense line therefore the possibility of T-DNA insertion effects cannot be ruled out.

In addition to reduced cytokinin sensitivity, Plakidou-Dymock et al. also reported that 25% of their antisense lines exhibited a distinct phenotype they label «Dainty». This phenotype was characterized by reduced cotyledon and leaf expansion and the production of a single flowering stem at the 5–8 leaf stage. Experience in our laboratory suggests that characteristics of the «Dainty» phenotype may be a general plant response to sub-optimal growth conditions. We have analyzed a large number of individual plants from many independent transgenic lines but have failed to observe this phenotype in any of our antisense plants. It is possible, however, that slight differences in our growth conditions have affected plant growth and morphology in such a way as to mask the «Dainty» phenotype.

Aside from the plant hormones already tested, there are a number of other potential candidate ligands including jasmonic acid, fusicoccin, pathogen elicitors and a wide range of as yet undefined signaling molecules which could include polyamines or lipid messengers. Despite the controversy over its importance in plant systems (Assman 1995), cAMP is even a possibility based on the low but significant homology between GCR1 and the Dictyostelium cAMP receptors. Finally, and in spite of our evidence to the contrary, a role for GCR1 in cytokinin signaling can not be ruled out and needs further investigation.

The evidence to suggest GCR1 is a G-protein coupled receptor is still fairly limited and yet also very promising. The two most important areas for further research are in the identification of a ligand and the demonstration of a coupling to heterotrimeric G-proteins. There is still much work to be done in determining the function of GCR1 and establishing its importance in plant development.

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References


