The Trihelix Transcription Factor GTL1 Regulates Ploidy-Dependent Cell Growth in the Arabidopsis Trichome

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Leaf trichomes in Arabidopsis thaliana develop through several distinct cellular processes, such as patterning, differentiation, and growth. Although recent studies have identified several key transcription factors as regulating early patterning and differentiation steps, it is still largely unknown how these regulatory proteins mediate subsequent trichome development, which is accompanied by rapid cell growth and branching. Here, we report a novel trichome mutation in Arabidopsis, which in contrast with previously identified mutants, increases trichome cell size without altering its overall patterning or branching. We show that the corresponding gene encodes a GT-2-LIKE1 (GTL1) protein, a member of the trihelix transcription factor family. GTL1 is present within the nucleus during the postbranching stages of trichome development, and its loss of function leads to an increase in the nuclear DNA content only in trichomes that have completed branching. Our data further demonstrate that the gtl1 mutation modifies the expression of several cell cycle genes and partially rescues the ploidy defects in the cyclin-dependent kinase inhibitor mutant siamese. Taken together, this study provides the genetic evidence for the requirement of transcriptional regulation in the repression of ploidy-dependent plant cell growth as well as for an involvement of GTL trihelix proteins in this regulation.

INTRODUCTION

Plant trichomes are highly specialized epidermal cells implicated in several important functions, including transpiration control, freezing tolerance, and protection against insects, diseases, and UV light (Johnson, 1975; Mauricio and Rausher, 1997). Trichomes are also of special commercial interest because they have a unique secondary metabolism, allowing the production and secretion of various useful natural compounds (Wang et al., 2008; Besser et al., 2009). Leaf trichomes in Arabidopsis thaliana develop through several distinct cellular processes, and over the last decade, they have become an ideal model system to study various developmental processes, such as cell patterning, differentiation, and morphogenesis, at the single cell level (Hulskamp et al., 1994).

Trichomes are the first epidermal cells that undergo cell specification in the Arabidopsis leaf primordia (Hulskamp et al., 1994; Larkin et al., 1996). The subsequent gradual development of leaf trichomes, ranging from their initiation at the leaf base to maturation at the leaf tip, can be easily followed on a single, young leaf (Hulskamp et al., 1994; Szymanski and Marks, 1998). From several mutagenesis screens in the past, a vast number of mutations affecting trichome development have been isolated (Hulskamp et al., 1994; Marks, 1997), and their molecular and genetic analyses have helped dissecting the pathways that underlie different phases of trichome development. Trichome patterning is governed by positive and negative regulators of trichome initiation. Mutations affecting the positive regulators TRANSPARENT TESTA GLABRA1 (TTG1) or GLABRA1 (GL1) result in impaired trichome initiation (Oppenheimer et al., 1991; Galway et al., 1994), and the corresponding TTG1 and GL1 loci encode WD40 protein and MYB-related transcription factors, respectively (Oppenheimer et al., 1991; Larkin et al., 1994; Walker et al., 1999). Strong ttg1 and gl1 mutants fail to develop large epidermal trichome precursor cells with polyploid nuclei, suggesting that endoreduplication cycles are a prerequisite for trichome initiation (Hulskamp et al., 1999). Further studies have shown that the basic helix-loop-helix transcription factor GL3 and its close homolog ENHANCER OF GLABRA3 (EGL3) are also positive regulators of trichome initiation. Single mutants for GL3 and EGL3 exhibit a small or no reduction in trichome number, whereas the gl3 egl3 double mutants are completely trichome-less, demonstrating that GL3 and EGL3 function in a partially redundant manner (Payne et al., 2000; Zhang et al., 2003). GL3, together with TTG1 and GL1, is known to activate the expression of downstream target genes, including an HD-Zip transcription factor GL2 and a WRKY transcription factor TTG2, which also act as positive regulators of trichome initiation (Rerie et al., 1994; Johnson et al., 2002; Ishida et al., 2007; Morohashi et al., 2007; Zhao et al., 2008). By contrast, the TRIPTYCHON (TRY) gene encodes a single-repeat MYB protein that acts as a negative...
regulator of trichome initiation (Schellmann et al., 2002). In try mutants, trichome initiation in cells adjacent to trichomes is not repressed, leading to the formation of trichome clusters. Recent studies have shown that five other single-repeat MYB homologs, CAPRICE (CPC), ENHANCER OF TRY AND CPC1 (ETC1), ETC2, ETC3/CPC-LIKE-MYB3, and TRICHOMELESS1 (TCL1), also participate in trichome development, and all, except TCL1, which is required for the stem and pedicel trichome formation, act redundantly with TRY in leaf trichome initiation (Esch et al., 2004; Kirik et al., 2004a, 2004b; Schellmann et al., 2007; Wang et al., 2007; Tominaga et al., 2008; Wester et al., 2009).

After cell fate specification, trichome cells enter a highly complex differentiation program that proceeds through two major growth phases (Melaragno et al., 1993; Hulskamp et al., 1994; Schnittger et al., 1998). During the first phase, cells undergo several rounds of endocycles, leading to a final ploidy of 3C in most trichomes and substantial increase in cell size. The first growth phase is also defined by radial expansion and epidermal outgrowth, which leads to two successive branching events (Folkers et al., 1997). The branching process is then followed by the second growth phase, which is defined by rapid cell expansion mainly driven by vacuolization (Hulskamp et al., 1994; Szymanski et al., 1999). Previous genetic studies suggest that the typical three-dimensional branching pattern of trichomes is regulated by several intersecting genetic pathways that modify ploidy, cell size, and/or cytoskeletons (Hulskamp et al., 1994; Folkers et al., 1997; Perazza et al., 1999; Sugimoto-Shirasu et al., 2002; Ilgenfritz et al., 2003). In addition to the trichome patterning defects, try mutants exhibit overbranched, over-endoreduplicated trichomes with an overall increase in trichome cell size (Hulskamp et al., 1994; Schnittger et al., 1998). By contrast, gl3 mutants display decreased ploidy, decreased trichome size and under-branching (Hulskamp et al., 1994), suggesting that TRY and GL3 also participate as negative and positive regulators, respectively, in the trichome development beyond the initial patterning and differentiation stage. Furthermore, several other mutations, such as kaktus (kak), rastafari (rfl), polychome (pym), and spindly (spy), that also cause alterations in trichome ploidy, size, and branching have been identified (Hulskamp et al., 1994; Perazza et al., 1999), highlighting the strong coupling between ploidy, cell size, and branching in trichome development.

The tight coupling between endocycling and trichome development is also supported by several lines of experimental evidence demonstrating that the up- or downregulation of key cell cycle genes directly modifies the trichome patterning and/or morphogenesis. For example, mutations in the SIAMESE (SIM) gene, encoding a plant-specific cyclin-dependent kinase (CDK) inhibitor, result in clustered multicellular trichomes with individual nuclei having reduced ploidy levels (Walker et al., 2000; Churchman et al., 2006). The SIM proteins function within the D-type cyclin (CYC)-CDKA1 complex, which normally promotes the progression of mitotic cycles (Schnittger et al., 2002), and the interaction between SIM and the CYCD-CDKA complex appears to result in the repression of mitotic cycles, thus allowing an entry into the endocycle (Churchman et al., 2006). CDKA1 is also known to form a regulatory complex with an A-type cyclin, CYCA2;3, in developing trichomes to repress the progression of endocycles (Imai et al., 2006). Correspondingly, mutations in CYCA2;3 cause an increase in ploidy and trichome over-branching (Imai et al., 2006). Another key regulator implicated in the regulation of endocycling is the CCS52/Fizzy-Related (FZR) family protein, which functions as an activator of anaphase-promoting complex (Cebolla et al., 1999; Lammens et al., 2008; Vanstraelen et al., 2009). A recent study demonstrates that at least one of the Arabidopsis homologs, CCS52A1/FZR2, is involved in the promotion of trichome endocycling (Larson-Rabin et al., 2009).

In this study, we identified a novel trichome mutant in Arabidopsis, which unlike all other previously described trichome mutants, only affects trichome size without altering their overall patterning or branching. We demonstrate that the corresponding gene encodes a plant-specific trihelix transcription factor, GT-2-LIKE1 (GTL1), and that it functions as a negative regulator of ploidy-dependent cell growth during postbranching phases of trichome development.

RESULTS

Cauliflower Mosaic Virus 35S Promoter–Driven Expression of Full-Length or Truncated GTL1 cDNA Leads to an Increase in Trichome Cell Size

In a screen of the Arabidopsis FOX (full-length cDNA overexpression) collection (Ichikawa et al., 2006) for gain-of-function mutants that display aberrant sizes of trichome cells, we identified a transgenic line, F26519, that shows substantial increase in trichome cell size (Figure 1A). The observed phenotype segregated dominantly within the original T2 population (43 seedlings with large trichomes and 14 seedlings with wild-type trichomes, n = 57, χ2 = 0.003, P > 0.9), and PCR genotyping using primers specific for FOX vectors revealed cosegregation of the genotype and the phenotype, suggesting that the observed phenotype is caused by the overexpression of F26519 cDNA in the FOX vector. Furthermore, the cauliflower mosaic virus 35S promoter (CaMV35S)–driven expression of F26519 cDNA in wild-type background recapitulated the large trichome phenotype among all selected transformants (Figure 1A, R26519), confirming the identity of the causal gene. Sequencing analysis revealed that F26519 cDNA corresponds to a truncated version of the Arabidopsis GTL1 cDNA that lacks 750 bp in the central region and 4 bp in the C-terminal region. These deletions are likely to be an artifact caused during the construction of the full-length cDNA library (Seki et al., 2002), rather than the products derived from alternative splicing in vivo, since the borders of the deleted cDNA fragments do not match conventional euakaryotic splice sites. Consistently, our RT-PCR using RNA from 2-week-old wild-type seedlings amplified a 2010-bp cDNA that corresponds to the annotated full-length GTL1.

GTL1 belongs to the GT-2 gene family, a subfamily of the plant-specific trihelix transcription factor family (Smalle et al., 1998; Zhou, 1999). The Arabidopsis genome encodes 29 trihelix proteins, each of which contains at least one trihelix DNA binding domain, and depending on additional conserved motifs, they are classified into several subfamilies (Gao et al., 2009). In addition to GTL1, six other trihelix proteins, including previously described
GT-2, DF1, and PETAL LOSS (PTL), are designated within the GT-2 subfamily in Arabidopsis. Phylogenetic analysis of the five genes most closely related in the GT-2 family demonstrates that GT-2, GTL1, and DF1 form a small clade, while two other homologs encoded by At5g28300 and At5g47660 are more distantly related (Smalle et al., 1998; Nagano et al., 2001; Figure 2; see Supplemental Data Set 1 online). All five proteins, however, share common features with highly conserved N- and C-terminal trihelix DNA binding domains, which are unique to the GT-2 family, and another well-conserved domain of unknown function in the central region (Figures 1B and 2A). Further sequence analysis using a bioinformatic tool (http://www.at.embnet.org/toolbox/9aatad/) also identified, in the C-terminal region of the GTL1 protein, a potential 9–amino acid transactivation domain (DLVMRELIQ, Figure 1B), which gives a 100% match to 9–amino acid transactivation domains previously identified in eukaryotic and viral transcription factors (Piskacek et al., 2007). Alignment of the deduced amino acid sequences of wild-type GTL1 and GTL1^F26519 proteins revealed that the deletions in GTL1^F26519 proteins cause a truncation of 250 amino acids within the central region and a frame shift in the C-terminal region, resulting in a triple amino acid substitution from PED to KTL and a premature translational stop codon that removes the C-terminal 82 amino acids (Figure 1B). These large truncations are expected to remove the entire central domain and the putative transactivation domain of GTL1 proteins, while leaving their two trihelix domains intact.

Intriguingly, our RT-PCR analysis using RNA isolated from trichome-enriched plant materials (Jakoby et al., 2008) revealed that while the CaMV35S promoter–driven expression of the GTL1^F26519 cDNA causes ~50-fold overexpression of GTL1^F26519, it also leads to strong downregulation of endogenous GTL1 expression with only ~10% of wild-type GTL1 transcripts present in F26519 trichomes (Figure 1C). Furthermore, we found that the CaMV35S promoter–driven expression of full-length GTL1 cDNA also results in the reduction of total GTL1 levels in trichomes (Figure 1D) and increased trichome cell size.
Figure 2. GTL1 Belongs to the Arabidopsis GT-2 Family.

(A) Protein alignment of GTL1 and its four closest homologs in Arabidopsis. Orange boxes highlight the N- and C-terminal trihelix DNA binding domains, and a blue box indicates the conserved central domain. Note that the sequence of the N-terminal trihelix DNA binding domain is highly conserved among GTL1, GT-2, and DF1 but to a lesser extent to the products of the loci At5g28300 and At5g47660.

(B) The phylogenetic tree, generated using MEGA version 4 (Tamura et al., 2007), shows that GTL1, GT-2, and DF1 form a subclade among the GT-2 family proteins. Bootstrap values (1000 trials) above 90% are shown on branches. Bar represents 0.1 substitutions per site.

(C) Sequence identity of the N- and C-terminal trihelix DNA-binding motifs between AtGTL1, AtGT2 and AtDF1

|          | AtGTL1 / AtGT2 | AtGTL1 / AtDF1 | AtGT2 / AtDF1 |
|----------|----------------|----------------|---------------|
| N-terminal| 77%            | 71%            | 80%           |
| C-terminal| 73%            | 74%            | 84%           |
of 36 selected T1 transformants showed large trichome phenotypes. These results suggest that 35S-driven expression of full-length GTL1 or GTL1<sup>236519</sup> may cause knockdown phenotypes with respect to trichome development.

**GTL1 Represses Trichome Cell Growth**

To further investigate the function of GTL1 in plant development, we identified one Wisconsin DsLox transposon insertion allele, gtl1-1 (WiscsDslox413-416C9), and two SALK T-DNA insertion alleles, gtl1-2 (SALK_005965) and gtl1-3 (SALK_005966), for the GTL1 locus (see Supplemental Figure 1A online). Both gtl1-2 and gtl1-3 mutants contain a T-DNA insertion in the first exon, disrupting the N-terminal trihelix domain, while the gtl1-1 mutant contains a transposon insertion in the second exon, disrupting the conserved central domain. The progenies of heterozygous parental lines exhibited classic Mendelian segregations for the gtl1 phenotypes (11:35 for gtl1-1 x gtl1-2, χ² = 0.014, P > 0.9; 12:35 for gtl1-2 x gtl1-3, χ² = 0.004, P > 0.9; 11:37 for gtl1-3 x gtl1-1, χ² = 0.055, P > 0.8), indicating that all three alleles represent recessive loss-of-function mutations. RT-PCR analysis using primers spanning the GTL1 coding sequence further demonstrates that the full-length GTL1 transcripts are not detectable in all three homozygous mutants (see Supplemental Figure 1B online).

Our phenotypic observation reveals that homozygous mutations for all three alleles lead to an increase in trichome size indistinguishable from original F26519 plants (Figures 1A and 3A). Further quantitative analysis using trichomes isolated from the fourth and fifth leaves of 24-d-old plants indeed demonstrates that the trichome branch length is increased to very similar degrees in gtl1-1, gtl1-2, and F26519 (Figure 3B). Although all previously described large trichome mutants, including <i>try</i>, <i>kak</i>, <i>rfi</i>, and <i>pym</i>, also have over-branching phenotypes, gtl1 trichomes do not show significant alterations in their branching patterns (Figure 3A), and, as in the wild type, the majority of trichomes possess three branches (Figure 3C). Quantification of epidermal cell numbers and trichome numbers in individual leaves also demonstrates that the gtl1 mutations do not affect their relative frequencies (Table 1). These results suggest that GTL1 is involved in the regulation of trichome cell growth through pathways uncoupled from those mediating trichome initiation or branching. The third and fourth leaves of 21-d-old gtl1 plants, which are still expanding, appear to be slightly smaller than those of the wild type (Table 1), but after their full development, we do not find significant differences in their sizes (see Supplemental Figure 2A online).

**An Increase in Trichome Cell Size Is Accompanied by an Increase in Nuclear DNA Content in gtl1 Mutants**

Many recent studies have shown that trichome cell size is often positively linked with their ploidy level (Hulkamp et al., 1994; Perazza et al., 1999; Sugimoto-Shirasu et al., 2005; Breuer et al., 2007). To test whether an increase in trichome cell size in gtl1 is accompanied by an increase in trichome ploidy, we visualized the nuclear DNA of fully mature trichomes isolated from the fourth and fifth leaves of 24-d-old plants using 4',6-diamidino-2-phenylindole (DAPI) staining. As shown in Figure 3D, our fluorescence microscopy shows that the DAPI-stained nuclear size in these trichomes is significantly increased in gtl1-1, gtl1-2, and F26519 trichome cells compared with wild-type trichomes at the equivalent developmental stage. Quantitative analysis of the nuclear DNA content indeed demonstrates that endoreduplication proceeds beyond wild-type levels in gtl1-1, gtl1-2, and F26519 trichome cells (Figure 3E). Our flow cytometry analysis using the whole third and fourth leaves of 26-d-old wild-type, gtl1-1, and gtl1-2 plants did not reveal major differences in the overall ploidy distributions (see Supplemental Figure 2B online), further supporting that GTL1 mainly functions in trichome development in leaves.

**GTL1 Is Required for the Repression of Endocycles after Trichomes Complete Branching**

Our data show a clear uncoupling of cell growth and endocycling from branching in gtl1, and one possible explanation for this phenotype is that GTL1 is required for the repression of cell growth and/or endocycling only after trichomes complete branching. To test this hypothesis, we measured the DNA content of DAPI-stained nuclei in young trichomes that have completed branching but not their subsequent cell growth (Figure 4A). If GTL1 functions only after the completion of trichome branching, gtl1 trichomes should not undergo an additional round of endocycling before they form three branches. Consistent with this idea, the gtl1 trichomes have comparable amounts of nuclear DNA relative to wild-type trichomes at the equivalent developmental stage (Figure 4B). These observations suggest that GTL1 is required for endocycle repression after trichomes complete their branching.

**GTL1 Is a Nuclear Protein Expressed during the Postbranching Stages of Trichome Development**

To investigate whether the expression pattern of the GTL1 gene supports its predicted function in postbranching stages of trichome development, we first generated transgenic lines carrying <i>GTL1pro:β-glucuronidase (GUS)</i> reporter constructs. All examined transgenic lines, derived from at least 10 independent T1 transformants, show strong GUS expression in developing trichomes (Figure 5A). Interestingly, we do not detect any GUS signal in small trichomes that have just initiated or in those that are still undergoing the branching process at the base of developing young leaves. The GUS signal becomes visible, however, in large trichomes with three branches and appears to peak as trichomes reach their final size. To test whether GTL1 proteins also show developmental-specific expression patterns, we expressed GTL1:green fluorescent protein (GFP) or GFP:GTL1 fusion proteins under the same GTL1 promoter. We recovered multiple independent transgenic lines that show full complementation of the gtl1 mutant phenotypes by expression of either GTL1:GFP or GFP:GTL1 proteins, indicating that both N and C terminus fusion proteins are functional in planta. Our fluorescence microscopy further demonstrates that, as with its promoter activity, GFP:GTL1 proteins are expressed only after trichomes complete their second branching event (Figure 5B), supporting our hypothesis that GTL1 acts in postbranching.
processes of trichome development. Once trichomes complete their full growth, GTL1 expression declines and eventually ceases since we do not detect any GFP signals in older trichomes (Figure 5C). As expected for a putative transcription factor, we found that GFP:GTL1 proteins are localized exclusively within the nucleus (Figure 5D).

We also explored whether GFP:GTL1 proteins are expressed in any other cell types in leaves, but we did not detect any GFP signals in cells other than trichomes under our standard growth conditions. By contrast, we did detect some weak nuclear accumulation of GFP:GTL1 in developing roots and petals (see Supplemental Figure 3 online). Interestingly, GFP:GTL1 proteins do not accumulate in proliferating cells at the root meristem, and its expression is induced as cells start expanding (see Supplemental Figures 3A and 3B online). We found that GFP:GTL1 proteins are also expressed in growing root hairs (see...
Table 1. Comparison of Adaxial Epidermal Cell Number and Trichome Number in the Wild Type (Col), gtl1-1, and gtl1-2

|                      | Col       | gtl1-1   | gtl1-2   |
|----------------------|-----------|----------|----------|
| Average size of third and fourth true leaves (mm²) | 28.97 ± 2.73 | 24.28 ± 2.43 | 23.77 ± 3.10 |
| Size of adaxial epidermal cells (μm²) | 3869 ± 1294 | 3295 ± 1378 | 3188 ± 1618 |
| Estimated total number of adaxial cells | 7487 | 7369 | 7456 |
| Number of trichomes/leaf | 34.75 ± 5.00 | 33.13 ± 3.19 | 34.05 ± 4.30 |
| Ratio adaxial cells/trichome | 215.5 | 222.4 | 219.0 |

All measurements were carried out on the third and fourth leaves of 3-week-old seedlings. Indicated values are mean ± SD

a = 12 for the wild-type, gtl1-1, and gtl1-2.

b Cell areas of 200 epidermal cells, excluding stomatal guard cells, from at least eight leaves were measured for each genotype.

c To estimate the total number of adaxial cells, the average size of the third and fourth true leaves was divided by the average size of adaxial epidermal cells.

Supplemental Figures 3C and 3D online). Within young petals, GFP:GTL1 proteins are present in a gradient fashion, with its highest expression level being detected at the tip of petals (see Supplemental Figures 3E and 3F online). In fully expanded petals, GFP:GTL1 proteins can be found in all epidermal cells (see Supplemental Figures 3E and 3F online).

The Expression and Function of GTL1 Require the Progression of Trichome Differentiation, Which Is Largely Dependent on TTG1 and GL2

Trichome cell fate is specified at an early stage by genetic pathways that involve several transcription factors, such as GL1-3, TTG1, and TRY (Oppenheimer et al., 1991; Galway et al., 1994; Larkin et al., 1994; Rerie et al., 1994; Walker et al., 1999). The expression of GTL1 during much later stages of trichome development suggests that GTL1 may act downstream of these early regulatory pathways. To test this hypothesis, we first generated double mutants between gtl1-1 and ttg1-10 or gl2-130213. Mutations in the TTG1 gene result in a dramatic decrease in the number of trichome initiation sites, and only a few trichomes develop at the leaf margins (Larkin et al., 1994). Phenotypic analysis of gtl1-1 ttg1-10 double mutants demonstrates that the ttg1-10 mutation is epistatic to the gtl1-1 mutation, with their double mutants showing ttg1-10–like patterning defects (Figure 6A). Mutations in gl2 do not affect specification of trichome initiation sites but severely impair perpendicular epidermal outgrowth and endoreduplication (Rerie et al., 1994). The gl2-130213 mutants, like ttg1-10, are epistatic to the gtl1-1 mutation since neither cell size nor ploidy of gtl1-1 gl2-130213 trichomes is increased compared with those of gl2-130213 trichomes (Figures 6A to 6D).

To further explore the functional relationship between GTL1 and TTG1 or GL2, we introduced the GTL1pro:GUS reporter construct into the ttg1-10 or gl2-130213 mutant backgrounds and asked whether these mutations modify the promoter activity of the GTL1 gene. As shown in Figure 6E, GTL1 promoter activity is completely abolished by the ttg1-10 or gl2-130213 mutations in almost all leaf epidermal cells of 16-d-old plants, although some GUS signals can be detected in ttg1-10 trichomes that occasionally form at the leaf margins. The leaves of 24-d-old gl2-130213 plants sometimes develop one or two unbranched trichomes, and we detected GUS signals in a fraction of these unbranched trichomes (Figure 6F).

While our data are largely consistent with our hypothesis that GTL1 acts downstream of TTG1 and GL2, the GUS activity detected in the ttg1-10 and gl2-130213 trichomes suggests that the expression of GTL1 is not absolutely dependent on TTG1 or GL2 function. An alternative scenario that can explain these observations is that GTL1 expression requires a certain stage of trichome development, which usually depends on TTG1 or GL2. As the ttg1-10 and gl2-130213 mutant phenotypes show (Figure 6A), both of these mutations are leaky, and trichomes that manage to initiate in the absence of TTG1 or GL2 appear to proceed to the developmental stage where GTL1 expression is induced. We found that the trichomes in gtl1-1 ttg1-10 double mutants are slightly larger and have higher ploidy than those in ttg1-10 (Figure 6A; see Supplemental Figure 4 online), indicating that the GTL1 proteins expressed in ttg1-10 trichomes do function to repress cell growth and endocycles. These results support the idea that the progression of trichome development to a certain stage is the prerequisite for the expression and function of GTL1.

The gtl1 gl3 and gtl1 try Mutants Display Additive Trichome Cell Size and Ploidy Phenotypes

The two patterning transcription factors, GL3 and TRY, also act as positive and negative regulators of ploidy-dependent trichome cell growth and branching, respectively. (Hulskamp et al., 1994; Folkers et al., 1997; Schnittger et al., 1998; Peraza et al., 1999). Accordingly, leaf trichomes in gl3 mutants are reduced in ploidy, are smaller in size, and are under-branched, while try mutations cause larger, over-branched trichomes with increased ploidy. To test the genetic relationship between GTL1, GL3, and TRY, we generated the corresponding double mutant combinations. As shown in Figure 7, mature trichomes of gtl1-1 gl3-7454 double mutants display intermediate phenotypes between their parental lines with respect to trichome cell size and ploidy (Figures 7A and 7C) but gl3-like phenotypes with respect to trichome branching (Figures 7A and 7D), further supporting an involvement of GTL1 in the regulation of cell growth and ploidy. Similarly, the gtl1-1 try-29760 double mutants show additive phenotypes for trichome cell size and ploidy (Figures 7A to 7C) while their over-branched phenotype resembles that of single try-29760 mutants (Figures 7A and 7D). The expression of TRY is invoked from a very early stage of trichome development (Schellmann et al., 2002). Consistent with this, young
three-branched trichomes in try-29760 possess significantly larger nuclei than those in wild-type or gt1-1 plants (Figure 4B), indicating that try-29760 trichomes already undergo one additional round of endocycling before they complete branching. The average nuclear DNA content of gt1-1 try-29760 trichomes at these early developmental stages resembles that of try-29760 (Figure 4B), further supporting the requirement of GTL1 in later stages of trichome development.

The gl3 and try mutations also disrupt trichome initiation and/or patterning, leading to alterations in trichome number and/or spacing (Schnittger et al., 1999; Payne et al., 2000). The gt1-1 mutation does not appear to enhance or rescue either of these phenotypes in gl3-7454 or try-29760 plants (Figure 7E), further demonstrating that GTL1 does not play profound roles in trichome initiation or patterning.

The gt1 Mutation Modifies the Expression of Cell Cycle Genes and Partially Rescues the Ploidy Defects in sim1 Mutants

Modified activities of core cell cycle regulators often have strong downstream consequences on trichome development (Ishida et al., 2008). It is thus plausible that the primary function of GTL1 is to modify the expression of cell cycle genes, preventing the progression of endocycles and trichome cell growth beyond wild-type levels. To explore this possibility, we first tested whether the phenotypes observed in gt1 trichomes associate with the misexpression of genes involved in cell cycle regulation. As shown in Figure 8, our quantitative RT-PCR analysis using RNA isolated from young trichomes of 28-d-old plants reveals that the expression of several cell cycle genes, many of which are implicated in trichome development, is modified moderately but significantly in gt1-1 trichomes. The strongest upregulation, of up to fourfold, is found for the expression of CCS52A2/FZR1, a positive regulator of endocycle progression in plants (Lammens et al., 2008; Vanstraelen et al., 2009), while the expression of its close homolog, CCS52A1/FZR2, which promotes endocycles in trichomes (Larson-Rabin et al., 2009), is only mildly increased (Figure 8). A plant-specific small CDK inhibitor protein, SIM, is required for the repression of endocycles in trichomes, and its
loss-of-function mutation leads to the development of multicellular trichomes with reduced ploidy (Walker et al., 2000; Churchman et al., 2006). The level of SIM transcripts is not significantly changed, while the expression of its interacting proteins, CDKA;1 and CYCD4;1, is reduced by 25 to 35% compared with the wild type (Figure 8). Downregulation of CYCA2;3 is previously shown to cause extended endocycling (Imai et al., 2006), and, consistent with this, the CYCA2;3 transcript level is reduced by 25% in gtl1-1 trichomes (Figure 8). Cell cycle proteins required for the licensing of DNA replication, including CDT1a and CDC6a, are expressed in developing trichomes, and overexpression of these proteins promotes an additional round of endocycling in trichomes (Castellano et al., 2001). We found that the transcript levels of CDT1a, its close homolog CDT1b, and CDC6a are moderately increased in gtl1-1 (Figure 8).

To further investigate the possibility that GTL1 functions in endocycle control, we introduced the gtl1-1 mutation into the sim-1 mutant background and tested whether the function of GTL1 interferes with that of SIM. Although many trichomes in sim-1 are multicellular (Walker et al., 2000), their overall size is comparable with that of wild-type trichomes (Figure 9A). By contrast, the majority of trichomes in the gtl1-1 sim-1 double mutants appear to be slightly larger than those in sim-1 (Figure 9A), although we cannot accurately measure the trichome size of gtl1-1 sim-1 plants due to the irregular branching patterns of trichome in these plants (Churchman et al., 2006). As previously
Figure 7. The gtl1-1 g3-7454 and gtl1-1 try-29760 Mutants Display Additive Trichome Cell Size and Ploidy Phenotypes.

(A) Bright-field microscopy of the third true leaves of 15-d-old wild-type (Col), g3-7454 (Col), try-29760 (Col), gtl1-1, gtl1-1 g3-7454, and gtl1-1 try-29760 mutants. Bar = 2 mm.

(B) Quantitative analysis of the nuclear DNA content in fully mature wild-type, g3-7454, try-29760, gtl1-1, gtl1-1 g3-7454, and gtl1-1 try-29760 trichomes isolated from fourth and fifth leaves of 24-d-old plants. Vertical and horizontal bars represent mean values and SD (n = 120 from at least 10 different plants), respectively. Nuclear DNA content is normalized relative to DNA content in guard cell nuclei.

(C) Total branch length of wild-type (Col), g3-7454 (Col), try-29760 (Col), gtl1-1, gtl1-1 g3-7454, and gtl1-1 try-29760 trichomes. The length of individual trichome branches was measured using trichomes isolated from fourth and fifth leaves of 24-d-old plants, and their sum was calculated for each trichome (n = 60 trichomes from at least five different plants per genotype).

(D) Distribution of trichome branch numbers in wild-type (Col), g3-7454 (Col), try-29760 (Col), gtl1-1, gtl1-1 g3-7454, and gtl1-1 try-29760 trichomes. Branch numbers were counted for all trichomes on the first pair of leaves from 24-d-old plants (n = 450 trichomes from at least 15 different plants per genotype).

(E) Average number of trichomes on the third and fourth leaves of 24-d-old seedlings (n = 30 from at least 15 different plants per genotype).
reported, the DNA content of individual nuclei in sim-1 trichomes is strongly reduced compared with that in wild-type trichomes (Walker et al., 2000; Figure 9B). We found that the average nuclear DNA content in gtl1-1 sim-1 trichomes is significantly greater than in wild-type trichomes (Figure 9B), demonstrating that the gtl1-1 mutation can partially bypass the endocycle defects caused by the sim-1 mutation. These results strongly support our hypothesis that GTL1 participates in the regulation of successive endocycling in trichome development.

DISCUSSION

GTL1 Is a Repressor of Cell Growth and Endocycling in Trichome Development

We have demonstrated that a novel Arabidopsis trihelix protein, GTL1, participates in the repression of ploidy-dependent cellular growth in trichome development. Recent molecular and genetic studies have uncovered the transcriptional networks involved in trichome patterning and differentiation, but the mechanism underlying subsequent growth and development is still poorly understood. Two trichome patterning transcription factors, TRY and GL3, are also implicated in the control of cell growth and branching (Hulskamp et al., 1994; Folkers et al., 1997; Schnittger et al., 1998; Perazza et al., 1999), indicating that some of the early acting transcriptional regulators also participate in trichome development beyond the differentiation stage. Our data show that the expression of GTL1 largely depends on the early patterning/differentiation genes, TTG1 and GL2, strongly suggesting that GTL1 acts as part of the regulatory mechanisms linking

Figure 8. The Expression of Cell Cycle Genes Is Modified in gtl1-1 Trichome Cells.

The quantitative PCR analysis of CCS52A1/FZR2, CCS52A2/FZR1, SIM, CDKA1, CYCA2-3, CDT1a, CDT1b, and CDC6a expression in wild-type and gtl1-1 trichomes. The CCS52A1/FZR2, CCS52A2/FZR1, and SIM genes, labeled in green, and CDKA1, CYCD4-1, and CYCA2-3, labeled in red, are implicated in the promotion and repression, respectively, of endocycles in plants. Those labeled in blue (CDT1a, CDT1b, and CDC6a) participate in the licensing of DNA replication. RNA was isolated from trichomes of young leaves of 28-d-old seedlings. Mean expression levels of four technical repeats in gtl1-1, relative to those of the wild type (Col), are shown with s.d.

Figure 9. The gtl1 Mutation Partially Rescues the Ploidy Defects in sim-1 Trichomes.

(A) Bright-field microscopy of the sixth true leaves of 28-d-old wild-type (Col), gtl1-1, sim-1 (Col), and gtl1-1 sim-1 mutants. Bar = 2 mm.
(B) Quantitative analysis of the nuclear DNA content in wild-type, gtl1-1, sim-1, and gtl1-1 sim-1 trichomes isolated from the fourth and fifth leaves of 28-d-old plants. The values on the y axis represent total number of nuclei counted for each genotype. Nuclear DNA content is normalized relative to that in guard cell nuclei.
early trichome differentiation to final growth and development. Given that we occasionally detect some GTL1 expression in ttg1 or gl2 trichomes, TTG1 or GL2 itself is not likely to be the absolute requirement for GTL1 expression. Our data, instead, suggest it is the progression of trichome development to a certain stage that induces the expression of GTL1. We do not yet know what specifies the developmental stage to allow GTL1 expression, but at least our data suggest that the level of trichome branching is not the prerequisite for this, since unbranched or under-branched trichomes can express GTL1 in ttg1 or gl2.

A key difference between GTL1 and previously identified trichome growth regulators, such as KAK, RFI, PYM, and SPY, is that GTL1 represses trichome cell growth without altering its branching pattern. The tight coupling between cell size and branching in many trichome mutants previously led to the idea that the pathways regulating these two cellular features are closely linked. Our new data, however, clearly demonstrate that at least part of these regulatory pathways can be uncoupled. One possible mechanism that can uncouple trichome cell growth from branching is that trichome cells continue to have growth potential when they reach their normal maximum size while their branching potential ceases much earlier during their development (Figure 10). Unlike TRY and GL3, which are both expressed throughout trichome development, GTL1 is expressed only after trichome cells complete branching, allowing its specific repression of trichome cell growth at the late stage of trichome development. In agreement with this idea, young three-branched trichomes in gtl1 have not undergone additional rounds of endocycling as opposed to those in try, which already possess significantly greater nuclear DNA content than the wild type. Thus, TRY and GTL1 appear to exert their function at different stages of trichome development, with TRY acting early, while cells are still undergoing branching and GTL1 only after cells complete branching. It is worth noting that 35S promoter–driven overexpression of GL1 is previously reported to increase trichome ploidy levels without modifying its branching (Larkin et al., 1994; Schnittger et al., 1998). However, this is only in the context of overexpression studies, and in combination with other mutations, GL1 overexpression does lead to altered trichome branching. We therefore believe that the mechanism underlying these events is probably distinct from that of GTL1.

How 35S:GTL1 or 35S:GTL1F26519 expression leads to the trichome phenotype resembling the loss-of-function mutants is not clear, but our data show that 35S-driven expression of either GTL1 or GTL1F26519 leads to the downregulation of full-length GTL1 expression (Figure 1). It is plausible that the ectopic expression of a growth repressor in cell types essential for the plant survival (e.g., meristem cells) leads to lethality, preventing the recovery of transgenic plants with high GTL1 expression. It is intriguing that 35S:GTL1F26519 expression, by contrast, allows ~50-fold accumulation of GTL1F26519 cDNA in F26519 trichomes. Overexpression of these heavily truncated variants

**Figure 10.** A Schematic Model Illustrating How GTL1 May Function in Trichome Development.

Two trichome patterning genes, GL3 and TRY, expressed throughout trichome development, function as a positive and negative regulator, respectively, of endoreduplication, cell growth, and branching. How they participate in these postinitiation processes is not well defined, but previous genetic studies suggest that TRY acts as a negative regulator of GL3 function in the regulation of cell growth. By contrast, GTL1, expressed only after trichomes complete branching, specifically represses endoreduplication and cell growth toward the end of trichome development. The uncoupling of cell growth and branching in gtl1 implies that the growth potential of trichome cells is maintained until much later stages than their branching potential, allowing the specific repression of cell growth at the time of GTL1 expression.
may cause dominant-negative or potentially gain-of-function phenotypes, and these possibilities should be investigated in future studies.

Transcriptional Regulation of Growth Repression and Its Link to Endocycling

The loss of GTL1 function permits trichome cell growth beyond wild-type levels, and this phenotype can be caused by either faster rates of cell growth and/or extended periods of cell growth compared with the wild type. Although technical limitations do not allow clear judgments of these two possibilities, the temporally restricted GTL1 expression after the completion of trichome branching and the gtl1 growth phenotypes observed at the corresponding developmental stage support the latter. These data suggest that normal trichome development in Arabidopsis requires previously uncharacterized regulatory steps to actively terminate their cell growth. We further show that the enhanced cell growth in gtl1 is coupled with additional cycles of endoreduplication and that the expression of several cell cycle genes implicated in endocycle control is modified in gtl1. We cannot yet distinguish whether these altered patterns of gene expression are the cause or downstream consequence of the enhanced endocycles, but these observations raise the possibility that GTL1 participates in the regulation of cell cycle gene expression to terminate endocycles. An involvement of GTL1 in cell cycle regulation is further substantiated by our observation that the gtl1 mutation can partially rescue the ploidy defects caused by the sim1 mutation. Our data of gtl1 sim1 double mutants, as well as of gtl1 ttg1 or gtl1 gi3 double mutants, show that GTL1 can repress early cycles (i.e., 4C-to-32C) of endoreduplication, suggesting that GTL1 can act as a general repressor of endocycles upon its expression. Identification of direct targets of GTL1 and their functional analyses should help us to gain further insights into how trichome cell growth is regulated and how endoreduplication contributes to this process.

An important next question is whether other plant cell types also require similar transcriptional regulation to repress their growth. Because many plant cell types have unique, and often very constant, final cell sizes, the extent of their growth and/or the timing of growth cessation are likely to be tightly controlled. Although we know very little about these controls, one recent study reports that a basic helix-loop-helix transcription factor, BIGPETALp, is required to limit petal cell growth in Arabidopsis (Szecsi et al., 2006), implying a more ubiquitous involvement of transcriptional regulators in the repression of plant cell growth. Our data show that GTL1 proteins are also expressed in growing petals and roots. Under our standard growth conditions, we do not see any obvious developmental defects in these organs, but, as discussed below, this could be due to the potential functional redundancies among GT-2 family genes.

Role of GT-2 Family Trihelix Proteins in Plant Development

Because trihelix transcription factors, also referred to as GT proteins because of their binding to GT motifs, are found only in plants, they are thought to have functional roles in plant-specific processes. The Arabidopsis genome contains at least seven GT-2 family proteins, which are distinct from other GT proteins because of their duplicated trihelix domains. Earlier studies have demonstrated that GT-2 proteins from rice (Oryza sativa) and its closely related homolog, DF1, from pea (Pisum sativum) bind to the promoter region of light-regulated genes, implying their involvement in the light response (Dehesh et al., 1990; Nagano et al., 2001). GTL1 is closely related to both GT-2 and DF1, sharing 70 to ~80% identity in amino acid sequence in both N- and C-terminal trihelix domains (Figure 2C), suggesting that they may have some overlapping functions. The loss-of-function mutants of GT-2 and DF1 do not display obvious morphological defects under our standard growth conditions (our unpublished data), and it will be of interest in future studies to explore the genetic and functional relationships between GT-2 and DF1. Interestingly, it has been previously shown that another GT-2 homolog in Arabidopsis, PTL, which has less homology to GT-2 compared with DF1 or GTL1, sharing 40 to ~60% identity in amino acid sequence in the trihelix domains, is involved in the repression of petal growth in developing flowers (Brewer et al., 2004). Furthermore, 35S promoter–driven expression of PTL causes strong inhibition of seedling growth, indicating that PTL can act as a general growth repressor. How PTL represses growth at the cellular level, for instance whether it retards cell proliferation or cell growth, is not currently known, but these observations raise an interesting possibility that one of the primary functions of GT-2 family trihelix proteins might be to repress plant growth in a tissue- or cell-specific manner.

METHODS

Plant Material and Growth Conditions

The original FOX mutant collection, and the gi3-7454 and try-29760 mutants (Col background), were described previously (Ichikawa et al., 2006; Ishida et al., 2007; Tominaga et al., 2008). The three gtl1 mutant alleles and gi3-130213 (SALK_130213) were obtained from the ABRC Stock Center (Ohio State University), and ttg1-10 (Col background; Larkin et al., 1994) and sim1-1 (Col background; Walker et al., 2000) were provided by John Larkin (Louisiana State University). Plants were either germinated and grown on plates containing Murashige and Skoog salts, pH 5.7, 1% sucrose (w/v), and 0.5% phytagel (w/v) or on a 1:1 mixture of soil Supermix A (SAKATA) and vermiculite (VS Kako) in continuous light at 22°C.

Amino Acid Sequence Alignment and Phylogenetic Analysis

Amino acid sequences of the full-length proteins were aligned using the online tool MULTALIN with default settings of the following parameters: gap penalty value, −1; gap length value, −1; extremity gap penalty, no penalty; scoring method, absolute (Corpet, 1988). The phylogenetic tree was constructed using the neighbor-joining algorithm MEGA version 4 (Tamura et al., 2007) with 1000 bootstrap trials performed.

Genotype Determination of Mutants

The original Arabidopsis thaliana FOX line (F26519) was genotyped with a pair of oligonucleotides, GS17 5′-GTACGTATTTTTACACAAATCCAAAC-3′ and GS18 5′-GATCTCACTTTAAGAACATTTATGCACCA-3′, designed for the FOX vector. The T-DNA/transposon mutant lines for GTL1 were genotyped for the relevant inserts using the following oligonucleotide pairs: WF627-F1 5′-TTCTCGTCATGACCTCATG-3′
Total RNA was extracted from aerial parts of young seedlings or leaf trichomes using the Qiagen RNeasy plant mini kit. For extraction of total trichome RNA, trichomes of the fifth to seventh true leaf were collected from 3-week-old plants as previously described (Jakoby et al., 2008). Extracted RNA was treated with DNasel (Takara Bio) following the manufacturer’s instructions, and subsequent reverse transcription was performed using the Invitrogen Superscript III kit. Transcript levels were determined by quantitative real-time PCR using the SYBR Green Supermix kit (Toyobo). The following oligonucleotides were used for RT-PCR analysis of the full-length GTL1 and the reference gene ACT2 (adenylyl-phosphoribosyl-transferase 1) expression in wild-type and gtl1 mutants: GTL1F 5'-ATGAGGACAAAGAGGAGG-3’ and GTL1R 5’-TTTACTGACCATGGTCAAGAAGG-3’; and ACT2F 5’-GGAATAAGGCGACTGAGATGTTG3’ and ACT2R 5’-TTCCGAGCTCTCCAGTAC-3’. Quantitative PCR analysis of the cell cycle genes was performed using primer sets listed in Supplemental Table 1 online.

**RNA Extraction and Gene Expression Analyses**

Total RNA was extracted from aerial parts of young seedlings or leaf trichomes using the Qiagen RNeasy plant mini kit. For extraction of total trichome RNA, trichomes of the fifth to seventh true leaf were collected from 3-week-old plants as previously described (Jakoby et al., 2008). Extracted RNA was treated with DNasel (Takara Bio) following the manufacturer’s instructions, and subsequent reverse transcription was performed using the Invitrogen Superscript III kit. Transcript levels were determined by quantitative real-time PCR using the SYBR Green Supermix kit (Toyobo). The following oligonucleotides were used for RT-PCR analysis of the full-length GTL1 and the reference gene ACT2 (adenylyl-phosphoribosyl-transferase 1) expression in wild-type and gtl1 mutants: GTL1F 5'-ATGAGGACAAAGAGGAGG-3’ and GTL1R 5’-TTTACTGACCATGGTCAAGAAGG-3’; and ACT2F 5’-GGAATAAGGCGACTGAGATGTTG3’ and ACT2R 5’-TTCCGAGCTCTCCAGTAC-3’. Quantitative PCR analysis of the cell cycle genes was performed using primer sets listed in Supplemental Table 1 online.

**GUS Staining and Microscopy**

To investigate the expression of GTL1::GUS reporter constructs, whole seedlings were fixed in acetic acid for 30 min at –30°C and then incubated in GUS staining solution for 5 to 12 h at 37°C as previously described (Malamy and Benfey, 1997). Stained plant tissue was washed in 70% ethanol for 4 h and cleared in chloral hydrate:H2O:glycerol (8:2:1) overnight at 4°C. GUS signal was visualized using a Leica MZ 16 FA microscope equipped with a digital Leica DFC 500 camera.

**Fluorescence Microscopy and Ploidy Measurements**

The in vivo expression pattern and subcellular localization of the GTL1::GFP::GT2 plants were documented using an Olympus BX51 fluorescence microscope equipped with a digital Olympus DP70 camera and Olympus DP Manager software (version 1.2.1.107). The same equipment was used to record DAPI-stained nuclei in leaf trichomes (Zhang and Oppenheimer, 2004). Fluorescent signals were measured with ImageJ (version 1.37; NIH) as previously described (Schnittger et al., 1998; Sugimoto-Shirasu et al., 2005). Flow cytometry analysis was performed using the ploidy analyzer PA-I (Partec) as described previously (Sugimoto-Shirasu et al., 2002). At least 10,000 nuclei isolated from third and fourth leaves of 24-d-old seedlings were used for each ploidy measurement.

**Accession Numbers**

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: GTL1, At1g33240; GT2, At1g76890; DF1, At1g76880; At1g28300; and At1g47660.

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure 1.** Structure of the GTL1 Gene Indicating the Position of T-DNA/Transposon Insertions.

**Supplemental Figure 2.** The *gtl1-1* Leaves Do Not Display Major Alterations in the Ploidy Distribution.

**Supplemental Figure 3.** GTL1 Is Expressed in Expanding Roots and Leaves.

**Supplemental Figure 4.** Trichomes That Develop in *gtl1-1 ttg1-10* Leaves Are Slightly Larger and Possess Higher Ploidy Than Those in *ttg1-10* Leaves.

**Supplemental Data Set 1.** Protein Sequences Used to Generate the Phylogeny in Figure 2B.

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REFERENCES

Besser, K., Harper, A., Welsby, N., Schauvinhold, I., Slocombe, S., Li, Y., Dixon, R.A., and Broun, P. (2009). Divergent regulation of terpenoid metabolism in the trichomes of wild and cultivated tomato species. Plant Physiol. 149: 499–514.

Breuer, C., Stacey, N.J., West, C.E., Zhao, Y., Chory, J., Tsukaya, H., Azumi, Y., Maxwell, A., Roberts, K., and Sugimoto-Shirasu, K. (2007). BNA4, a novel component of the plant DNA topoisomerase VI complex, is required for endoreduplication in Arabidopsis. Plant Cell 19: 3655–3668.

Breuer, P.B., Howles, P.A., Dorian, K., Griffith, M.E., Ishida, T., Azumi, Y., Maxwell, A., Roberts, K., and Sugimoto-Shirasu, K. (2007). BIN4, a novel component of the plant DNA topoisomerase VI complex, is required for endoreduplication in Arabidopsis. Plant Cell 19: 3655–3668.

Castellano, M.M., del Pozo, J.C., Ramirez-Parra, E., Brown, S., and Gutierrez, C. (2001). Expression and stability of Arabidopsis CDC6 are associated with endoreplication. Plant Cell 13: 2671–2686.

Cebolla, A., Vinardell, J.M., Kiss, E., Olah, B., Roudier, F., Kondorosi, A., and Kondorosi, E. (1999). The mitotic inhibitor ccss2 is required for endoreduplication and ploidy-dependent cell enlargement in plants. EMBO J. 18: 4476–4484.

Churchman, M.L., et al. (2006). SIAMESE, a plant-specific cell cycle regulator, controls endoreplication onset in Arabidopsis thaliana. Plant Cell 18: 3145–3157.

Clough, S.J., and Bent, A.F. (1998). Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16: 735–743.

Corpet, F. (1989). Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. 16: 1081–1090.

Dehesh, K., Bruce, W.B., and Quail, P.H. (1990). A trans-acting factor that binds to a GT-motif in a phytrome gene promoter. Science 250: 1397–1399.

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.

Folkers, U., Berger, J., and Hulskamp, M. (1997). Cell morphogenesis of trichomes in Arabidopsis: Differential control of primary and secondary branching by branch initiation regulators and cell growth. Development 124: 3779–3786.

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. 168: 740–754.

Gao, M.-J., Lydiate, D., Li, X., Li, H., Gjetvaj, B., Hegedus, D.D., and Rozwadowski, K. (2009). Repression of seed maturation genes by a trihelix transcriptional repressor in Arabidopsis seedlings. Plant Cell 21: 54–71.

Hulskamp, M., Misra, S., and Jurgens, G. (1994). Genetic dissection of trichome cell development in Arabidopsis. Cell 76: 555–566.

Hulskamp, M., Schnittger, A., and Folkers, U. (1999). Pattern formation and cell differentiation: trichomes in Arabidopsis as a genetic model system. Int. Rev. Cytol. 186: 147–178.

Ichikawa, T., et al. (2006). The FOX hunting system: An alternative gain-of-function gene hunting technique. Plant J. 48: 974–985.

Igenfrutz, H., Bouyer, D., Schnittger, A., Mathur, J., Kirik, V., Schwab, B., Chua, N.H., Jurgens, G., and Hulskamp, M. (2003). The Arabidopsis STICHEL gene is a regulator of trichome branch number and encodes a novel protein. Plant Physiol. 131: 643–655.

Imai, K.K., Ohashi, Y., Tsuge, T., Yoshizumi, T., Matsu, M., Oka, A., and Aoyama, T. (2006). A-type cyclin CYCA2;3 is a key regulator of ploidy levels in Arabidopsis endoreduplication. Plant Cell 18: 382–396.

Ishida, T., Hattori, S., Sano, R., Inoue, K., Shirano, Y., Hayashi, H., Shibata, D., Sato, S., Kato, T., Tabata, S., Okada, K., and Wada, T. (2007). Arabidopsis TRANSPARENT TESTA GLABRA2 is directly regulated by R2R3 MYB transcription factors and is involved in regulation of GLABRA2 transcription in epidermal differentiation. Plant Cell 19: 2531–2543.

Ishida, T., Kurata, T., Okada, K., and Wada, T. (2008). A genetic regulatory network in the development of trichomes and root hairs. Annu. Rev. Plant Biol. 59: 365–386.

Jakoby, M.J., Falkenhan, D., Mader, M.T., Brininstool, G., Wischnitzki, E., Platz, N., Hudson, A., Hulskamp, M., Larkin, J., and Schnittger, A. (2008). Transcriptional profiling of mature Arabidopsis trichomes reveals that NOECK encodes the MIXTA-like transcriptional regulator MYB106. Plant Physiol. 148: 1583–1602.

Johnson, C.S., Kolevski, B., and Smyth, D.R. (2002). TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. Plant Cell 14: 1359–1375.

Johnson, H.B. (1975). Plant pubescence - Ecological perspective. Bot. Rev. 41: 233–258.

Kirk, V., Simon, M., Huelskamp, 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.

Kirk, V., Simon, M., Wester, K., Schiefelbein, J., and Hulskamp, M. (2004b). ENHANCER OF TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichome development of Arabidopsis. Plant Mol. Biol. 56: 389–398.

Lammens, T., Boudolf, V., Kheibarshekan, L., Zalmas, L.P., Gaamouche, T., Maes, S., Vanstraalen, M., Kondorosi, E., La Thangue, N.B., Govaerts, W., Inze, D., and De Veylder, L. (2008). Atypical E2F activity restrains APC/CCS52A2 function obligatory for endocycle onset. Proc. Natl. Acad. Sci. USA 105: 14721–14726.

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., Young, N., Prigge, M., and Marks, M.D. (1996). The control of trichome spacing and number in Arabidopsis. Development 122: 997–1005.

Larson-Rabin, Z., Li, Z., Masson, P.H., and Day, C.D. (2009). FZR2/CCS52A1 expression is a determinant of endoreduplication and cell expansion in Arabidopsis. Plant Physiol. 149: 874–884.

Malamy, J., and Benfey, P. (1997). Organization and cell differentiation in lateral roots of Arabidopsis thaliana. Development 124: 33–44.

Marks, M.D. (1997). Molecular genetic analysis of trichome development in Arabidopsis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48: 137–163.

Mauricio, R., and Rausher, M.D. (1997). Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. Evolution 51: 1435–1444.
Melaragno, J.E., Mehrotra, B., and Coleman, A.W. (1993). Relationship between endopolyploidy and cell size in epidermal tissue of Arabidopsis. Plant Cell 5: 1661–1668.

Morohashi, K., Zhao, M., Yang, M., Read, B., Lloyd, A., Lamb, R., and Grotewold, E. (2007). Participation of the Arabidopsis bHLH factor GL3 in trichome initiation regulatory events. Plant Physiol. 145: 736–746.

Nagano, Y., Inaba, T., Furuhashi, H., and Sasaki, Y. (2001). Trihelix DNA-binding protein with specificities for two distinct cis-elements: Both important for light-downregulated and dark-inducible gene expression in higher plants. J. Biol. Chem. 276: 22238–22243.

Nakagawa, T., Kurose, T., Hino, T., Tanaka, K., Kawamukai, M., Niwa, Y., Toyooka, K., Matsuoka, K., Jinbo, T., and Kimura, T. (2007). Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. J. Biosci. Bioeng. 104: 34–41.

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.

Perazza, D., Herzog, M., Hulskamp, M., Brown, S., Dorne, A.M., and Payne, C.T., Zhang, F., and Lloyd, A.M. (2002). TRIPTYCHON and CAPRICE mediate lateral inhibition during formation in the root and shoot of Arabidopsis. Biochem. Soc. Trans. 29: 146–148.

Schellmann, S., Gregor, M., Nemethova, M., Grabner, M., Kovarik, P., and Piskacek, M. (2007). Nine-aminoc-acid transactivation domain: Establishment and prediction utilities. Genomics 89: 756–768.

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., Hulskamp, M., and Uhrig, J. (2007). Epidermal pattern formation in the root and shoot of Arabidopsis. Biochem. Soc. Trans. 35: 146–148.

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

Schnittger, A., Folkers, U., Schwab, B., Jurgens, G., and Hulskamp, M. (1999). Generation of a spacing pattern: the role of triptychon in trichome patterning in Arabidopsis. Plant Cell 11: 1105–1116.

Schnittger, A., Jurgens, G., and Hulskamp, M. (1998). Tissue layer and organ specificity of trichome formation are regulated by GLABRA1 and TRIPTYCHON in Arabidopsis. Development 125: 2283–2289.

Schnittger, A., Schobinger, U., Bouyer, D., Weinl, C., Stierhof, Y.D., and Hulskamp, M. (2002). Ectopic D-type cyclin expression induces not only DNA replication but also cell division in Arabidopsis trichomes. Proc. Natl. Acad. Sci. USA 99: 6410–6415.

Seki, M., et al. (2002). Functional annotation of a full-length Arabidopsis cDNA collection. Science 296: 141–145.

Smalle, J., Kurepa, J., Haegeman, M., Gielen, J., Van Montagu, M., and Van Der Straeten, D. (1998). The trihelix DNA-binding motif in higher plants is not restricted to the transcription factors GT-1 and GT-2. Proc. Natl. Acad. Sci. USA 95: 3318–3322.

Sugimoto-Shirasu, K., Roberts, G.R., Stacey, N.J., McCann, M.C., Maxwell, A., and Roberts, K. (2005). RHL1 is an essential component of the plant DNA topoisomerase VI complex and is required for ploidy-dependent cell growth. Proc. Natl. Acad. Sci. USA 102: 18736–18741.

Sugimoto-Shirasu, K., Stacey, N.J., Corsar, J., Roberts, K., and McCann, M.C. (2002). DNA topoisomerase VI is essential for endoreduplication in Arabidopsis. Curr. Biol. 12: 1782–1786.

Szczes, J., Joly, C., Bordji, K., Varaud, E., Cock, J.M., Dumas, G., and Bendahmane, M. (2006). BIGPETALP1, a bHLH transcription factor is involved in the control of Arabidopsis petal size. EMBO J. 25: 3912–3920.

Szymanski, D.B., and Marks, M.D. (1998). GLABROUS1 overexpression and TRIPTYCHON alter the cell cycle and trichome cell fate in Arabidopsis. Plant Cell 10: 2047–2062.

Szymanski, D.B., Marks, M.D., and Wick, S.M. (1999). Organized F-actin is essential for normal trichome morphogenesis in Arabidopsis. Plant Cell 11: 2331–2347.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596–1599.

Tominaag, R., Iwata, M., Sano, R., Inoue, K., Okada, K., and Wada, T. (2008). Arabidopsis CAPRICE-LIKE MYB 3 (CPL3) controls endoreduplication and flowering development in addition to trichome and root hair formation. Development 135: 1335–1345.

Vanstraelen, M., Balaban, M., Da Ines, O., Cultrone, A., Lammens, T., Boudolf, V., Brown, S.C., De Veylder, L., Mergaert, P., and Kondorosi, E. (2009). APC/CCCS52A complexes control meristem maintenance in the Arabidopsis root. Proc. Natl. Acad. Sci. USA 106: 11806–11811.

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.

Walker, J.D., Oppenheimer, D.G., Concienne, J., and Larkin, J.C. (2000). SIAMESE, a gene controlling the endoreduplication cell cycle in Arabidopsis thaliana trichomes. Development 127: 3931–3940.

Wang, G., Tian, L., Aziz, N., Broun, P., Dai, X., He, J., King, A., Zhao, P.P., and Dixon, R.A. (2008). Terpene biosynthesis in glandular trichomes of hop. Plant Physiol. 148: 1254–1266.

Wang, S., Kwak, S.H., Zeng, Q., Ellis, B.E., Chen, X.Y., Schiefelbein, J., and Chen, J.G. (2007). TRICHOMELESS1 regulates trichome patterning by suppressing GLABRA1 in Arabidopsis. Development 134: 3873–3882.

Wester, K., Digiumi, S., Geier, F., Timmer, J., Fleck, C., and Hulskamp, M. (2009). Functional diversity of R3 single-repeat genes in trichome development. Development 136: 1487–1496.

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.

Zhang, X., and Oppenheimer, D.G. (2004). A simple and efficient method for isolating trichomes for downstream analyses. Plant Cell Physiol. 45: 221–224.

Zhao, M., Morohashi, K., Hatlestad, G., Grotewold, E., and Lloyd, A. (2008). The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. Development 135: 1991–1999.

Zhou, D.X. (1999). Regulatory mechanism of plant gene transcription by GT-elements and GT-factors. Trends Plant Sci. 4: 210–214.
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*Arabidopsis* Trichome

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