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TRICHOMELESS1 regulates trichome patterning by suppressing *GLABRA1* in *Arabidopsis*

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The patterning of epidermal cell types in *Arabidopsis* is a simple and useful model for studying the molecular basis of cell specification in plants. The distribution of different cell types in the *Arabidopsis* epidermis is regulated by a lateral inhibition mechanism that relies on interactions between transcription factors. However, it is unclear how temporal- or organ-specific differences in epidermal patterning are achieved. Here we identify TRICHOMELESS1 (TCL1) as a new and major single-repeat MYB-type transcription factor that negatively regulates trichome formation in the inflorescence epidermis. A dominant mutant with elevated expression of *TCL1* has a glabrous (trichomeless) phenotype, whereas a loss-of-function mutation in *TCL1* uniquely confers ectopic trichome formation on inflorescence stem and pedicels. Genetic analyses demonstrate that TCL1 and CAPRICE work synergistically to regulate trichome patterning on these organs. Interestingly, overexpression of TCL1 specifically suppresses the expression of *GLABRA1* (*GL1*), a crucial component in the trichome initiation complex, whereas loss-of-function of *TCL1* enhances *GL1* expression. Chromatin immunoprecipitation results show that TCL1 can be recruited to the cis-acting regulatory elements of *GL1*. These results provide the first molecular and genetic evidence that an R3 MYB may negatively regulate trichome cell specification in a novel manner by directly suppressing the transcription of *GL1*.

KEY WORDS: Lateral inhibition, MYB, Pattern formation, Trichome, TRICHOMELESS1

INTRODUCTION

The patterning of epidermal cell types in *Arabidopsis* has become one of the best models for studying the molecular basis of cell specification in plants (Schiefelbein, 2003; Pesch and Hülskamp, 2004; Serna, 2005; Schellmann et al., 2007). Trichome patterning in *Arabidopsis* is controlled by several transcription factors. According to their effect on trichome initiation, these transcription factors can be divided into two groups: positive regulators and negative regulators. Positive regulators include the WD-repeat protein TRANSPARENT TESTA GLABRA1 (TTG1) (Galway et al., 1994; Walker et al., 1999), the R2R3 MYB-type transcription factor GLABRA1 (GL1) (Oppenheimer et al., 1991), the basic helix-loop-helix (bHLH) transcription factors GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) (Payne et al., 2000; Zhang et al., 2003) and the homeodomain protein GLABRA2 (GL2) (Rerie et al., 1994; Masucci et al., 1996). The negative regulators include several small single-repeat R3 MYB transcription factors, such as TRIPTYCHON (TRY) (Schnittger et al., 1999; Schellmann et al., 2002), CAPRICE (CPC) (Wada et al., 1997; Wada et al., 2002) and ENHANCER OF TRY AND CPC 1 and 2 (ETC1 and ETC2) (Esch et al., 2004; Kirik et al., 2004a; Kirik et al., 2004b).

These positive and negative regulators work together to control trichome initiation and patterning in *Arabidopsis*. The R2R3 MYB-type transcription factor GL1, a bHLH transcription factor (GL3 or EGL3), and TTG1 form a complex to induce the expression of *GL2*, which in turn induces trichome formation in shoots (reviewed by Schiefelbein, 2003; Pesch and Hülskamp, 2004). The small MYB transcription factors TRY, CPC, ETC1 and ETC2, inhibit the trichome cell type in the shoot, presumably by competing with GL1 for binding GL3, thereby limiting the transcriptional activity of the trichome initiation and patterning activator complex (Hülskamp et al., 1994; Wada et al., 1997; Schellmann et al., 2002; Esch et al., 2003).

Root hair patterning is largely controlled by the same components, except that GL1 is replaced by another R2R3 MYB-type transcription factor, WEREWOLF (WER), to form a complex with TTG1 and GL3/EGL3 to induce *GL2* expression (Bernhardt et al., 2003; Bernhardt et al., 2005; Lee and Schiefelbein, 1999). However, the positive regulators for trichome initiation function to inhibit root hair initiation, whereas negative regulators for trichome initiation function to promote root hair initiation (Schiefelbein, 2003; Pesch and Hülskamp, 2004).

We report here the identification and functional analysis of a new negative regulator for trichome initiation and patterning, TRICHOMELESS1 (TCL1). TCL1 represents a previously unknown member of the single-repeat R3 MYB transcription factor family. We demonstrate that overexpression of *TCL1* completely abolishes trichome formation on all organs examined, whereas a loss-of-function mutation in *TCL1* confers unique, ectopic trichome formation along inflorescence stems and pedicels. These trichome phenotypes have not been reported in mutants of any other members of the single-repeat R3 MYB transcription factor family. Furthermore, we provide genetic evidence that TCL1 and CPC work synergistically to regulate trichome formation on inflorescence stems and pedicels. In an unexpected finding, we show that TCL1 is likely to act by negatively regulating *GL1* expression. This provides new insight into the organ-specific control of epidermal patterning and suggests the existence of a novel regulatory loop in trichome patterning.

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MATERIALS AND METHODS

Plant materials and growth conditions

The *try* mutant, *try_29760*, is in the Columbia-0 (Col-0) ectopic background (Esch et al., 2003). The *cpc* mutant is in the WS ecotypic background (Wada et al., 1997). Double mutants were generated by crossing single mutants, examining the F2 progeny for putative mutant phenotype, and confirming their double-mutant status by genotyping in F2 and subsequent generations. Similarly, *tcl1-1 cpc try* triple mutants were generated by crossing *tcl1-1 cpc* with *tcl1-1 try* double mutants. Plants were grown at 23°C with 14/10 hour photoperiod at approximately 120 μmol m^{-2} s^{-1}.

For seedlings used for phenotypic and RT-PCR analyses, seeds were surface-sterilized and grown on Murashige & Skoog (MS) basal medium with vitamins (Plantmedia, Dublin, OH) and 1% (w/v) sucrose, solidified with 0.6% (w/v) phytoagar (Plantmedia).

Isolation of the *tcl1-1D* mutant and molecular cloning of *TCL1*

A dominant mutant with glabrous leaves was isolated from an activation-tagged mutant population (~10,000 plants) produced in a *gpa1-2* mutant background (Ullah et al., 2001), and designated as *tcl1-1D*.

A plasmid rescue technique was used to clone the *TCL1* gene locus. Genomic DNA (20 μg) isolated from the *tcl1-1D* mutant was digested with *Pst*I, which left the right-border of the T-DNA intact. The digestion products were purified, ligated, and transformed into *Escherichia coli* DH5α. The transformants were selected on LB plates containing 100 μg/ml ampicillin. Two independent colonies were selected and the plasmid DNA was sequenced using T-DNA left-border (5′-TTGACAGTGACGACAAATCG-3′) and right-border (5′-ATGTGATATCTAGATCCGAAAC-3′) primers. The *tcl1-1D* phenotypes were subsequently confirmed by recapitulation experiments.

Isolation of the loss-of-function allele *tcl1-1*

A T-DNA insertion mutant allele of *TCL1*, SALK_055460, was identified from the SALK T-DNA Express Database (http://signal.salk.edu/cgi-bin/tdnaexpress). In this allele, the T-DNA insertion site is in the second intron of the *TCL1* gene. The insertion was confirmed by PCR and sequencing using a *TCL1*-specific primer (5′-ATGGATAACACAAACCGTC-3′) and the T-DNA-specific primer JMLB1 (5′-GGCAATCAGCTGTTGCCCGTCTCACTGGTG-3′), and the mutant allele was designated *tcl1-1*. Loss of detectable full-length *TCL1* transcript in the *tcl1-1* mutant was verified by RT-PCR.

Plasmid construction

To generate the *35S:HA-TCL1* construct, the full-length open reading frame (ORF) of *TCL1* (gene locus At2g30432) was amplified by RT-PCR using RNA from 10-day-old light-grown *Arabidopsis* seedlings. The PCR fragment was then cloned in frame with an N-terminal HA tag into the pUC19 vector under the control of the double *35S* enhancer promoter of CaMV (Tiwari et al., 2003; Wang et al., 2005). To generate the *35S:TCL1-VP16* construct, *VP16* was amplified by PCR using *GD-VP16* (Tiwari et al., 2003) as template and fused in frame with *TCL1* under the control of the CaMV *35S* promoter. The *TCL1-GFP* construct was cloned by fusing *TCL1* in frame with *GFP*, then subcloning this into the pUC19 vector under the control of the *TCL1* promoter (a fragment that covers the region –1535 to +1 of the start codon of *TCL1*). The P_{TCL1}*:GUS* construct was cloned by replacing the P_{AtOPF1} promoter in P_{AtOPF1}*:GUS* (Wang et al., 2007) with the *TCL1* promoter. Corresponding constructs in pUC19 were digested with *Eco*RI, then subcloned into the binary vector pPZP211 or pPZP221 for plant transformation (Hajdukiewicz et al., 1994).

Phylogenetic analysis

The phylogenetic tree for TCL1, TRY, CPC, ETC1, ETC2 and At4g01060 (Fig. 2D) was generated using AliBee - Multiple Alignment software, release 2.0 (http://www.genebee.msu.su/services/malign_reduced.html).

Plant transformation and selection of transgenic plants

Plants of about 5 weeks of age and with several mature flowers on the main inflorescence were transformed with various constructs via *Agrobacterium tumefaciens* GV3101 by the floral dip method (Clough and Bent, 1998). Phenotypes of transgenic plants were examined in the T1 generation, and confirmed in T2 up to T4 generations. For all transgenic plants, at least five transgenic lines with similar phenotypes were obtained.

Histochemical staining for β-glucuronidase (GUS) activity

Histochemical staining for GUS activity used the substrate 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-Gluc; Rose Scientific, Edmonton, Alberta, Canada) and the general procedure described by Ulmasov et al. (Ulmasov et al., 1997).

Protoplast isolation, transfection and GUS activity assay

Protoplast isolation, transfection and the GUS activity assay were undertaken as described previously (Tiwari et al., 2003; Wang et al., 2005; Wang et al., 2007). GUS activities were measured using a Fluoroskan Finstruments Microplate Reader (MTX Lab Systems, Vienna, VA).

Microscopy

Trichomes and root hairs were analyzed and photographed under a Leica MZ6 microscope equipped with a digital camera. The pattern of epidermal cell types was determined as described previously (Lee and Schiefelbein, 2002; Kirik et al., 2004a; Kirik et al., 2004b). Root hair analysis used ~10-day-old seedlings grown vertically on Petri plates. For leaf trichome analysis, the first two leaves of soil-grown plants were used. For stem or pedicel trichome analysis, adult soil-grown plants were used.

The expression and localization of TCL1-GFP in transgenic plants expressing TCL1-GFP under the control of the *TCL1* promoter were examined in 4-day-old seedlings.

RNA isolation and RT-PCR

Total RNA was isolated from seedlings and from the various tissues/organs of adult plants using the Trizol reagent (Invitrogen Canada, Burlington, Ontario, Canada). cDNA was synthesized using 1 μg total RNA by oligo(dT)-primed reverse transcription, using the Omniscript RT Kit (Qiagen). *TCL1*-specific primers (5′-ATGGATAACACAAACCGTC-3′ and 5′-TCATTTGTGGGAGAAATAGTC-3′) were used to amplify the full-length ORF of *TCL1*. *GFP*-specific primers (5′-ATGGTGAGCAAGGGCGAGGAG-3′ and 5′-TTACTTGTACAGCTCGTCCATGCC-3′) were used to check the expression of *TCL1-GFP*. *HA*-specific (5′-TACCCTTACGATGTTCCTGATTAC-3′) and *TCL1*-specific (5′-TCATTTGTGGGAGAAATAGTC-3′) primers were used to check the expression of *HA-TCL1*. *TTG1*-specific primers (5′-ATGGATAATTCAGCTCCAG-3′ and 5′-TCAAACTCTAAGGAGCTGC-3′) were used to check the expression of *TTG1*. *GL1*-specific primers (5′-ATGAGAATAAGGAGAAG-3′ and 5′-CTAAAGGCAGTACTCAACATC-3′) were used to check the expression of *GL1*. *GL2*-specific primers (5′-ATGTCAATGGCCGTCGACATGTC-3′ and 5′-TCTCGCAGCTTCTCTAGTTCCC-3′) were used to check the expression of *GL2*. *GL3*-specific primers (5′-ATGGCTACCGGACAAAACAG-3′ and 5′-AAGGAACGGGAAGCAAACCACTGTG-3′) were used to check the expression of *GL3*. *ACTIN2* (amplified using 5′-CCAGAAGGATGCATATGTTGGTGA-3′ and 5′-GAGGAGCCTCGGTAAGAAGA-3′) was used as a control in all PCR reactions.

Chromatin immunoprecipitation (ChIP) assay

ChIP assay was conducted according to Lawrence et al. (Lawrence et al., 2004) and Wang et al. (Wang et al., 2007). Briefly, about 1.5 g of 10-day-old *35S:HA-TCL1* seedlings were cross-linked using 1% formaldehyde solution, ground with liquid nitrogen, and sonicated using a Branson sonifier for 4×10 seconds at 40% duty cycle and 20% power. Soluble chromatin was subject to ChIP using anti-HA antibodies (Abgent) or rabbit preimmune sera. Chromatin-antibody complexes were collected on salmon sperm DNA/protein A-agarose (Upstate). DNA-protein cross-links were reversed at 65°C overnight, and the DNA purified and used in PCR reactions. Primer pairs used for PCR were: GL1intronFW (5′-TGGACAGTTGAAGAAGACAACATC-3′) and GL1intronRV (5′-CACATCTCTTTAGCCCTATCAACG-3′), and GL1UTRFW (5′-TACACATAGGGACATACATATGCG-3′) and GL1UTRRV (5′-TAGTTTTGGTGTCGAAATTCCCGG-3′).

RESULTS

TRICHOMELESS1 encodes a single-repeat R3 MYB transcription factor

A dominant mutant with glabrous leaves was identified in an activation-tagged mutagenized population of *Arabidopsis*. The null mutant of the heterotrimeric G-protein α subunit, *gpa1-2* (Ullah et al., 2001), was used as the parental genotype. This glabrous mutant was named *trichomeless 1-1 Dominant* (*tcl1-1D*) (Fig. 1). Compared with wild type and its parent, the *tcl1-1D* mutant does not have any trichomes on the first pair of rosette leaves (Fig. 1A, Table 1), nor on successive leaves, inflorescence stems, cauline leaves or floral organs (data not shown). However, all other aspects of plant growth and development studied were unaffected in the *tcl1-1D* mutant.

A plasmid rescue procedure (Weigel et al., 2000) was used to identify the T-DNA insertion site in the *tcl1-1D* mutant. As shown in Fig. 2A, in the *tcl1-1D* mutants the T-DNA was inserted in chromosome 2 at a position that is 2115 bp upstream of the start codon of the gene locus At2g30432, and 1339 bp downstream of the stop codon of the gene locus At2g30440, with the four outward-facing *35S* enhancers oriented toward the gene locus At2g30432. RT-PCR analysis revealed that the transcript level of gene locus At2g30432 was elevated (Fig. 2B), indicating that the *TCL1* gene locus is most likely to be at At2g30432.

BLAST search analysis indicated that *TCL1* encodes a protein that is closely related to proteins encoded by the previously characterized genes *TRY*, *CPC*, *ETC1*, *ETC2*, and by an uncharacterized gene locus At4g01060 (Fig. 2C) (Kirik et al., 2004b; Serna and Martin, 2006). All these proteins have a conserved MYB region that is most closely related to the R3 MYB domains of the R2R3 MYB gene family members in plants (Wada et al., 1997; Schellmann et al., 2002; Esch et al., 2004; Kirik et al., 2004a; Kirik et al., 2004b). Therefore, TCL1 represents a previously unknown member of the single-repeat R3 MYB transcription factor family. Results from phylogenetic analysis suggested that TCL1 is more closely related to CPC than to TRY (Fig. 2D).

To confirm that the phenotypes observed in the *tcl1-1D* mutant were caused by elevated expression of *TCL1*, we transformed wild-type Columbia (Col) plants with a binary vector containing the N-terminal HA-tagged full-length ORF of *TCL1* expressed from the strong *35S* promoter of the cauliflower mosaic virus (*35S:HA-TCL1*). As expected, overexpression of *TCL1* recapitulated the *tcl1-1D* phenotypes (Fig. 1B). The transcript level of *TCL1* in the transgenic lines was confirmed by RT-PCR using *HA*-specific and *TCL1*-specific primers (Fig. 1B).

TCL1 does not appear to affect root hair formation and patterning. The number and pattern of root hair cells in the *tcl1-1D* mutant or in plants overexpressing *TCL1* were indistinguishable from those of wild-type plants (Fig. 1C, and see Table S1 in the supplementary material). Because overexpression of the other single-repeat R3 MYBs (including CPC, TRY, ETC1 and ETC2) using the *35S* promoter induces ectopic root hair cells (Wada et al., 1997; Schellmann et al., 2002; Kirik et al., 2004a; Kirik et al., 2004b), this result indicates that the TCL1 protein may differ functionally from other R3 MYBs.

Loss-of-function mutation in *TCL1* promotes trichome formation on inflorescence stems and pedicels

To further analyze the function of *TCL1* in trichome formation, we took a reverse genetic approach to seek loss-of-function alleles of *TCL1*. By searching the SALK T-DNA Express Database (http://signal.salk.edu/cgi-bin/tdnaexpress) (Alonso et al., 2003), we found one mutant allele, SALK_055460, in which *TCL1* is interrupted by a T-DNA insertion within its second intron (Fig. 3A). The presence of the T-DNA at the expected location was further verified by sequencing, and plants homozygous for the T-DNA insertion at this locus were isolated by PCR-based screening (data not shown). This mutant allele was named *tcl1-1*. The expression of *TCL1* was undetectable in *tcl1-1* mutants by RT-PCR (Fig. 3B), indicating that *tcl1-1* is likely to be a loss-of-function mutant allele of *TCL1*.

Because trichome initiation in the *tcl1-1D* mutant was suppressed, we expected to see an increase in trichome initiation or altered trichome patterning in the *tcl1-1* mutant. We first checked trichome production on rosette leaves. However, both trichome initiation and patterning in the *tcl1-1* mutant were indistinguishable from those in wild-type plants (Table 1). Interestingly, we observed a dramatic increase in trichome

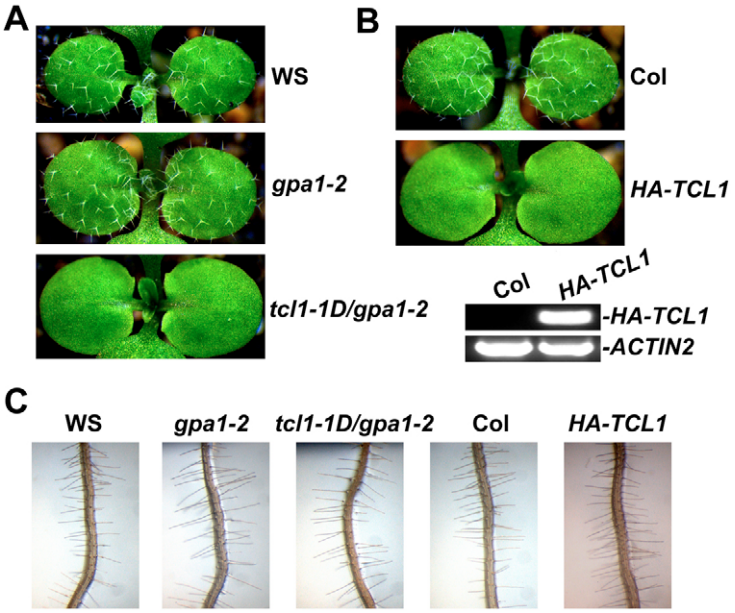

Fig. 1. *tcl1-1D* is a gain-of-function dominant glabrous mutant. (**A**) Trichomes on leaves of WS (top) and *gpa1-2* mutant (middle), and glabrous leaves of *tcl1-1D/gpa1-2* dominant mutant (bottom). (**B**) Trichomes on leaves of Col (top) and glabrous leaves of transgenic *Arabidopsis* plants overexpressing *HA-TCL1* (bottom). The overexpression of *TCL1* was confirmed by RT-PCR (below) using a pair of primers – one *HA*-specific, the other *TCL1*-specific. Expression of *ACTIN2* provided a control. (**C**) Root hair production in WS, *gpa1-2*, Col, in the *tcl1-1D/gpa1-2* dominant mutant and in a transgenic plant overexpressing *HA-TCL1*.

Table 1. Leaf trichome production in wild-type, mutant and transgenic lines

Genotype	Number of trichomes per leaf	Frequency of trichome clusters (%)
WT (Col)	50.7±10.2	0
WT (WS)	39.1±5.4	0
tcl1-1D	0±0*	0
35S:HA-TCL1	0.1±0.1*	0
P_{TCL1}*:TCL1-GFP/tcl1-1*	49.7±11.7	0
tcl1-1	55.8±9.2	0
cpc	83.7±16.6*	0.1
try	44.1±8.2	7.1*
tcl1-1 cpc	86.9±12.5*	0.2
tcl1-1 try	45.8±8.5	8.3*
cpc try	181±24*	85*
tcl1-1 cpc try	164±28*	81*

Values indicate mean±s.d. of at least ten rosette leaves for each line.
*, P<0.05, relative to the corresponding wild-type line.

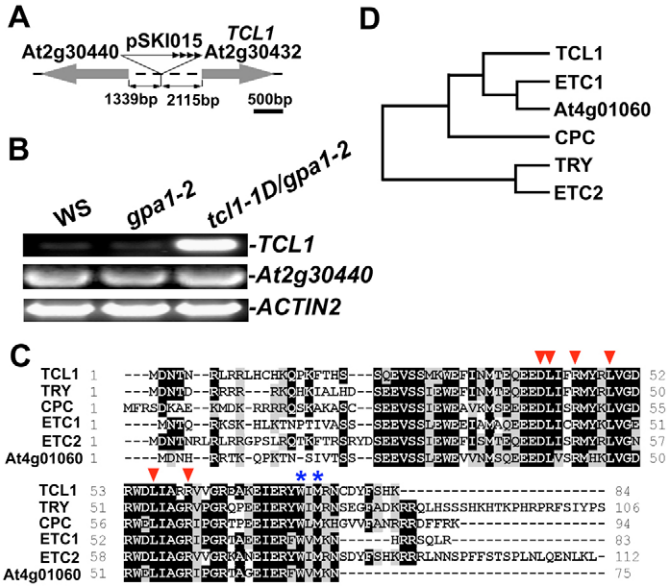

Fig. 2. Molecular cloning of *TCL1*. (**A**) Diagram illustrating the insertion site of the activation-tagged T-DNA in the *tcl1-1D/gpa1-2* mutant. The orientation of the four *35S*-enhancer repeats in the T-DNA situated 2115 bp upstream of the start codon of *TCL1* (At2g30432) is indicated by arrows. (**B**) RT-PCR indicating transcript level of *TCL1* in wild-type, *gpa1-2* and *tcl1-1D/gpa1-2 Arabidopsis* plants. RNA was isolated from 10-day-old light-grown seedlings. *ACTIN2* provided a control. (**C**) The TCL1 protein is similar to other single-repeat R3 MYB transcription factors TRY, CPC, ETC1, ETC2 and At4g01060. Identical amino acids are shaded in black, similar amino acids in gray. The amino acid signature [D/E]Lx2[R/K]x3Lx6Lx3R (Zimmermann et al., 2004) that is required for interacting with R/B-like bHLH transcription factors is indicated by arrowheads. Asterisks indicate the amino acids within the MYB domain that are crucial for cell-to-cell movement (Kurata et al., 2005). (**D**) Phylogenetic analysis of TCL1, TRY, CPC, ETC1, ETC2 and At4g01060.

formation on the inflorescence stems in the *tcl1-1* mutant (Fig. 3C, Fig. 4). In wild-type plants, in addition to a decline in adaxial trichome production on successive cauline leaves (Telfer et al., 1997), a decline of trichome production on successive inflorescence stem internodes was also observed (Gan et al., 2006). We found that no matter how many internodes (usually 3-4) Col wild-type plants produced on the main inflorescence stem, trichome production was restricted to the region below the first flower on the main inflorescence stem. Only very few, or no trichomes were formed on the internode just adjacent to the first flower (Fig. 3C, Fig. 4). However, in the *tcl1-1* mutant, the number of trichomes on the main inflorescence stem internodes was dramatically increased (Fig. 3C, Fig. 4). Moreover, trichomes also formed beyond the site of the first flower branch (Fig. 3C, Fig. 4). Trichome distribution on the internodes of lateral branches was similar to that of the main inflorescence stem (data not shown). Such ectopic trichome formations on the inflorescence stems have not been reported for mutants of any other members of the single-repeat R3 MYB transcription factor family.

In addition to the ectopic formation of trichomes on inflorescence stems, *tcl1-1* mutants also produced trichomes on pedicels (Fig. 3C, Fig. 4). No trichomes were found on the pedicels of wild-type plants (Fig. 3C). Similar to the pattern on inflorescence internodes, trichome production declined on successive pedicels in the *tcl1-1* plants (Fig. 4). Again, ectopic trichome formation on pedicels has not been reported in mutants of any other members of the single-repeat R3 MYB transcription factor family. Mutations in *ETC2*, one member of this gene family, confer ectopic formation of trichomes on the leaf petioles but not on the inflorescence stems or pedicels (Kirik et al., 2004b). Based on these results, we conclude that TCL1 has a unique role in regulating epidermal cell patterning by controlling trichome cell specification on the inflorescence stems and pedicels.

To confirm that the phenotype we observed in *tcl1-1* mutants was due to the loss-of-function of *TCL1*, we transformed *tcl1-1* mutants with a binary vector containing the full-length ORF of *TCL1* fused in frame with *GFP*, driven by *TCL1*'s own promoter (P_{TCL1}*:TCL1-GFP*). We used a genomic DNA fragment that covers the region –1535 to +1 of the start codon of *TCL1* to provide putative regulatory sequences for *TCL1*. When expressed from this putative regulatory region, the *TCL1* ORF was able to complement the *tcl1* mutant (Fig. 3C), indicating that this putative regulatory sequence is sufficient for normal *TCL1* expression, and that the TCL1-GFP fusion protein is most likely functional. The complete rescue of the *tcl1-1* phenotype by P_{TCL1}*:TCL1-GFP* shows that the trichome phenotype in the *tcl1-1* mutant is indeed due to the loss-of-function of *TCL1*.

Microscopic examination of the P_{TCL1}*:TCL1-GFP* transgenic plants revealed that TCL1 is localized in the nucleus of epidermal cells, but TCL1-GFP fluorescence could also be detected in regions near to, or at, the plasma membrane (Fig. 3D).

Synergistic effect between *TCL1* and *CPC* on trichome formation

Four single-repeat R3 MYB proteins, CPC, TRY, ETC1 and ETC2, have been shown to repress trichome initiation in a redundant manner (Schellmann et al., 2002; Esch et al., 2004; Kirik et al., 2004a; Kirik et al., 2004b). Overexpression of *TCL1* also repressed trichome initiation (Fig. 1A), implying that TCL1 might function redundantly with other single-repeat MYB proteins in regulating trichome formation. Because TRY and CPC are the best characterized of the known single-repeat R3 MYB transcription factors, we focused on testing functional redundancy between TCL1 and TRY or CPC.

We generated double mutants between *tcl1-1* and *try* or *cpc*. As shown in Table 1, *tcl1-1 try* double mutants have no significant difference in the number of trichomes or trichome clusters on their

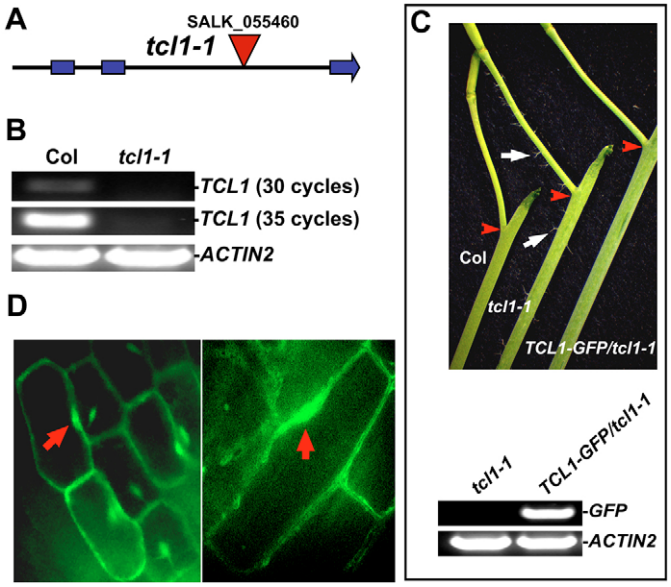

Fig. 3. Loss-of-function allele of *TCL1*. (**A**) Diagram illustrating the T-DNA insertion site in the *tcl1-1* mutant. The T-DNA is inserted in the second intron of *TCL1*. (**B**) Expression of *TCL1* in wild-type and *tcl1-1* mutant *Arabidopsis* plants. Expression of *ACTIN2* provided a control. (**C**) Comparison of trichome formation on the first pedicel and the internode subtending the site of the first flower on the main inflorescence of wild type (left), *tcl1-1* mutant (middle) and *tcl1-1* mutant transformed with P_{TCL1}:*TCL1-GFP* (right). Arrowheads indicate the site of the first flower/siliques on the main inflorescence stem. Arrows indicate trichomes formed on the main inflorescence stem and pedicel of the *tcl1-1* mutant. Beneath is shown the results from RT-PCR in which *GFP*-specific primers were used to examine the expression of *TCL1-GFP* in P_{TCL1}:*TCL1-GFP/tcl1-1* plants. Expression of *ACTIN2* provided a control. (**D**) TCL1-GFP fluorescence in the epidermal cells of the lower part of the hypocotyl in a 4-day-old P_{TCL1}:*TCL1-GFP* seedling. Shown are differential interference contrast (left) and confocal (right) images of hypocotyl epidermal cells. Arrows point to the nucleus of a cell.

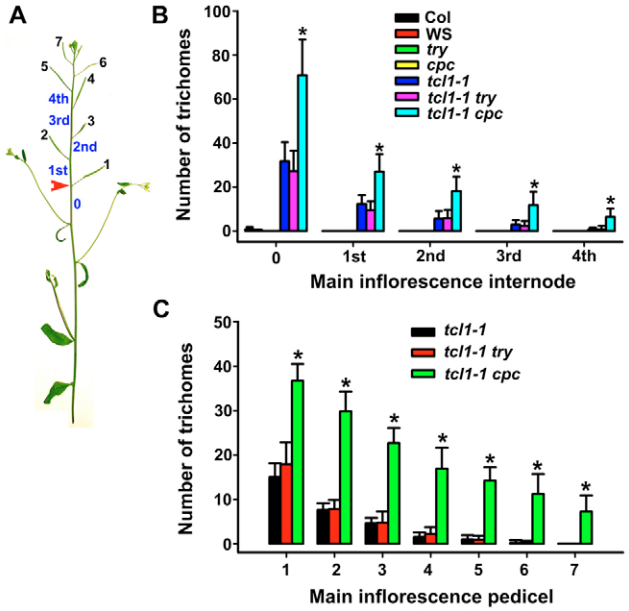

Fig. 4. Trichomes on the main inflorescence stem and pedicels of *tcl1-1 cpc* double mutants. (**A**) The regions that were used to score trichome density. Arrowhead indicates the site of the first flower/silique on the main inflorescence stem. The positions before (0) and after (first to fourth) the site of the first flower/silique on the main inflorescence stem are indicated. These five positions were used to score the number of trichomes on the main inflorescence stem. The first seven siliques (numbered 1 to 7) on the main inflorescence stem were used to examine the number of trichomes on pedicels. (**B**) Trichome density on the internode before (0) and after (first to fourth) the site of the first flower on the main inflorescence stem of wild type and mutants. Shown are means±s.e. of at least nine plants. *, significantly different from *tcl1-1* mutants, $P<0.05$. (**C**) Trichome density on pedicels on the main inflorescence. No trichome was found on pedicels in wild type, *try*, or *cpc* single mutants. Shown are means±s.e. of at least nine plants. *, significantly different from *tcl1-1* mutants, $P<0.05$.

rosette leaves, as compared with the *try* single mutant. Furthermore, the *tcl1-1 try* double mutant had no significant change in root epidermal cell pattern (see Table S1 in the supplementary material). Similarly, *tcl1 cpc* double mutants were indistinguishable from the *cpc* single mutant in leaf trichome formation or in H- and N-root epidermal cell specification (Table 1, and see Table S1 in the supplementary material).

We next examined trichome formation on the inflorescence stems and pedicels in adult plants of *tcl1-1 try* and *tcl11-1 cpc* double mutants to determine whether TRY and CPC have overlapping function with TCL in trichome cell specification on these organs. As shown in Fig. 4, the number of trichomes on the inflorescence stem and pedicels in *tcl1-1 try* double mutants was statistically equivalent to that in the *tcl1-1* single mutant. However, the number of trichomes on the inflorescence stem was increased 2- to 5-fold in *tcl1-1 cpc* double mutants, as compared with the *tcl1-1* single mutant. The number of trichomes on the pedicels in *tcl1-1 cpc* double mutants increased even more dramatically. For example, a >10-fold increase in trichome number was observed in the sixth flower/silique pedicel on the main inflorescence stem of *tcl1-1 cpc* double mutants, as compared with *tcl1-1* single mutants (Fig. 4C). Furthermore, a significant number of trichomes were also found in the seventh pedicel along the main inflorescence stem of *tcl1-1 cpc* double mutants, whereas no trichomes were found at the same site in the *tcl1-1* single mutant. These results indicate that TCL1 and CPC can work synergistically to regulate trichome formation on the inflorescence stem and pedicels. Because the number of trichomes on the inflorescence stems and pedicels in *try* or *cpc* single mutants do not differ from those in wild-type, these results confirmed that TCL1 is the major player of the single-repeat R3 MYB transcription factor family in regulating trichome cell specification on inflorescence stem and pedicels.

We generated *tcl1-1 cpc try* triple mutants and found that they did not differ significantly from the *cpc try* double mutants in terms of the number of trichomes and trichome clusters on their rosette leaves (Table 1), or in root epidermal cell pattern (see Table S1 in the supplementary material). As expected, *tcl1-1 cpc try* triple mutants formed trichome clusters on the inflorescence stems beyond the site of the first flower branch, and on pedicels (Fig. 5). These results support a predominant role for TCL1, compared to that of other members of the single-repeat R3 MYB transcription factor family, in regulating trichome cell specification on inflorescence stem and pedicels.

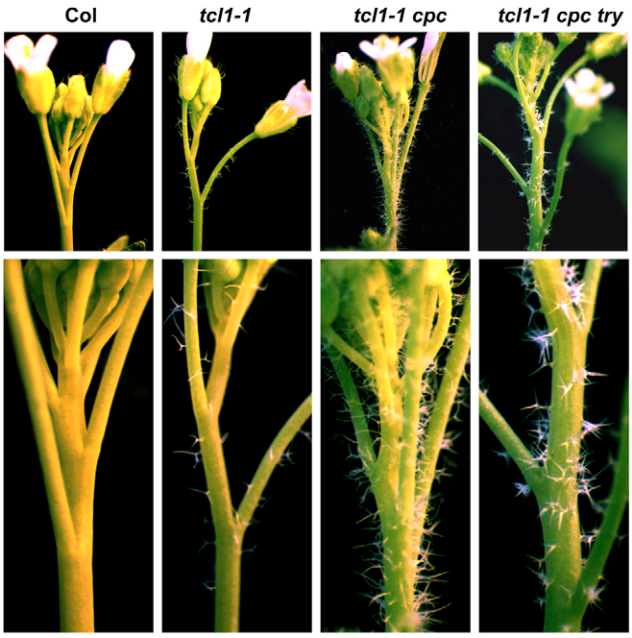

Fig. 5. Trichomes on the main inflorescence stem and pedicels of *tcl1-1 cpc try* triple mutants. Upper panels show the top portions of the main inflorescence stem of wild-type (Col), *tcl1-1*, *tcl1-1 cpc*, and *tcl1-1 cpc try* mutants. Bottom panels are magnified images of the inflorescence stem.

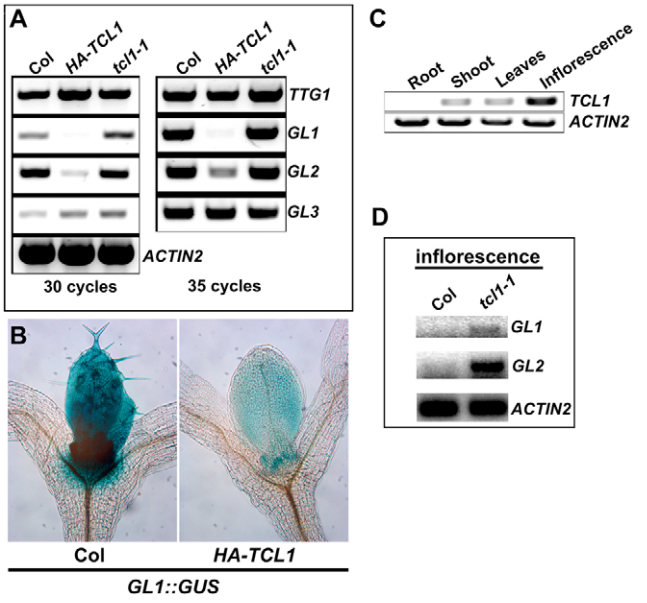

Fig. 6. TCL1 suppresses the expression of *GL1*. (**A**) RT-PCR analysis of *TTG1*, *GL1*, *GL2* and *GL3* transcripts in *Arabidopsis* seedlings overexpressing *HA-TCL1*. Expression of *ACTIN2* provided a control. (**B**) Suppression of *GL1::GUS* reporter by *TCL1*. Shown are the expression of *GL1::GUS* reporter in wild-type (Col) and in *35S:HA-TCL1* backgrounds. In the *GL1::GUS* reporter, the expression of *GUS* was driven by a chimerical genomic sequence consisting of the immediate 5′ upstream sequence, the first intron, and the 3′-UTR region of *GL1* (Wang et al., 2004). (**C**) RT-PCR analysis of *TCL1* transcript in various tissues/organs. Expression of *ACTIN2* provided a control. (**D**) RT-PCR analysis of *GL1* and *GL2* transcripts in the developing inflorescence of *tcl1-1* mutants. Expression of *ACTIN2* provided a control.

TCL1 suppresses the expression of *GL1*

To gain insight into the mechanism by which *TCL1* affects trichome initiation and patterning, we examined the expression of the known trichome initiation and patterning positive regulators *TTG1* (Galway et al., 1994; Walker et al., 1999), *GL1* (Oppenheimer et al., 1991), *GL3* (Payne et al., 2000; Zhang et al., 2003; Bernhardt et al., 2003; Bernhardt et al., 2005) and *GL2* (Rerie et al., 1994; Masucci et al., 1996) in *TCL1* overexpression plants and in *tcl1-1* mutants. As expected, the expression of *GL2* was reduced in *TCL1* overexpression plants (Fig. 6A), presumably because TCL1, like TRY and CPC, can bind to GL3, thus blocking the interaction between GL1 and GL3, an interaction that is required to form the GL1-GL3-TTG1 activator complex. According to one current model, inhibition of the formation of this complex would result in the suppression of expression of the trichome initiation positive regulator *GL2* (Larkin et al., 2003; Schiefelbein, 2003; Schellmann et al., 2007). Unexpectedly, however, the *GL1* transcript level was also dramatically reduced in plants overexpressing *TCL1*. The transcript level of *TTG1* and *GL3*, the other two members in the proposed activator complex, was not affected (Fig. 6A), indicating that such a repression is *GL1*-specific. Consistent with this, we found that the expression of a *GL1::GUS* reporter was dramatically reduced in plants overexpressing *TCL1* (Fig. 6B). These results raise the possibility that TCL1 affects trichome formation by directly suppressing *GL1* expression. In this scenario, the reduced expression of *GL2* in plants overexpressing *TCL1* might be a consequence of reduced expression of *GL1*, because a reduced availability of GL1 would decrease the transcriptional activity of the overall activator complex. To investigate this further, we examined the expression levels of *GL1* and *GL2* in the developing inflorescence of *tcl1-1* mutants by RT-PCR, because *TCL1* was expressed at a relatively higher level in inflorescence than in other tissues/organs (Fig. 6C). *GL1* and *GL2* transcripts were found to be increased in the *tcl1-1* mutant, as compared with the wild type (Fig. 6D), supporting the possibility that TCL1 negatively regulates *GL1* in the developing inflorescence epidermis.

GL1 is a target gene of TCL1

The hypothesis that TCL1 might directly target *GL1* was tested further. Because TCL1 is a single-repeat R3 transcription factor that does not contain an apparent transcription-activation domain, we first tested whether TCL1 itself could alter reporter gene expression in a protoplast transient-expression system (Tiwari et al., 2003; Wang et al., 2005). As expected, the TCL1 protein alone could not activate or repress reporter gene expression when it was recruited to the promoter region (*Gal4*) of the *GUS* reporter gene (Fig. 7A,B). However, when TCL1 was fused with a heterologous activator domain, VP16, the TCL1-VP16 fusion protein was able to function efficiently as an activator (Fig. 7A,B). We therefore generated transgenic plants overexpressing this TCL1-VP16 fusion protein.

We reasoned that if *GL1* is the target for TCL1, one would expect that the transcription of *GL1* would be elevated in *35S:TCL1-VP16* plants owing to the direct binding of TCL1 to the cis-acting regulatory elements of *GL1* and the concurrent activation of *GL1* transcription by VP16. In addition, we would predict that the *35S:TCL1-VP16* plants should phenocopy plants overexpressing *GL1*. Indeed, multiple transgenic lines of *35S:TCL1-VP16* plants displayed dramatically reduced number of trichomes on their leaves and inflorescence stems, some of which had glabrous stems (Fig. 7C), thus phenocopying plants overexpressing *GL1* (Larkin et al.,

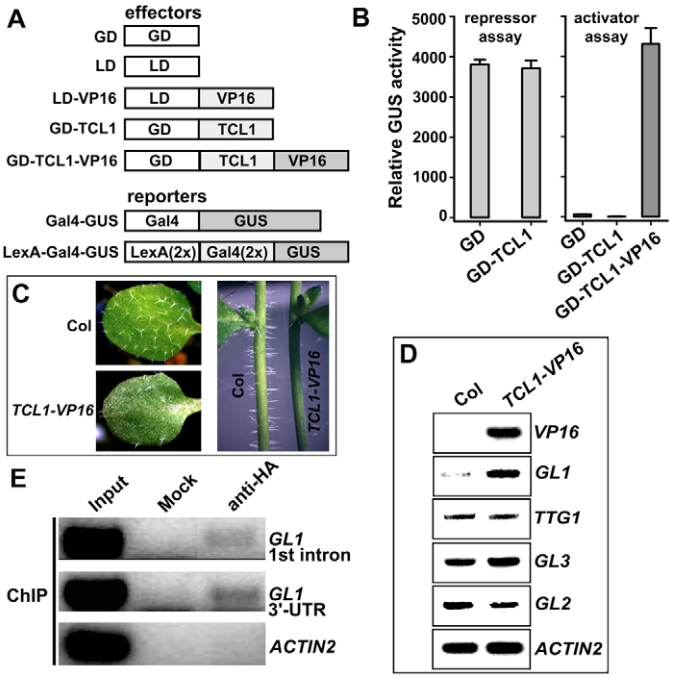

Fig. 7. *GL1* is a target gene of TCL1. (**A**) Schematic showing the effectors and reporter constructs used in the *Arabidopsis* protoplast transfection assays. (**B**) TCL1-VP16 transactivator fusion protein activates the expression of the reporter. Effectors and reporters were cotransfected into protoplasts derived from *Arabidopsis* rosette leaves. GUS activity was assayed after protoplasts were incubated in the dark for 20-22 hours. (**C**) Leaves and stem trichome phenotypes of transgenic plants overexpressing *TCL1-VP16*. (**D**) RT-PCR analysis of the level of *GL1*, *TTG1*, *GL3* and *GL2* transcripts in plants overexpressing TCL1-VP16. Expression of *ACTIN2* provided a control. (**E**) Chromatin immunoprecipitation (ChIP) assay. ChIP was performed with *35S:HA-TCL1* plants using anti-HA antibodies. Rabbit preimmune serum was used as a mock control. Primer sets specific for the first intron or the 3′-UTR region of *GL1* were used in PCR reactions. *ACTIN2* provided a control.

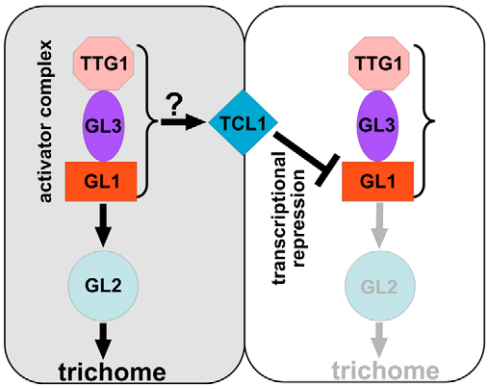

Fig. 8. Model of action of TCL1 in trichome patterning in *Arabidopsis*. *GL1* encodes an R2R3 MYB-type transcription factor. *GL3* encodes a bHLH-related transcription factor. *TTG1* encodes a WD40 domain-containing protein. *GL2* encodes a homeobox transcription factor. *TCL1* encodes a single-repeat R3 MYB transcription factor. Other known single-repeat MYB transcription factors, including TRY, CPC, ETC1 and ETC2, are not shown in the model. TTG1, GL3 and GL1 form an activator complex, whereas TCL1, TRY, CPC, ETC1 and ETC2 act as repressors that were proposed to bind GL3, thus limiting the interaction between GL1 and GL3, an interaction that is required to form the TTG1-GL3-GL1 activator complex that regulates the transcription of *GL2*. As shown in this study, TCL1 can directly suppress the expression of *GL1*, thereby limiting the transcriptional activity of the TTG1-GL3-GL1 activator complex. It is unclear whether the TTG1-GL3-GL1 activator complex can promote the transcription of *TCL1*. Arrows indicate positive regulation and the blunt-ended line indicates negative regulation.

1994). Consistent with this, we detected elevated levels of *GL1* transcript (in contrast to largely unchanged levels of *TTG1* and *GL3* transcripts) in these transgenic lines (Fig. 7D). Taken together, these results support a working model in which TCL1 controls trichome patterning by suppressing the expression of *GL1* (Fig. 8).

To test this directly, we used a chromatin immunoprecipitation (ChIP) assay to determine the association of TCL1 protein with the cis-acting regulatory sequence of *GL1*. *35S:HA-TCL1* plants and anti-HA antibodies were used. We detected a specific PCR product of the expected size amplified using primers specific to the first intron or the 3′-UTR region of *GL1* (Fig. 7E). Both of these regions have been previously shown to be required for the proper expression of *GL1* (Larkin et al., 1993; Wang et al., 2004). As a mock control, we used rabbit preimmune sera, and we did not detect any specific PCR products using the same sets of primers (Fig. 7E). Therefore, we conclude that *GL1* is indeed a target gene of TCL1.

DISCUSSION

We report the identification and functional analysis of a previously unknown member of the single-repeat R3 MYB transcription factor family, TCL1. Several lines of evidence support the hypothesis that TCL1, like TRY, CPC, ETC1 and ETC2 (Hülskamp et al., 1994; Wada et al., 1997; Schellmann et al., 2002; Esch et al., 2004; Kirik et al., 2004a; Kirik et al., 2004b), acts as a negative regulator for trichome initiation. First, the dominant mutant *tcl1-1D* isolated from an activation-tagged population displayed a glabrous phenotype, owing to *TCL1* overexpression (Fig. 1, Table 1). Second, ectopic overexpression of *TCL1* under the control of the CaMV *35S* promoter in wild-type plants resulted in the loss of trichomes (Fig. 1, Table 1). Finally, a loss-of-function mutation in *TCL1* conferred ectopic trichome formation on the inflorescence stems and pedicels (Figs 3, 4, 5). We provide genetic evidence that TCL1 is the major player of the single-repeat R3 MYB proteins in regulating trichome formation on the inflorescence stems and pedicels (Figs 4, 5), but it does not affect root epidermal cell fate (Fig. 1, and see Table S1 in the supplementary material). Furthermore, we provide evidence that TCL1 negatively regulates trichome patterning in a novel manner by directly suppressing the expression of *GL1* (Figs 6, 7).

TCL1 is a major negative regulator for trichome patterning on the inflorescence stems and pedicels

In wild-type plants, trichome initiation decreases on successive internodes with only very few or no trichomes on the internode subtending the site of the first flower on the main inflorescence stem (Gan et al., 2006), and no trichomes were found on the main inflorescence stem above the site of the first flower (Figs 3, 4). In the *tcl1-1* mutant, the number and patterning of trichomes on leaves were indistinguishable from those of wild type (Table 1). However, the number of trichomes was significantly increased in the internodes of *tcl1-1* mutants, and trichomes were also formed

beyond the site of the first flower (Figs 3, 4). Loss-of-function of *TCL1* also results in trichome formation on pedicels, which normally do not bear any trichomes (Figs 3, 4, 5). Because these trichome phenotypes on inflorescence stems and pedicels have not been reported in mutants of other members of the single-repeat R3 MYB transcription factor family, our data support the notion that TCL1 is the predominant member of this family for the regulation of trichome formation on these organs.

Although TCL1 functions as a trichome initiation repressor, as do TRY, CPC, ETC1 and ETC2 (Hülskamp et al., 1994; Wada et al., 1997; Schellmann et al., 2002; Esch et al., 2004; Kirik et al., 2004a; Kirik et al., 2004b), our results suggest that TCL1 might have a unique biochemical role in regulating epidermal cell specification because TCL1 does not appear to affect root hair formation and patterning. The position-dependent specification of root epidermal cells is not altered in either the gain- or loss-of-function mutants of *TCL1* (Fig. 1, and see Table S1 in the supplementary material), whereas overexpression of the other single-repeat R3 MYBs tested, including CPC, TRY, ETC1 and ETC2, induces ectopic root hair cells (Wada et al., 1997; Schellmann et al., 2002; Kirik et al., 2004a; Kirik et al., 2004b). These results imply significant differences in the biochemical properties of these single-repeat R3 MYBs. Our results from genetic analysis of double and triple mutants among *tcl1-1*, *cpc* and *try*, suggested that TCL1 and CPC, but not TRY, have a synergistic effect on trichome formation on inflorescence epidermis (Figs 4, 5). These findings are consistent with the promoter-swap assays. We found that the expression of *TCL1* under the control of the *CPC* promoter in a *cpc* mutant background (P_{CPC}*:HA-TCL1/cpc*) could partially rescue the root hair phenotype of the *cpc* mutant while simultaneously repressing trichome initiation on leaves (see Fig. S1 in the supplementary material). On the other hand, trichome clusters, a phenotypic trait of the *try* mutant (Esch et al., 2003), were still present on the leaves of plants expressing *TCL1* under the control of the *TRY* promoter in a *try* mutant background (P_{TRY}*:HA-TCL1/try*), although their frequency was significantly reduced (see Fig. S1 the in supplementary material). These results suggest that the TCL1 protein is not fully interchangeable with TRY in controlling trichome patterning on rosette leaves, and that TCL1 is functionally similar, but not identical, to CPC in the developmental context of regulation of epidermal cell specification. These results support the notion that the functional specificity of TCL1 largely derives from the protein coding sequence, rather than from its promoter activity.

The phenotype of ectopic trichome formations on the inflorescence stems observed in *tcl1-1* mutants has also been reported for plants overexpressing *GIS* (Gan et al., 2006). *GIS* encodes a transcription factor of the C2H2 family, and acts upstream of the trichome initiation complex (Gan et al., 2006). Recently, it has been found that GIS and two other C2H2 transcription factors, ZFP8 and GIS2, play partially redundant and essential roles in inflorescence trichome initiation and are regulated by the plant hormones, gibberellins and cytokinins (Gan et al., 2007). It is unclear whether TCL1 can work together with GIS to regulate trichome formation on inflorescence epidermis.

Although TCL1 is a major negative regulator for trichome patterning on the inflorescence stems and pedicels, it is possible that TCL1 might also have a role in leaf epidermal development. For example, *TCL1* transcript can be detected in the leaf (Fig. 6C), and overexpression of *TCL1* represses trichome formation (Fig. 1). A higher-order combination of mutations in single-repeat R3 MYBs might help clarify the exact role of TCL1 in leaf trichome formation.

A possible molecular mechanism for the action of TCL1 in the regulation of trichome patterning

Previous analyses in yeast two-hybrid assays have demonstrated that TRY, CPC, ETC1 and ETC2, can interact with GL3 to limit the interaction between GL1 and GL3 (Payne et al., 2000; Esch et al., 2003; Esch et al., 2004; Kirik et al., 2004b; Zimmermann et al., 2004), thus inhibiting the formation of an activator complex between TTG1, GL1 and GL3/EGL3, that is required for the activation of *GL2* transcription (Schiefelbein, 2003). Our results showed that *GL2* expression was reduced in plants overexpressing *TCL1* (Fig. 6), which could be explained if TCL1 operates by the same mechanism. However, overexpression of *TCL1* also suppressed the expression of *GL1* (Fig. 6). The suppression of *GL1* by TCL1 appears to be specific to this member of the activator complex, because the expression levels of the other two components in the activator complex, *TTG1* and *GL3*, were largely unaffected by overexpression of *TCL1* (Fig. 6). One possible scenario is that TCL1 directly suppresses the expression of *GL1* and, thereby, indirectly causes a reduction in *GL2* expression (Fig. 8). This possibility is supported by the elevated levels of *GL1* and *GL2* transcripts in the developing inflorescence of *tcl1-1* mutants (Fig. 6). Furthermore, the notion that *GL1* might be a potential target gene for TCL1 was supported by the observation that plants overexpressing a TCL1-VP16 fusion protein, shown to be a transactivator in the protoplast transfection assay, have elevated expression levels of *GL1* (but largely unchanged levels of *TTG1* and *GL3*) and display phenotypes similar to plants overexpressing *GL1* (Fig. 7). An increase in GL1 level in response to TCL1-VP16 overexpression presumably alters the titration balance among components of the GL1-GL3-TTG1 activator complex, thereby disrupting efficient formation of the activator complex and, subsequently, its transcriptional activity. Finally, our ChIP assay demonstrates that TCL1 can be recruited to the cis-acting regulatory elements of *GL1*, suggesting that *GL1* is indeed a target gene of TCL1. A study on *GL1* promoter activity and GL1 protein localization at a subcellular level in a *tcl1* mutant background might strengthen the proposed model, and is worth further investigation.

Little is known about the regulation of *GL1* expression. Here we provide evidence that TCL1 might act to directly control *GL1* expression. It is possible that, in addition, TCL1 competes with GL1 for binding to GL3, so as to inhibit the formation of the activator complex, as suggested for the other single-repeat R3 MYB transcription factors. It is worth noting that TCL1 has the amino acid signature [D/E]Lx2[R/K]x3Lx6Lx3R (Zimmermann et al., 2004) that is required for interacting with R/B-like bHLH transcription factors (Fig. 2C). TCL1 also has the conserved amino acids in the MYB domain (Kurata et al., 2005) that are crucial for cell-to-cell movement (Fig. 2C). Using transgenic plants expressing a TCL1-GFP fusion protein, we found that the TCL1-GFP can be detected in epidermal cells (Fig. 3D). Using transgenic plants expressing a *TCL1* promoter-*GUS* fusion construct (P_{TCL1}*:GUS*), we found that P_{TCL1}*:GUS* was widely expressed (see Fig. S2 in the supplementary material). A detailed study on *TCL1* mRNA expression pattern and TCL1 protein subcellular localization might help clarify the cell-to-cell movement ability of TCL1.

TCL1 is unique among the single-repeat R3 MYB proteins in that overexpression of TCL1 does not affect root epidermis cell fate, which does not depend on *GL1* function. However, we found that overexpression of CPC could also dramatically suppress the expression of *GL1* (see Fig. S3 in the supplementary material). Therefore, it is possible that other single-repeat R3 MYBs, such as CPC, can also function in a similar manner to TCL1 to directly suppress the transcription of *GL1* and regulate trichome patterning.

It is worth noting that a similar mechanism has been proposed for root hair patterning in which CPC (single-repeat MYB) negatively regulates *WER* (R2R3 MYB) expression in hair-forming cells (Lee and Schiefelbein, 2002). WER has been proposed to be functionally equivalent to GL1 (Lee and Schiefelbein, 2001).

In summary, we identified TCL1 as a previously unknown member of the single-repeat R3 MYB transcription factor family. We provide evidence that TCL1 is a negative regulator of trichome initiation and patterning, and that TCL1 has a specific role in regulating epidermal cell specification on inflorescence stems and pedicles. This provides new insight into the way that organ-specific regulation of epidermal patterning might be achieved using a common mechanism and related transcription factor molecules. Furthermore, we show that TCL1 is likely to negatively regulate trichome formation in a novel manner, by directly suppressing the expression of *GL1* (Fig. 8). This suggests the existence of a novel regulatory loop in trichome patterning, and offers a fine-tuning mechanism for the interaction between the negative regulators and the activator complex.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/134/21/3873/DC1

References

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R. et al. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**, 653-657.

Bernhardt, C., Lee, M. M., Gonzalez, A., Zhang, F., Lloyd, A. and Schiefelbein, J. (2003). The bHLH genes *GLABRA3* (*GL3*) and *ENHANCER OF GLABRA3* (*EGL3*) specify epidermal cell fate in the *Arabidopsis* root. *Development* **130**, 6431-6439.

Bernhardt, C., Zhao, M., Gonzalez, A., Lloyd, A. and Schiefelbein, J. (2005). The bHLH genes *GL3* and *EGL3* participate in an intercellular regulatory circuit that controls cell patterning in the *Arabidopsis* root epidermis. *Development* **132**, 291-298.

Clough, S. J. and Bent, A. F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 736-743.

Esch, J. J., Chen, M., Sanders, M., Hillestad, M., Ndkium, S., Idelkope, B., Neizer, J. and Marks, M. D. (2003). A contradictory *GLABRA3* allele helps define gene interactions controlling trichome development in *Arabidopsis*. *Development* **130**, 5885-5894.

Esch, J. J., Chen, M. A., Hillestad, M. and Marks, M. D. (2004). Comparison of *TRY* and the closely related *At1g01380* gene in controlling Arabidopsis trichome patterning. *Plant J.* **40**, 860-869.

Galway, M. E., Masucci, J. D., Lloyd, A. M., Walbot, V., Davis, R. W. and Schiefelbein, J. W. (1994). The *TTG* gene is required to specify epidermal cell fate and cell patterning in the *Arabidopsis* root. *Dev. Biol.* **166**, 740-754.

Gan, Y., Kumimoto, R., Liu, C., Ratcliffe, O., Yu, H. and Broun, P. (2006). GLABROUS INFLORESCENCE STEMS modulates the regulation by gibberellins of epidermal differentiation and shoot maturation in *Arabidopsis*. *Plant Cell* **18**, 1383-1395.

Gan, Y., Liu, C., Yu, H. and Broun, P. (2007). Integration of cytokinin and gibberellin signalling by Arabidopsis transcription factors GIS, ZFP8 and GIS2 in the regulation of epidermal cell fate. *Development* **134**, 2073-2081.

Hajdukiewicz, P., Svab, Z. and Maliga, P. (1994). The small, versatile *pPZP* family of Agrobacterium binary vectors for plant transformation. *Plant Mol. Biol.* **25**, 989-994.

Hülskamp, M., Misra, S. and Jürgens, G. (1994). Genetic dissection of trichome cell development in *Arabidopsis*. *Cell* **76**, 555-566.

Kirik, V., Simon, M., Hülskamp, M. and Schiefelbein, J. (2004a). The *ENHANCER OF TRY AND CPC1* gene acts redundantly with *TRIPTYCHON* and *CAPRICE* in trichome and root hair cell patterning in *Arabidopsis*. *Dev. Biol.* **268**, 506-513.

Kirik, V., Simon, M., Wester, K., Schiefelbein, J. and Hülskamp, M. (2004b). *ENHANCER* of *TRY* and *CPC 2*(*ETC2*) reveals redundancy in the region-specific control of trichome development of *Arabidopsis*. *Plant Mol. Biol.* **55**, 389-398.

Kurata, T., Ishida, T., Kawabata-Awai, C., Noguchi, M., Hattori, S., Sano, R., Nagasaka, R., Tominaga, R., Koshino-Kimura, Y., Kato, T. et al. (2005). Cell-to-cell movement of the CAPRICE protein in Arabidopsis root epidermal cell differentiation. *Development* **132**, 5387-5398.

Larkin, J. C., Oppenheimer, D. G., Pollock, S. and Marks, M. D. (1993). Arabidopsis *GLABROUS1* gene requires downstream sequences for function. *Plant Cell* **5**, 1739-1748.

Larkin, J. C., Oppenheimer, D. G., Lloyd, A. M., Paparozzi, E. T. and Marks, M. D. (1994). Roles of the *GLABROUS1* and *TRANSPARENT TESTA GLABRA* genes in Arabidopsis trichome development. *Plant Cell* **6**, 1065-1076.

Larkin, J. C., Brown, M. L. and Schiefelbein, J. (2003). How do cells know what they want to be when they grow up? Lessons from epidermal patterning in Arabidopsis. *Annu. Rev. Plant Biol.* **54**, 403-430.

Lawrence, R. J., Earley, K., Pontes, O., Silva, M., Chen, Z. J., Neves, N., Viegas, W. and Pikaard, C. S. (2004). A concerted DNA methylation/histone methylation switch regulates rRNA gene dosage control and nucleolar dominance. *Mol. Cell* **13**, 599-609.

Lee, M. M. and Schiefelbein, J. (1999). WEREWOLF, a MYB-related protein in *Arabidopsis*, is a position-dependent regulator of epidermal cell patterning. *Cell* **99**, 473-483.

Lee, M. M. and Schiefelbein, J. (2001). Developmentally distinct MYB genes encode functionally equivalent proteins in *Arabidopsis*. *Development* **128**, 1539-1546.

Lee, M. M. and Schiefelbein, J. (2002). Cell pattern on the Arabidopsis root epidermis determined by lateral inhibition with feedback. *Plant Cell* **14**, 611-618.

Masucci, J. D., Rerie, W. G., Foreman, D. R., Zhang, M., Galway, M. E., Marks, M. D. and Schiefelbein, J. W. (1996). The homeobox gene *GLABRA2* is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana*. *Development* **122**, 1253-1260.

Oppenheimer, D. G., Herman, P. L., Sivakumaran, S., Esch, J. and Marks, M. D. (1991). A myb gene required for leaf trichome differentiation in *Arabidopsis* is expressed in stipules. *Cell* **67**, 483-493.

Payne, C. T., Zhang, F. and Lloyd, A. M. (2000). *GL3* encodes a bHLH protein that regulates trichome development in *Arabidopsis* through interaction with *GL1* and *TTG1*. *Genetics* **156**, 1349-1362.

Pesch, M. and Hülskamp, M. (2004). Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation. *Curr. Opin. Genet. Dev.* **14**, 422-427.

Rerie, W. G., Feldmann, K. A. and Marks, M. D. (1994). The *GLABRA2* gene encodes a homeo domain protein required for normal trichome development in *Arabidopsis*. *Genes Dev.* **8**, 1388-1399.

Schellmann, S., Schnittger, A., Kirik, V., Wada, T., Okada, K., Beermann, A., Thumfahrt, J., Jürgens, G. and Hülskamp, M. (2002). TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in *Arabidopsis*. *EMBO J.* **21**, 5036-5046.

Schellmann, S., Hülskamp, M. and Uhrig, J. (2007). Epidermal pattern formation in the root and shoot of Arabidopsis. *Biochem. Soc. Trans.* **35**, 146-148.

Schiefelbein, J. (2003). Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot. *Curr. Opin. Plant Biol.* **6**, 74-78.

Schnittger, A., Folkers, U., Schwab, B., Jürgens, G. and Hülskamp, M. (1999). Generation of a spacing pattern: The role of *TRIPTYCHON* in trichome patterning in Arabidopsis. *Plant Cell* **11**, 1105-1116.

Serna, L. (2005). Epidermal cell patterning and differentiation throughout the apical-basal axis of the seedling. *J. Exp. Bot.* **56**, 1983-1989.

Serna, L. and Martin, C. (2006). Trichomes: different regulatory networks lead to convergent structures. *Trends Plant Sci.* **11**, 274-280.

Telfer, A., Bollman, K. M. and Poethig, R. S. (1997). Phase change and the regulation of trichome distribution in *Arabidopsis thaliana*. *Development* **124**, 645-654.

Tiwari, S. B., Hagen, G. and Guilfoyle, T. (2003). The roles of auxin response factor domains in auxin-responsive transcription. *Plant Cell* **15**, 533-543.

Ullah, H., Chen, J. G., Young, J. C., Im, K. H., Sussman, M. R. and Jones, A. M. (2001). Modulation of cell proliferation by heterotrimeric G protein in *Arabidopsis*. *Science* **292**, 2066-2069.

Ulmasov, T., Murfett, J., Hagen, G. and Guilfoyle, T. J. (1997). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* **9**, 1963-1971.

Wada, T., Tachibana, T., Shimura, Y. and Okada, K. (1997). Epidermal cell differentiation in *Arabidopsis* determined by a Myb homolog, CPC. *Science* **277**, 1113-1116.

Wada, T., Kurata, T., Tominaga, R., Koshino-Kimura, Y., Tachibana, T., Goto, K., Marks, M. D., Shimura, Y. and Okada, K. (2002). Role of a positive

regulator of root hair development, *CAPRICE*, in *Arabidopsis* root epidermal cell differentiation. *Development* **129**, 5409-5419.

Walker, A. R., Davison, P. A., Bolognesi-Winfield, A. C., James, C. M., Srinivasan, N., Blundell, T. L., Esch, J. J., Marks, M. D. and Gray, J. C. (1999). The *TRANSPARENT TESTA GLABRA1* locus, which regulates trichome differentiation and anthocyanin biosynthesis in *Arabidopsis*, encodes a WD40 repeat protein. *Plant Cell* **11**, 1337-1350.

Wang, S., Wang, J. W., Yu, N., Li, C. H., Luo, B., Gou, J. Y., Wang, L. J. and Chen, X. Y. (2004). Control of plant trichome development by a cotton fiber MYB gene. *Plant Cell* **16**, 2323-2334.

Wang, S., Tiwari, S. B., Hagen, G. and Guilfoyle, T. J. (2005). AUXIN RESPONSE FACTOR7 restores the expression of auxin-responsive genes in mutant Arabidopsis leaf mesophyll protoplasts. *Plant Cell* **17**, 1979-1993.

Wang, S., Chang, Y., Guo, J. and Chen, J. G. (2007). Arabidopsis Ovate Family Protein 1 is a transcriptional repressor that suppresses cell elongation. *Plant J.* **50**, 858-872.

Weigel, D., Ahn, J. H., Blazquez, M. A., Borevitz, J. O., Christensen, S. K., Fankhauser, C., Ferrandiz, C., Kardailsky, I., Malancharuvil, E. J., Neff, M. M. et al. (2000). Activation tagging in Arabidopsis. *Plant Physiol.* **122**, 1003-1013.

Zhang, F., Gonzalez, A., Zhao, M., Payne, C. T. and Lloyd, A. (2003). A network of redundant bHLH proteins functions in all TTG1-dependent pathways of *Arabidopsis*. *Development* **130**, 4859-4869.

Zimmermann, I. M., Heim, M. A., Weisshaar, B. and Uhrig, J. F. (2004). Comprehensive identification of Arabidopsis thaliana MYB transcription factors interacting with R/B-like BHLH proteins. *Plant J.* **40**, 22-34.

Table S1. Root hair and non-hair cell specification in the root epidermis of wild-type, mutant and transgenic lines

Genotype	Hair cells in epidermis (%)	H cell position		N cell position	
		Hair cells (%)	Non-hair cells (%)	Hair cells (%)	Non-hair cells (%)
WT (Col)	42.1±3.6	98.0±1.7	2.0±1.7	0.8±1.0	99.2±1.0
WT (WS)	41.6±4.0	93.8±2.2	6.2±2.2	0.5±0.5	99.5±0.5
tcl1-1D	41.2±4.4	90.2±3.7	9.8±3.7	1.0±1.2	99.0±1.2
35S:HA-TCL1	40.9±3.9	91.2±2.3	8.8±2.3	2.3±2.0	97.7±2.0
P_{TCL1}:TCL1-GFP/tcl1-1	42.2±4.7	96.8±1.9	3.2±1.9	0±0	100±0
tcl1-1	41.4±5.3	93.1±3.2	4.1±3.2	0±0	100±0
cpc	13.8±3.4*	23.4±4.9*	76.6±4.9*	0±0	100±0
try	41.4±3.1	96.5±3.5	3.5±3.5	1.0±0.6	99.0±0.6
tcl1-1 cpc	14.4±3.0*	22.9±5.2*	77.1±5.2*	0±0	100±0
tcl1-1 try	39.8±3.3	95.7±3.3	4.3±3.3	1.6±2.0	98.4±2.0
cpc try	0±0*	0±0*	100±0*	0±0	100±0
tcl1-1 cpc try	0±0*	0±0*	100±0*	0±0	100±0

Values indicate mean±s.d. of at least ten roots for each line. In all strains, approximately 40% of epidermal cells are in the H position.
*, $P<0.05$, relative to the corresponding wild-type line.