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Trichome Development in *Arabidopsis thaliana*. I. T-DNA Tagging of the *GLABROUS1* Gene

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Progeny from a transformed *Arabidopsis* plant (produced by the *Agrobacterium*-mediated seed transformation procedure) were found to be segregating for an altered trichome phenotype. The mutant plants have normal leaf trichomes but completely lack trichomes usually found on the stem. The mutation is tightly linked to a T-DNA insert. Complementation analysis with genetically characterized trichome mutants revealed that the new mutation is an allele of the *GL1* locus. The new trichome mutant has been designated *gl1-43*. DNA gel blot analysis indicated that the insert site contains a complex array of at least four tandemly linked T-DNA units oriented as both direct and inverted repeats. A genomic library, constructed using DNA from *gl1-43* plants, was used to clone DNA that flanks the left end of the T-DNA insert. The availability of DNA from the region interrupted by the insert has allowed initial characterization of the wild-type *GL1* gene and will permit the eventual cloning and sequencing of this developmentally interesting gene.

INTRODUCTION

The molecular mechanisms of cellular differentiation in plants are poorly understood. Since numerous efforts are currently underway to isolate and characterize genes required for various developmental pathways from several plant species (e.g., Bowman, Smyth, and Meyerowitz, 1989; Hake, Vollbrecht, and Freeling, 1989; McCarty et al., 1989), this situation should change. *Arabidopsis* is playing a major role in these studies because of the availability of morphological mutants (McKelvie, 1962; Koornneef et al., 1983; Kranz and Kirchheim, 1987; Haughn and Somerville, 1988) and because of the feasibility of isolating developmentally important genes via chromosome walking or gene tagging (Meyerowitz and Pruitt, 1985; Guzman and Ecker, 1988; Feldmann et al., 1989).

As a model for comparison to more complex forms of plant cell differentiation, we are studying the molecular mechanism of development of plant hairs (trichomes) on *Arabidopsis*. The trichomes of *Arabidopsis* are composed of single cells that differentiate from protodermal cells on immature leaves and stems. This process begins when a cell starts to rapidly enlarge relative to its neighbors (J. Esch and M.D. Marks, manuscript in preparation). The expansion occurs perpendicular to the leaf or stem surface and results in a spike-like cell (branched or unbranched; see Figure 1) that is up to 500 μm in length.

Trichome differentiation is an attractive model system for two reasons. First, certain attributes of the developmental process make it amenable to study. Normal or altered development can easily be monitored because trichome formation occurs on the surface of the plant. In addition, since loss of trichomes is not detrimental to the plant (unlike the loss of other cell types), it has been possible to induce mutations that block normal trichome development. Second, the differentiation of trichomes follows the same sequence of events observed in the differentiation of other cell types that are involved in the formation of complex organs and tissues. For example, trichome formation can be compared to the formation of the procambium and its subsequent differentiation into the vascular system. Procambium formation is initiated with the differentiation of a fraction of the residual meristem cells into procambial cells (Esau, 1977). As growth in the stem continues, the procambial cells divide and differentiate into phloem and xylem cells. This process requires drastic changes in cell shape. In a similar fashion, trichome differentiation also involves only a fraction of the protodermal cells of immature leaves and stems and also requires drastic changes in cell shape. Similar types of molecular signals and responses may be common to both developmental programs.

The availability of six nonallelic, genetically characterized mutants of *Arabidopsis* that exhibit alterations in trichome formation further enhances the utility of the model. Although the trichome mutations (all recessive) initially served only as genetic markers, their potential use for studying cellular differentiation has recently been recog-

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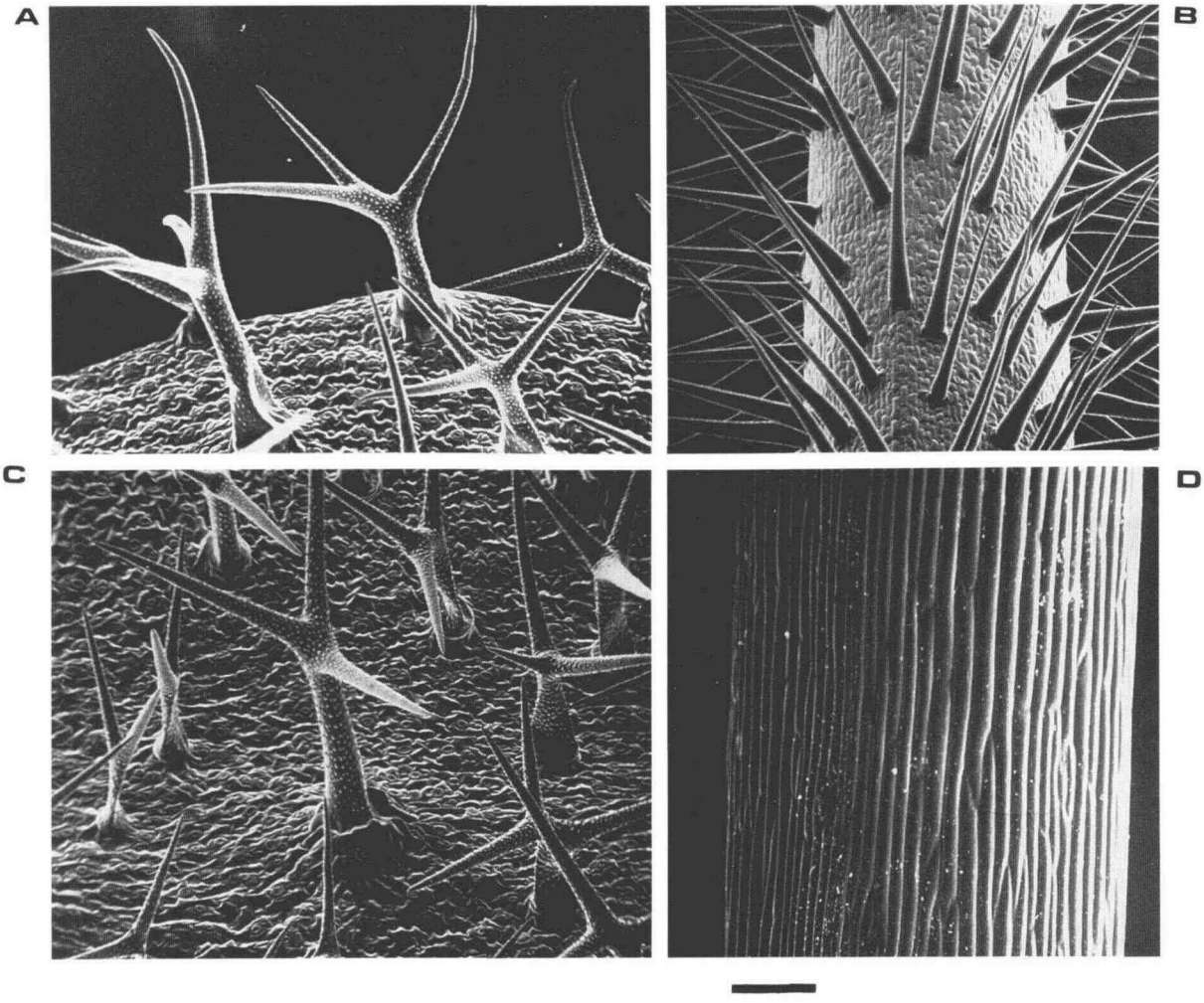

Figure 1. Scanning Electron Microscope Analysis of Leaf and Stem Trichomes on Wild-Type and Mutant WS Plants.

Samples were either unfixed [**(A)** *and* **(B)**] *or fixed in gluteraldehyde and layered with gold after critical point drying* [**(C)** *and* **(D)**].
(A) and **(B)** Normal leaf and stem tissue from geographical race WS plants.
(C) and **(D)** Leaf and stem tissue from stem trichome mutant.
(A), **(B)**, and **(C)** Bar = 90 μm.
(D) Bar = 58 μm.

nized (Haughn and Somerville, 1988). Particularly valuable is the fact that trichome development in the various mutants is affected at different stages, resulting in several different trichome phenotypes. These mutant phenotypes include the following: (1) an absence of almost all trichomes on mutants designated *gl1* (*glabrous1*; Koornneef, Dellaert, and van der Veen, 1982) and *ttg* (transparent testa, glabrous; Koornneef, 1981), (2) a reduction in trichome number as well as a reduction in the branch number of leaf trichomes on mutants designated *gl2* and *gl3* (*glabrous2* and *glabrous3*; Koornneef et al., 1982), and (3) the presence of trichomes that have enlarged abnormally on mutants designated *dis1* and *dis2* (*distorted1* and *distorted2*; Feenstra, 1978). Understanding the molecular mechanism of the alterations in these mutants may provide valuable information on the kinds of mechanisms involved in the initiation and control of cellular differentiation in plants.

There are two reasonable strategies for isolating the trichome genes. One approach is to do a chromosome walk to the genes. Both the availability of abundant and closely spaced restriction fragment length polymorphism markers and the small size of the genome make this feasible in *Arabidopsis* (Chang et al., 1988). A second possibility is to use insertional mutagenesis to tag the genes. We have developed an *Agrobacterium*-mediated

seed transformation procedure (Feldmann and Marks, 1987) that has generated morphological mutations shown to be induced by T-DNA inserts (Feldmann et al., 1989). In this paper, we present the characterization of an insertionally induced mutant that results in a new trichome phenotype. The mutant has normal leaf trichomes, but completely lacks the trichomes usually found on the stem. We show that the mutant contains a complex T-DNA insert, that the insert is very tightly linked to the trichome mutation, and that the mutation is allelic to the genetically characterized *gl1* gene. We also present details concerning the cloning of genomic DNA flanking the T-DNA insert and discuss strategies that will allow the ultimate isolation of the wild-type *GL1* gene.

RESULTS

Isolation and Preliminary Characterization of a Stem Trichome Mutant

A trichome mutant was identified during an initial screen of 136 families of plants that were descendants of unrelated transformed plants generated by the seed transformation procedure (Feldmann and Marks, 1987; Feldmann et al., 1989). All of the families segregated for the T-DNA marker gene, neomycin phosphotransferase II (NPT II), as shown by segregation for resistance to kanamycin. A number of the families were also segregating for a variety of altered phenotypes. In addition to the family segregating for the trichome mutation, families segregating for reduced stature, altered floral structures, and embryo and seedling lethality were also observed.

The trichome mutant was isolated from family number 43. In the initial screen, 10 members of this family were grown in an individual pot and only one member exhibited the mutant phenotype. (Seeds were collected from all individuals for further analysis.) The mutant had normal trichomes on the rosette leaves but completely lacked those normally found on the stem (Figure 1). No evidence of trichome initiation on the stems of the mutant was observed. The mutation did not affect root hairs, growth rate, or seed set (as determined by visible inspection). To confirm that we were observing a stable mutation in the family, another 143 members were examined for the presence of stem trichomes. Within this population, 115 had normal stem trichomes and 28 lacked stem trichomes (χ^2 (3:1) = 2.4 $P > 0.01$). Thus, the trait appears to be determined by a single recessive allele. To ascertain the number of T-DNA inserts in the family, the ratio of kanamycin-resistant to kanamycin-sensitive (kan^r to kan^s) members was found. In a sample of 327 plants there were 298 kan^r to 29 kan^s individuals (χ^2 (15:1) = 3.83 $P > 0.05$), which indicates the presence of two T-DNA inserts. The presence of two inserts was further demonstrated by DNA gel blot analysis (Figure 2).

Progeny resulting from self-fertilization of the 10 plants in the initial screen were tested for kanamycin resistance and for the presence of stem trichomes (Table 1). Three groups of progeny from normal plants (43-2, -4, and -5) were 100% kan^s and all members of these groups had normal stem trichomes. Five groups (43-1, -3, -6, -7, and -8) were either 100% kan^r or were segregating for kanamycin resistance and were also segregating for the lack of stem trichomes. One group (43-9) was segregating 3 kan^r:1 kan^s and at the same time contained normal stem trichomes. This latter group was shown to contain the T-DNA fragments that did not cosegregate with the stem trichome mutation (lane x, Figure 2). The progeny from the original stem trichome mutant (43-10) were homozygous for the trait and were 100% kan^r.

Linkage of T-DNA Insert to Mutant Trait

As a test of linkage of the altered trichome phenotype to a T-DNA insert, DNA gel blot analysis on DNA isolated from individual plants was performed. Sixty-four individuals lacking stem trichomes were selected from a population that was segregating for both the stem trichome mutation and kanamycin resistance. The population of plants used in the study had been grown on vermiculite without selection for kanamycin resistance. The DNA was cut with

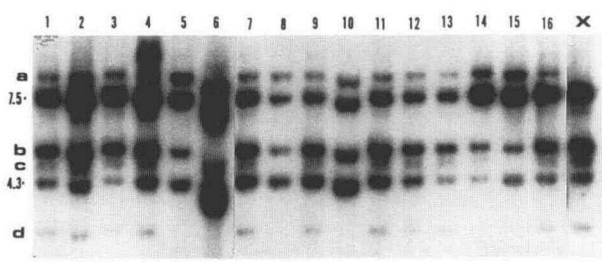

Figure 2. Representative DNA Gel Blot Hybridization Analysis Showing Cosegregation of a T-DNA Insert with the Trichome Mutation.

DNA was isolated from individual plants exhibiting the trichome mutation (lanes 1 to 16) and from progeny of a kan^r plant that had normal trichomes (plant 43-9; lane x). The DNA was cleaved with HindIII and treated as described in Methods before being hybridized to a ^{32}P-labeled plasmid that contained sequences homologous to the right border region of the T-DNA unit (pNEONOS; described in Feldmann and Marks, 1987). Sizes (in kilobases) of the HindIII fragments derived from the internal region of the T-DNA unit are shown (see Figure 3).

(a) 8.5-kb fragment found in all plants expressing the trichome mutation.

(b) Fragment of the appropriate size to be derived from an inverted repeat about the right border of the T-DNA unit.

(c) and **(d)** Fragments associated with the T-DNA insert that does not cosegregate with the trichome mutation.

Table 1. Analysis of Progeny from Members of Family 43

Plant	kan^r:kan^s [a]	Trichome +:trichome −[b]
43-1	55:0	16:7
43-2	All kan^s	30:0
43-3	34:2	18:5
43-4	All kan^s	20:0
43-5	All kan^s	25:0
43-6	57:9	12:5
43-7	37:5	9:3
43-8	50:0	18:3
43-9	84:31	15:0
43-10	42:0	0:24

[a] Progeny were grown on agar-solidified medium containing 50 μg/mL kanamycin, and the ratio of plants that survived to those that died was ascertained.

[b] Progeny were grown without selection in pots of vermiculite, and the ratio of plants that contained stem trichomes to those that lacked them was determined.

HindIII and probed with a plasmid, pNeoNos, that contains sequences from the right border as well as from the internal region of the T-DNA. The probe hybridized to DNA from all of the plants, confirming the presence of a T-DNA insert(s) (Figure 2). All plants contained hybridizing 7.5-kb and 4.3-kb fragments that were derived from the internal region of the T-DNA unit and a 5.1-kb fragment that is the appropriate size to have been derived from a direct repeat of two T-DNA units (see below). Forty-one of the 64 plants contained two other fragments (4.8 kb and 3.0 kb) that were always found together. These fragments demonstrate the presence of a T-DNA insert that is not tightly linked to the trichome mutation. All 64 plants contained a unique 8.5-kb fragment that was formed by either a rearrangement of a T-DNA unit or by sequences from the right border of the T-DNA and the bordering plant DNA. In either case, the 8.5-kb fragment identifies a unique T-DNA insert that cosegregates with the stem trichome mutation. Mutant plants that contained only the cosegregating T-DNA insert were used for further analysis.

Characterization of the T-DNA Insert and Isolation of the Left Border

Once linkage between the trichome mutation and a T-DNA insert was established, a genomic library was constructed. DNA was isolated from stem trichome mutants and was cloned into the λ phage vector EMBL3. Because the T-DNA insert contained two regions of DNA sequence derived from pBR322, the library (unamplified) was screened with ^{32}P-labeled pSP64 (a pBR322 derivative; Promega, Madison, WI). DNA was isolated from 20 phage isolates and characterized by restriction enzyme mapping (Figure 3). None of the genomic clones was expected to contain the entire insert because the DNA gel blot data indicated that the insert was a tandem array of T-DNA units (each over 15 kb in length). Many of the clones contained an almost complete T-DNA unit (Figure 3A, 1–4). In addition, several clones contained an insert derived from a direct repeat of two T-DNA units (Figure 3A, 5–6). Four clones contained a portion of a T-DNA unit and sequences of unknown origin [as determined by the restriction enzyme mapping (Figure 3A, 7–10)].

To identify a clone containing a T-DNA:plant DNA junction, those clones potentially containing sequences from a non-T-DNA region (as suggested by the restriction maps) were used as probes in a DNA gel blot analysis on genomic DNA from mutant and nontransformed wild-type plants. One clone hybridized to DNA from wild-type plants (clone 10; Figure 3) and was shown via restriction enzyme analysis to contain the junction between a left T-DNA border and the interrupted plant DNA (Figure 4). Other clones

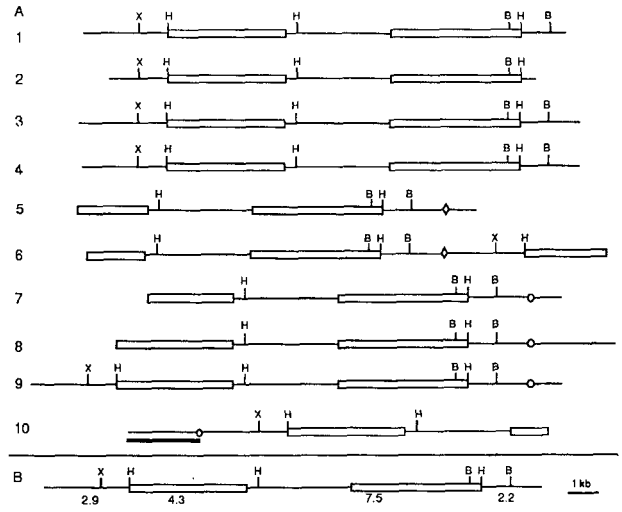

Figure 3. Restriction Maps of λ Clones Containing T-DNA Sequences.

(A) Representative restriction maps of clones isolated from a λ genomic library that was constructed using DNA from the trichome mutant. Lines 1 to 4, maps of clones containing a nearly complete T-DNA unit. Lines 5 and 6, clones containing a junction between a direct repeat of two T-DNA units (the diamond indicates position of the junction). Lines 7 to 10, clones that contain a region of DNA whose origin cannot be ascertained by restriction enzyme mapping (the circle indicates the junction between the T-DNA unit and the region of unknown origin). The bar under clone 10 shows the position 3-kb fragment of *Arabidopsis* DNA that flanks the left side of the insert. Sizes of fragments generated by cleavage of the T-DNA unit **(B)** with HindIII are shown. B, H, and X represent BamHI, HindIII, and XbaI restriction enzyme sites, respectively.
(B) Map of T-DNA unit in *Agrobacterium* used for the transformation. Boxed region represents sequences derived from pBR 322.

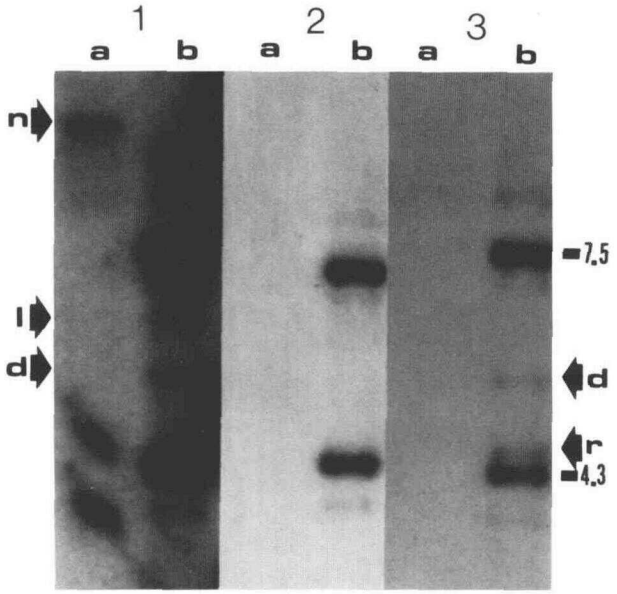

Figure 4. DNA Gel Blot Hybridization Analysis of DNA from *Normal and Mutant Plants* Using Clones Containing DNA of Unknown Origin as Probes.

DNA from wild-type (a) and mutant (b) plants was cleaved with HindIII, resolved on agarose, blotted onto nitrocellulose, and probed with (1) labeled clone 10 (Figure 3), (2) pBR322, or (3) clone 7 (Figure 3). Sizes of the HindIII fragments derived from the internal region of the T-DNA (Figure 3) are shown on the right. n indicates a fragment from a wild-type nontransformed plant that hybridized to clone 10. Shown are fragments of the appropriate size to be derived from d, a direct repeat of two T-DNA units, l, an inverted repeat about the left border, and r, an inverted repeat about the right border.

tested were shown to carry rearranged sequences derived from the pBR322 region (data not shown).

Plasmid pBR322 alone and two λ clones containing the pBR322 region plus only the right border or left border of the T-DNA unit (clones 7 and 10, respectively; Figure 3) were used as probes to study the structure of the T-DNA insert (Figure 4). pBR322 alone hybridized strongly to 7.5-kb and 4.3-kb HindIII fragments which are derived from the internal region of the insert and hybridized weakly to three other fragments (one of which appears to be the 8.5-kb fragment found in all mutant plants) that may have been formed by rearrangements in T-DNA units during insertion. The clone containing the right border also hybridized to a fragment of 4.4 kb (band r, Figure 4), which is the appropriate size to represent an HindIII fragment generated by an inverted repeat centered about the right end of the T-DNA, and to a fragment of 5.2 kb (bands d, Figure 4), which is of the appropriate size to represent a direct repeat of two T-DNA units. Hybridization of the left border probe (which also contained *Arabidopsis* DNA from the border of the insertion site) to the 5.2-kb fragment supports this latter conclusion. The left border probe also hybridized to a 5.8-kb fragment (band l, Figure 4), which was of the appropriate size to be derived from an inverted repeat of two T-DNA units centered about the left border, and to a 10-kb fragment that was subsequently shown to contain the left T-DNA:plant DNA junction (Figure 5). To account for these fragments, the insert must contain at least four complete or partial T-DNA units. Complex arrays of T-DNA units in plants transformed by *Agrobacterium* have previously been noted (Feldmann and Marks, 1987; Jorgensen, Snyder, and Jones, 1987).

A 3.0-kb EcoRI-SalI fragment from clone 10 (Figure 3) contains 2.9 kb of *Arabidopsis* sequence that flanks the left border of the T-DNA insert and approximately 75 bases of the left end of the T-DNA. This region was subcloned into pBS+ and used as a probe to obtain additional linkage data and to study the structure of the uninterrupted region in wild-type plants. The subclone was hybridized to DNA from descendants of an F1 plant derived from a cross between a trichome mutant and wild-type plant. Progeny from 16 F2 plants homozygous for the altered trichome trait, from eight heterozygous F2 plants, and from six homozygous normal F2 plants were used as the source of DNA. These families were, respectively, 100% kan^r, segregating 3 kan^r:1 kan^s, or 100% kan^s. The probe hybridized to a 10-kb HindIII fragment in DNA from plants that were either segregating for the trichome mutation or that were homozygous for the mutation (Figure 5). In addition, the probe hybridized to an 11-kb HindIII fragment in the heterozygous and homozygous normal plants (Figure 5). The fact that no cross-overs were observed that (1) resulted in trichome mutants having both of the fragments, (2) resulted in heterozygotes having only one fragment and not the other, or (3) produced a homozygous normal plant having the 10-kb fragment indicates that the probe is derived from a region of the genome linked to the mutation.

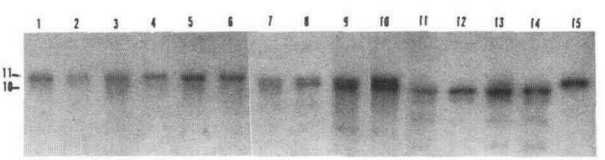

Figure 5. Representative DNA Gel Blot Hybridization Analysis of DNA from Plants with a Known Genotype.

DNA was isolated from different families of plants that were *GL1/GL1* (lanes 1 to 6), *GL1/gl1* (lanes 7 to 10), or *gl1/gl1* (lanes 11 to 14). Lane 15 contains DNA from a non-transformed normal plant. All plants contain a WS background. The DNA was cleaved with HindIII, resolved on an agarose gel, blotted to nitrocellulose, and hybridized to ^{32}P-labeled probe that was derived from the region flanking the T-DNA in clone 10. Sizes (in kilobases) of the DNA fragments are shown on the left.

Test for Allelism to Other Trichome Mutations

To test for allelism to other known trichome mutations, the stem trichome mutant was used as a recipient for pollen from either *gl1*, *gl2*, *gl3*, *dis1*, *dis2*, or *ttg* plants. F1 plants from the crosses between the stem trichome mutant and either *gl2*, *gl3*, *dis1*, *dis2*, and *ttg* plants had normal leaf and stem trichomes. However, the cross between the stem trichome mutant and *gl1* resulted in F1 plants with the stem trichome mutant phenotype. To confirm that the result was not due to inadvertent selfing of the stem trichome mutant, the F1 plants were allowed to self, and the F2 progeny were analyzed. Seventy-seven progeny segregated such that 60 had the stem trichome mutant phenotype and 17 had the *gl1* phenotype (characterized by an absence of trichomes on leaves and stems). These results indicate that the stem trichome mutation is an allele of *GL1* and dominant to *gl1*. The new allele has been designated *gl1-43*.

Because *gl1* and *gl1-43* plants have different phenotypes, a new test for linkage between the T-DNA insert and the stem trichome mutation could be conducted. Plants expressing *gl1* and *gl1-43* were crossed, and F2 progeny were assessed for kanamycin resistance and trichome phenotype. If the T-DNA insert induced the stem trichome mutation (these plants have normal leaf trichomes, whereas *gl1* plants do not), then all the kan^r plants would have normal leaf trichomes. Of 2785 plants that were grown on agar medium containing kanamycin, 2078 survived and 707 died (χ^2 (3:1) = 0.22 $P > 0.05$). All of the survivors had normal leaf trichomes. When the same population was assessed for the presence of leaf trichomes without selection on kanamycin, 181 had leaf trichomes (*gl1-43*/−) and 65 lacked leaf trichomes (*gl1*/*gl1*; χ^2 (3:1) = 0.27 $P > 0.05$).

DISCUSSION

The genetic evidence presented here is consistent with the hypothesis that a T-DNA insert has interrupted a gene required for the initiation of stem trichome formation. No evidence of a cross-over between the T-DNA insert and the mutation was observed. Complementation analysis indicated that the mutation is a new allele at the *GL1* locus that resulted in a novel trichome phenotype. Previously described mutants of *GL1* have exhibited an almost complete loss of trichomes on both leaves and stems, whereas the new mutant has normal leaf trichomes but lacks stem trichomes. The new allele was designated *gl1-43*.

After tight linkage had been established between the T-DNA insert and the trichome mutation, the next step was to isolate the *Arabidopsis* DNA-flanking insert. To isolate this region, a genomic library was constructed in EMBL3 with DNA from mutant plants. Since the T-DNA insert contained sequences from pBR322, pSP64 (derived from pBR322) was used as a probe to isolate DNA clones containing the T-DNA region. One clone containing the left T-DNA:genomic DNA junction was identified among the clones that hybridized to the probe. This clone will serve as a probe to isolate the corresponding uninterrupted sequence that should contain the intact *GL1* gene (see accompanying paper, Herman and Marks, 1989).

The phenotypic differences among the various independently derived *gl1* mutants is quite striking. The *gl1* mutant used in our complementation assay was in a Landsberg erecta background (*gl1-L*; Koornneef et al., 1982). *gl1-L* plants usually lack all trichomes; however, an occasional plant is observed that has a trichome on the leaf margin (approximately 1 in 500). In contrast, a *gl1* mutant in the Columbia background (*gl1-C*) lacks trichomes on the stem and leaf surfaces, but trichomes are found on the margins of most leaves. Because it is possible that these phenotypic differences are due to differences in the genetic backgrounds, both alleles are being back-crossed into a common genetic background. If the phenotypic differences are due to the allelic differences and not to differences in the genetic background, then both alleles will be isolated, sequenced, and compared with the wild-type sequence. This may provide important information on the molecular basis for the phenotypic differences and on the mechanism by which the product of *GL1* acts.

The *gl1-43* plants [with a Wassilewkija (WS) background] differ from *gl1-C* and *gl1-L* plants in having normal leaf trichomes and no stem trichomes. This difference does not appear to be due to genetic background because the *gl1-L* allele has been back-crossed three times into the WS background with no change in its phenotype. Several possible explanations exist to explain the phenotype of the *gl1-43* mutant at the molecular level. It is possible that the T-DNA insert in *gl1-43* disabled a promoter sequence required for stem expression. Alternatively, initiation of stem and leaf trichomes may be induced by different products that are produced through alternate splicing of the *GL1* transcript. The stem splicing pathway in *gl1-43* may be blocked. A comparison of the normal and interrupted genes should indicate whether one of these possibilities is correct.

The non-allelic mutant, *ttg* (transparent testa, glabrous), also lacks almost all trichomes (Koornneef et al., 1982). As with the *gl1* mutants, *ttg* plants occasionally have a few trichomes on the leaf margin. In addition to the absence of trichomes, *ttg* mutants also produce seeds with a transparent seed coat lacking the normal seed mucilage. The phenotypic alterations exhibited by *ttg* plants have led to the speculation that *ttg* may be a regulatory gene that affects several different pathways (Koornneef, 1981). Our scanning electron microscope analysis of both *ttg* and *gl1* mutants failed to reveal the presence of any epidermal

cells on the leaf or stem surface that show even the earliest stages of trichome development (J. Esch and M.D. Marks, manuscript in preparation). A search by others has not led to identification of additional nonallelic mutants that lack leaf or stem trichomes (Koornneef et al., 1982; Haughn and Somerville, 1988). This suggests that the number of nonessential genes required for the initiation of trichome differentiation may be quite small.

The information gained in the study of the action of *GL1* and other genes required for trichome formation should prove valuable in increasing our understanding of the molecular mechanisms that underlie cellular differentiation in plants.

METHODS

Genetic Material and Crosses

Seeds for the trichome mutants *gl1*, *gl2*, *gl3*, *dis1*, *dis2*, and *ttg* in the Landsberg erecta background were obtained from Martin Koornneef (Agricultural University, Wageningen, The Netherlands). The *gl1* mutant in the Columbia background was obtained from George Redei (University of Missouri, Columbia, MO). The transformed stem trichome mutant (*gl1-43*) had a WS background and was generated as previously described (Feldmann and Marks, 1987; Feldmann et al., 1989). Plants were grown on vermiculite or in Petri dishes on agar-solidified medium with or without kanamycin (50 μg/mL) as previously described (Feldmann and Marks, 1986, 1987). Crosses were made using the stem trichome mutant as female recipient and the other mutants as pollen donors. F1 plants were initially germinated on agar-solidified media containing kanamycin and then transferred to vermiculite. If the crosses between the stem trichome mutant and the other mutants resulted in F1 plants with normal leaf and stem trichome, then the causative mutations were considered to be nonallelic.

DNA Isolation and DNA Gel Blot Analysis

For DNA gel blot linkage analysis, DNA was isolated from individuals or descendants of individual plants by the methods of Weeks, Beerman, and Griffith (1986) as previously described (Feldmann and Marks, 1987). DNA was cleaved with HindIII and resolved on a 0.7% agarose gel. The DNA was blotted onto nitrocellulose and hybridized to plasmid pNeoNos (obtained from Mike Fromm and described in Feldmann and Marks, 1987) that was labeled with ^{32}P by nick translation using previously described conditions (Feldmann and Marks, 1987). After hybridization and washing, filters were exposed to Kodak XAR-5 film.

Construction and Screening of Genomic Libraries

DNA was isolated as described above from a family of plants that lacked stem trichomes and only contained the cosegregating T-DNA insert. The DNA was partially cleaved with Sau3A, treated with calf alkaline phosphatase, and resolved on a sucrose gradient as described by Maniatis, Fritsch, and Sambrook (1984). DNA from the 15-kb to 20-kb fraction was ligated into BamHI cleaved EMBL3 and packaged into bacteriophage λ using Packagene extracts (Promega, Madison, WI). The library was screened with pSP64 (Promega, Madison, WI), which contains sequences found in the interior of the T-DNA used to transform the plants. Hybridizing phage were subjected to several rounds of plaque purification and then used to infect 100-mL cultures for DNA isolation (Yamamoto and Alberts, 1970; Maniatis et al., 1982). Restriction maps of the phage DNA were derived from analysis of DNA that had been digested with single or combinations of restriction enzymes.

Identification and Cloning of DNA-Flanking T-DNA Insert

λ clones that contained T-DNA sequences and DNA of unknown origin were labeled with ^{32}P by nick translation (with a kit from Bethesda Research Laboratories, Gaithersburg, MD). The labeled DNA was hybridized to filters containing DNA from the stem trichome mutant and nontransformed WS plants. Clones that hybridized to DNA from nontransformed plants should contain sequences derived from the border of the T-DNA insert.

The region flanking the left side of the T-DNA insert was subcloned from λ clone 10 (Figure 3) into pBS+ (Stratagene, La Jolla, CA) and used as a probe for linkage analysis and characterization and isolation of the uninterrupted sequence from *GL1* plants.

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