Trichome Development in *Arabidopsis thaliana*. II. Isolation and Complementation of the *GLABROUS1* Gene

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We are using the formation of trichomes in *Arabidopsis thaliana* as a model system to study gene expression during cellular differentiation. To initiate the molecular characterization of this system, we tagged and isolated a gene that is specifically required for the development of the specialized trichome cell. We confirmed the identity of this gene, *GLABROUS1* (GL1), by complementation. These results demonstrate that a crucial gene in a plant developmental pathway can be successfully identified by complementation.

INTRODUCTION

Trichomes are the hairs that are present on the surface of the leaves and stems of many plants (Esau, 1965). In *Arabidopsis thaliana*, these trichomes are large, single cells that differentiate from individual protodermal cells in the developing epidermis of the leaves and stems (Rollins and Banerjee, 1975). We are using the process of trichome formation in *Arabidopsis* as a relatively simple model system to study the complex mechanisms that control gene expression during cellular differentiation. When compared with other cell types, the development of trichomes is an attractive model system for two reasons (Haughn and Somerville, 1988). First, the process can be easily monitored because it occurs on the accessible outer surface of the plant. Second, morphological mutants can be readily isolated and characterized because trichomes are not essential for the survival of the plant.

The accompanying paper (Marks and Feldmann, 1989) describes a new trichome mutant of *Arabidopsis* that was isolated from a population of plants that had been transformed by a seed transformation procedure (Feldmann and Marks, 1987; Feldmann et al., 1989). Several different genetic analyses showed that a T-DNA insert was tightly linked to this new trichome mutation. The mutant plants exhibited a unique phenotype in that they had normal leaf trichomes, but no stem trichomes. Crosses revealed that the new mutation is allelic to the previously described *glabrous1* (gl1) mutation, which is characterized by the absence of both leaf and stem trichomes (Koornneef, Dellair, and van der Veen, 1982). The new allele was designated gl1-43. To isolate the junction between the T-DNA and flanking plant DNA, a genomic library was constructed with DNA from gl1-43 and screened with a T-DNA probe. Restriction mapping and DNA gel blot hybridization identified one clone that contained sequences that flank the left border of the T-DNA insertion site.

In the experiments described here, we used the left border clone as a probe to isolate an uninterrupted sequence from a genomic library of wild-type *Arabidopsis* and subcloned it into a binary vector suitable for Agrobacterium-mediated transformation. The transformed shoots that regenerated from gl1 root tissue exhibited normal leaf and stem trichomes. An analysis of the progeny from one of the transformed plants showed that the presence of trichomes cosegregated with the kanamycin resistance gene on the T-DNA. These results confirm that we have isolated *GLABROUS1* (GL1), a gene with an unknown product that is required for a specific type of cellular differentiation. This work also demonstrates that T-DNA tagging and complementation can be used successfully to isolate and identify a crucial gene in a plant developmental pathway.

RESULTS

Isolation of a DNA Sequence Tightly Linked to the GL1 Locus

The accompanying paper reports the cloning of a DNA sequence that flanks a T-DNA insert that is tightly linked to the GL1 locus. This 2.8-kb clone, pBS43LG, was used as a probe to isolate the uninterrupted GL1 sequence from a wild-type *Arabidopsis* genomic library. When the library was screened with this probe, a hybridizing phage clone, designated NT2, was identified. After this clone was taken through three rounds of plaque purification, DNA was
isolated and characterized by restriction enzyme analysis (Figure 1). To identify the region that corresponded to the site of the T-DNA insertion into the gl1-43 mutant, a DNA gel blot hybridization analysis was performed. Fragments from two regions of the pBS43LG border clone were used as probes and hybridized to a filter containing restricted NT2 DNA cleaved with Scal (data not shown). One probe was a 1.0-kb Scal-Acl fragment from the left side of the border clone and the other probe was a 0.8-kb Scal-EcoRI fragment from the right side of the border clone directly adjacent to the T-DNA insertion site. The Scal-Acl probe only hybridized to the sequences immediately to the left side of the Scal site located 4.2 kb from the left end of the NT2 clone. The Scal-EcoRI fragment only hybridized to the sequences immediately to the right of the same Scal site. These results demonstrate that the second Scal site from the left end of the NT2 clone corresponds to the Scal site of the pBS43LG probe. The restriction map in Figure 1 shows that the NT2 clone has fragments of 4.2 kb and 9.5 kb flanking the site of the T-DNA insertion into the gl1-43 mutant. Because the NT2 genomic clone contained large regions of sequence flanking both sides of the gl1-43 T-DNA insertion site, it was used as a source of subclones for subsequent attempts to identify the GL1 gene by complementation.

Regeneration of Wild-Type and gl1 Root Explants

Before an Agrobacterium-mediated transformation protocol could be used for complementation experiments, it was necessary to determine whether the expression of the trichome phenotype was affected by the process of tissue culture. Shoots were regenerated from root explants taken from wild-type and from gl1 mutant seedlings in the Columbia and Landsberg backgrounds using modifications of the protocol described by Valvekens, Van Montagu, and Van Lijsebettens (1988). The shoots regenerated from the gl1 Columbia and the gl1 Landsberg root explants lacked leaf and stem trichomes (Figure 2A), the expected mutant phenotype. In contrast, the shoots regenerated from the wild-type Columbia and Landsberg root explants had normal leaf and stem trichomes (Figure 2B). Interestingly, there were discernible differences in the extent and in the time course of the regeneration response that were apparently a reflection of the genotypic variation in the root explants. For example, the Columbia root explants (both wild-type and gl1) yielded several more shoots per explant than the Landsberg root explants (data not shown). Also, shoot primordia were visible on the gl1 Columbia root explants at least a week before they became visible on the wild-type Columbia root explants.

Complementation of the gl1 Allele

For the initial transformation experiments, gl1 Columbia and gl1 Landsberg root explants were cocultivated with Agrobacterium strain LBA4404 bearing the constructs pBINNT2SE and pBINNT2SH. As a negative control, gl1 root explants were also transformed with Agrobacterium carrying pBI121, a plasmid derived from the binary vector pBIN19 that contains the β-glucuronidase (GUS) gene driven by the constitutive cauliflower mosaic virus 35S promoter (Jefferson, Kavanagh, and Bevan, 1987). Transformed calli were selected on shoot-inducing medium containing 50 mg/L kanamycin. After 10 days, small green calli were visible on the roots. Most of the calli derived from the gl1 Columbia root explants had normal leaf and stem trichomes (Figure 2C). Interestingly, the shoots transformed with both of the pBIN19 constructs from the NT2 genomic clone developed normal leaf and stem trichomes (Figures 2D and 2E). The shoots transformed with the pBI121 plasmid had GUS activity when they were tested with a fluorometric assay. However, these shoots exhibited the gl1 phenotype because they lacked leaf and stem trichomes (Figure 2C). When the transformation experiments were repeated using the pBINNT2SE and the pBI121 constructs in Agrobacterium strain C58, the same results were observed.

The transformed shoots were transferred to Magenta boxes to allow seed set. Seeds were collected from one of the plants that had been transformed with the pBINNT2SE construct and germinated on medium without kanamycin. Five of the progeny from this transformant lacked trichomes, whereas 28 displayed normal trichomes on the leaves and stems (Figure 2F). When the progeny
Figure 2. Complementation of the gl1 Mutation of Arabidopsis (Columbia) with the pBINNT2SE Construct Containing the GL1 Gene.

(A) Plantlets without leaf trichomes regenerating from a gl1 root explant.
(B) Plantlets with leaf trichomes regenerating from a wild-type root explant.
(C) Plantlets without leaf trichomes regenerating from a gl1 root explant transformed with the pBl121 construct that contains the GUS gene. The plantlets exhibit GUS activity.
(D) Plantlets with leaf trichomes regenerating from a gl1 root explant transformed with the pBINNT2SE construct.
(E) Surface view of a leaf from a transformed gl1 plantlet in (D) showing the branched trichomes characteristic of a wild-type leaf.
(F) Progeny from a gl1 plant transformed with the pBINNT2SE construct segregating for leaf trichomes. One seedling has trichomes on the first pair of true leaves, whereas the other seedling completely lacks leaf trichomes. (The cotyledons, which are also visible, do not develop trichomes.)
were transferred to medium supplemented with 50 mg/L kanamycin, the plants with trichomes grew rapidly and set seed. In contrast, the five plants without trichomes grew very slowly and eventually became chlorotic.

DISCUSSION

The proof that a gene has been isolated through the use of gene tagging is the ability of the uninterrupted sequence to restore gene activity. In the accompanying paper (Marks and Feldmann, 1989), we showed that a new trichome mutation allelic to gl7 was tightly linked to a T-DNA insert. The T-DNA "tag" was used to isolate the DNA flanking the insertion site. This flanking sequence was then used to isolate the uninterrupted sequence. In this paper, we showed that the uninterrupted sequence restored the ability of the gl7 plants to produce trichomes.

The fact that the insertional mutation was allelic to gl7 gave us flexibility in the source of tissue to be used for the complementation assay. The insertional mutant had normal leaf trichomes and was kanamycin-resistant. Because the binary vector pBIN19 carries the neomycin phosphotransferase gene that confers kanamycin resistance, we found it much more convenient to attempt to transform the known gl7 mutants because they are naturally sensitive to kanamycin. The use of the gl7 mutant also allowed the success of the experiment to be assessed at an earlier stage. Since the gl7 mutants lack leaf trichomes, complementation was confirmed by the expression of branched leaf trichomes on the surface of the young leaves in the regenerating shoots. The gl7 mutant in the Columbia background, which regenerated very efficiently under our tissue culture conditions, carries another mutation. This mutation, designated an, results in narrower leaves and is unlinked to gl7. The an phenotype served as a contamination control in the complementation experiments. The only plants in the laboratory that have the an mutation also have either the dis1 mutation that results in distorted trichomes or the gl7 mutation. All of the transformed plants in the complementation experiments expressed the an phenotype and normal trichomes. This confirms that the complementation results were valid and not due to wild-type seeds contaminating our gl7 stocks.

Two overlapping constructs from the genomic clone successfully complemented the gl7 mutation. These experiments allowed the GL7 gene to be localized to an 8.3-kb Sall-EcoRI subclone. We are now in the process of transforming smaller fragments from this subclone into Arabidopsis to determine the precise location of the gene. We are also attempting to isolate a cDNA clone homologous to the region to the left of the T-DNA insertion, and we have begun to sequence the 8.3-kb subclone. These analyses should allow us to determine the sequence of the GL7 gene.

Our successful identification of the GL7 gene by complementation should permit us to isolate other interesting genes that affect trichome differentiation. Besides GL7, there are five other distinct loci affecting trichome formation which have been characterized. The phenotypes of these mutants suggest that they contain blocks at different points in trichome differentiation. We are continuing to screen our transformed Arabidopsis population for trichome mutants and we have also initiated chromosome walks to some of the known loci.

The molecular characterization of GL7 and the other trichome genes should provide information on the complex mechanisms that control the differentiation of trichome cells. An understanding of this model system may lead to a better understanding of the process by which other types of plant cells differentiate.

METHODS

Genetic Material

Seeds of Arabidopsis thaliana ecotype Landsberg erecta and of the gl7 trichome mutant in the Landsberg erecta background were obtained from Dr. Maarten Koornneef (Agricultural University, Wageningen, The Netherlands). Seeds of Arabidopsis thaliana ecotype Columbia and of the gl7 trichome mutant in the Columbia background were obtained from Dr. George Redei (University of Missouri, Columbia, MO).

Genomic Clones and Subclones

All procedures, unless indicated otherwise, were performed using standard protocols as described by Maniatis, Frisch, and Sambrook (1982). The genomic library was constructed from partially digested Sau3A fragments of genomic DNA that had been isolated from wild-type Arabidopsis in the Wassilewskija background by the method of Weeks, Beerman, and Griffith (1986). These fragments were cloned into the bacteriophage vector EMBL3. The library was screened using the pBS43LG clone (Marks and Feldmann, 1989), labeled with 32P by nick translation, as a probe. Two overlapping fragments from the NT2 genomic clone, both including the T-DNA insertion site, were subcloned into the pBluescript KS+ vector (Stratagene, Inc.) to generate the constructs pKSNT2SH and pKSNT2SE. pKSNT2SE contains an 8.3-kb Sall-EcoRI fragment from the NT2 clone. pKSNT2SH contains a 10.6-kb Sall-HindIII fragment that includes the same region as the pKSNT2SE subclone and an additional 2.3-kb region (Figure 1).
Agrobacterium tumefaciens strain harboring a helper Ti plasmid pBINNT2SE and pBINNT2SH. The constructs were then transferred by direct transformation (An et al., 1988) into a competent Agrobacterium tumefaciens strain harboring a helper Ti plasmid to supply the virulence functions necessary for DNA transfer from the bacterium to the plant. Two Agrobacterium strains were used: LBA4404 containing the plasmid pAL4404 (Hoekema et al., 1983), and C58 containing the plasmid pGV3850 (Zambryski et al., 1983).

**Regeneration and Transformation of Root Explants**

Root explants were transformed and regenerated using modifications of the method described by Valvekens et al. (1988). The callus-inducing medium was R3 medium (Christianson, 1979), and the shoot-inducing medium was 0.05/7 medium (Christianson and Warnick, 1983). All hormones were added to the media before it was autoclaved at 121°C for 15 min.

Seeds were sterilized in pure Clorox for 2 min, washed four times with sterile distilled water, rinsed with 95% ethanol, and dried in a laminar flow hood. They were germinated on a solidified medium containing MS salts and vitamins, 3% sucrose, and 0.8% Difco agar. Roots were grown for at least 3 weeks under fluorescent light (100 μE/m²/sec²) on a 12-hr light/dark cycle at 22°C in Magenta boxes on a solidified medium containing MS salts and vitamins, 3% sucrose, and 0.8% Difco agar. Root explants about 1 cm in length were taken from these in vitro seedlings and incubated on solid callus-inducing medium for 3 days before cocultivation for 48 hr with Agrobacterium cells grown overnight in 3 mL of YEP medium (An et al., 1988).

**Measurement of β-Glucuronidase Activity**

The fluorometric assay of Jefferson (1987) was used to measure GUS activity in plants that had been transformed with the pBI121 binary vector.

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**REFERENCES**


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