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Note

Analysis of *TTG1* and CPC-like MYB genes during *Arabidopsis* epidermal cell differentiation

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Abstract The development of *Arabidopsis thaliana* epidermal cells includes the differentiation of trichomes and root hairs. The *TRANSPARENT TESTA GLABRA 1* (*TTG1*) gene encodes a WD40 protein that induces trichome differentiation and reduces root hair formation in *Arabidopsis*. The *CAPRICE* (*CPC*) gene family includes *CPC, ENHANCER OF TRY AND CPC1* (*ETC1*), *ENHANCER OF TRY AND CPC2* (*ETC2*), and *CPC LIKE MYB3* (*CPL3*), which encode R3-type MYB transcription factors that inhibit trichome differentiation and promote root hair formation. *CPC* expression is positively regulated by a transcriptional complex that includes TTG1. To determine whether *ETC1*, *ETC2*, and *CPL3* are also regulated by the TTG1 complex, we examined the functional relationship between *TTG1* and *CPC-like MYB* genes. Double mutant analysis showed that the *ttg1* mutant is epistatic to the *cpc*, *etc1*, *etc2*, and *cpl3* mutants in trichome cell fate determination but not in root hair development. In roots, the *cpc* mutant is epistatic to the *ttg1* mutant in root epidermal cell fate determination. Promoter-GUS analysis indicated that *TTG1* is necessary for the expression of *ETC1* and *CPL3*, but not for *ETC2* expression. These results indicate that TTG1 had a stronger effect on trichome formation than CPC-like MYBs. By contrast, CPC had a stronger effect on root hair formation than TTG1. Our results suggest that *ETC1* and *CPL3* are also regulated by the TTG1 complex as is the case for *CPC*; however, *ETC2* is not regulated by this complex. We concluded that *ETC2* does not have a role in trichome and root hair formation.

Key words: Arabidopsis, MYB, root hairs, trichomes, TTG1.

Trichomes and root hairs are single cell extensions that originate from leaf or root epidermal cells in *Arabidopsis thaliana*. Numerous regulatory factors involved in epidermal cell differentiation have been identified. The *TRANSPARENT TESTA GLABRA1* (*TTG1*) gene encodes a WD40-repeat protein that induces trichome differentiation and reduces root hair formation in *Arabidopsis* (Galway et al. 1994; Walker et al. 1999). The *CAPRICE* (*CPC*) gene encodes an R3-type myeloblastosis (MYB) transcription factor in *Arabidopsis* (Wada et al. 1997). Several other CPC-like MYBs are known: *ENHANCER OF TRY AND CPC1* (*ETC1*); *ENHANCER OF TRY AND CPC2* (*ETC2*); and *CPC LIKE MYB3/ENHANCER OF TRY AND CPC3* (*CPL3*/*ETC3*). Overexpression of these transcription factors inhibits trichome differentiation and promotes root hair formation in *Arabidopsis* (Esch et al. 2004; Kirik et al. 2004a, 2004b; Simon et al. 2007; Tominaga et al. 2008).

The R2R3-type MYB transcription factors, WEREWOLF (WER) and GLABRA 1 (GL1), are also involved in epidermal cell differentiation in *Arabidopsis* (Lee and Schiefelbein 1999; Oppenheimer et al. 1991). The TTG1, CPC-like MYB, WER, and GL1 proteins interact with the bHLH proteins, GLABRA 3 (GL3) and ENHANCER OR GLABRA 3 (EGL3), and act as a transcription regulatory complex of MYB-bHLH-WD40 in *Arabidopsis* epidermal cells (Bernhardt et al. 2003; Esch et al. 2003; Payne et al. 2000; Tominaga et al. 2008; Zhang et al. 2003). The WER-GL3/EGL3-TTG1 transcription complex activates the expression of *GLABRA 2* (*GL2*) and induces non-hair cell fate (Bernhardt et al. 2003; Hung et al. 1998; Lee and Schiefelbein 1999; Payne et al. 2000). Conversely, the CPC-like MYB-GL3/EGL3-TTG1 transcriptional complex is proposed to inactivate expression of *GL2* (Schiefelbein and Lee 2006; Tominaga-Wada and Wada 2014). Further, the WER-GL3/EGL3-TTG1 transcriptional complex positively regulates the expression of *CPC* and *ETC1* genes (Bernhardt et al. 2003; Lee and Schiefelbein 2002; Simon et al. 2007).

In the present study, we sought to elucidate the relationship for transcriptional regulation between *TTG1* and the *CPC-like MYB* genes *ETC1*, *ETC2*, and *CPL3* in *Arabidopsis*. Previously, *CPC* expression was reported to be enhanced in roots by a transcriptional complex that included TTG1 (Bernhardt et al. 2003). However, the precise change in expression of *ETC1*, *ETC2*, and *CPL3* induced by the TTG1 transcriptional complex has not

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been determined. To clarify the epistatic interactions during epidermal cell differentiation, we analyzed homozygous double mutant combinations of *ttg1 etc1*, *ttg1 etc2*, and *ttg1 cpl3*. To elucidate the relationship between *TTG1* and *CPC-like MYB* genes more precisely, we introduced the *ETC1::GUS*, *ETC2::GUS*, or *CPL3::GUS* transcriptional reporters into the *ttg1* mutant lines.

The *Arabidopsis thaliana* ecotype Columbia (Col-0), Landsberg *erecta* (Ler), and Wassilewskija (WS) were used as the wild type for these experiments. The *ttg1-1* (Ler background) (Koornneef et al. 1982), *ttg1-10* (Col-0 background) (Larkin et al. 1994), *cpc-1* (WS background) (Wada et al. 1997), *etc1-1* (Col-0 background), *etc2-2* (Col-0 background) (Tominaga-Wada et al. 2013), *cpl3-1* (Col-0 background) mutants, and the *35S::CPL3* (Col-0 background) transgenic plants (Tominaga et al. 2008) were used in the present study. Double mutants of *ttg1* and *cpc-like myb* mutants were screened from F2 progeny using PCR to identify homozygous *ttg1-10 etc1-1*, *ttg1-10 etc2-2*, *ttg1-10 cpl3-1*, *ttg1-1 cpc-1*, and *ttg1-1 cpl3-1* double mutants. *35S::CPL3* was introduced into *ttg1-1* mutant by a traditional cross and F2 seedlings were analyzed by PCR to identify *ttg1-1 35S::CPL3* lines. The *ETC1::GUS*, *ETC2::GUS*, and *CPL3::GUS* constructs (Tominaga et al. 2008) were introduced into the *ttg1-1*, and *ttg1-10* mutants by conventional crossing and F2 seedlings were analyzed by PCR.

Promoter::GUS plants were immersed in a solution containing 1 mM X-Gluc (5-bromo-4-chloro-3-indolyl-β-glucuronide), 1 mM $K_3Fe(CN)_6$, 1.0 mM $K_4Fe(CN)_6$, 100 mM NaPi (pH 7.0), 100 mM EDTA, and 0.1% Triton X-100. Primary roots of five-day-old seedlings were incubated at 37°C overnight. Aerial parts of two-week-old seedlings were incubated at 37°C for 3 h.

For the observation of seedling phenotypes, seeds were surface sterilized and sown on 1.5% agar plates using a method described previously (Okada and Shimura 1990). Seeded plates were incubated at 4°C for two days and then transferred to 22°C under continuous white light (50–100 μmol $m^{-2}s^{-1}$). For each mutant transgenic line, at least five two-week-old third leaves were observed for trichome formation, and at least ten individual five-day-old seedlings were assayed for root hair formation. Mutant and transgenic plants were observed using the Leica MZ16FA stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany). Images were recorded using a high-sensitivity CCD color camera system (Keyence VB 7010, Osaka, Japan).

Semi-quantitative RT-PCR analysis was performed. Total RNA was prepared from roots, shoots, stems, siliques, inflorescences, and rosette leaves using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). On-column DNase I digestion was performed during RNA purification following the protocol described in the RNeasy Mini Kit handbook. First-strand cDNA was synthesized from 1 μg total RNA in a 20 μl reaction mixture using the Prime Script RT Reagent Kit (Takara). Semi-quantitative RT-PCR reaction was conducted as described by Kurata et al. (2003). The *CPC* and *ETC2* fragments were amplified with RT128/RT129 and RT124/RT125 primer pairs, respectively (Tominaga-Wada and Nukumizu 2012). The *CPL3* and *EF* fragments were amplified with RT73/RT92 and EF1a-F/EF1a-R primer pairs, respectively (Tominaga et al. 2008). The *ETC1* fragment was amplified with the RT122 GCG ATC GTA AAT CTT TGT GTA CTA AG/RT123 CTC AGG AAC AAA ACT GCA GAA TTA C primer pair.

To investigate the interaction of the *TTG1* gene and the *CPC-like MYB* genes in epidermal cell development, plants carrying the *ttg1-10* and *ttg1-1* mutant alleles were crossed with *cpc-1*, *etc1-1*, *etc2-2*, or *cpl3-1* mutants, or the *35S::CPL3* transgenic line (Table 1). As previously reported, the *ttg1-10* single mutant has a dramatically reduced number of trichomes and a slightly increased number of root hairs, and the *ttg1-1* single mutant has no trichomes and a greatly increased number of root hairs compared to the wild type (Figure 1, Table 1). These observations were consistent with previous descriptions of the *ttg1* mutant alleles and suggest that between the two alleles, the *ttg1-1* allele presents a more severe phenotype than the *ttg1-10* allele (Larkin et al. 1994). The *ttg1-10 etc1-1, ttg1-10 etc2-2*, and *ttg1-10 cpl3-1* double mutants showed a dramatically reduced number of trichomes compared to wild type (Figure 1, Table 1). Based on these double mutant leaf phenotypes, *ttg1-10* is epistatic to *etc1-1*, *etc2-2*, and *cpl3-1* in trichome formation. However, compared to the *ttg1-10* single mutant, the *ttg1-10 etc1-1* and *ttg1-10 cpl3-1* double

Table 1. Number of leaf trichomes and root hairs on wild type and mutant seedlings.

Genotype	Trichome number/leaf	Root hair number/mm
Col-0	48.0±3.8	44.7±2.2
Ler	33.2±4.3	36.1±1.6
WS	51.6±6.1	37.0±1.2
ttg1-10 (Col-0)	2.0±1.4	47.4±2.4
ttg1-1 (Ler)	0	61.1±3.7
cpc-1 (WS)	81.0±4.0	5.7±1.1
etc1-1 (Col-0)	48.4±8.1	44.5±2.0
etc2-2 (Col-0)	49.0±5.0	47.0±1.6
cpl3-1 (Col-0)	59.6±2.3	31.3±2.0
35S::CPL3 (Col-0)	0±0	59.6±1.9
ttg1-10 etc1-1	7.3±1.6*	39.4±1.6**
ttg1-10 etc2-2	0.2±0.2	46.7±2.3
ttg1-10 cpl3-1	9.2±1.2**	29.6±2.7**
ttg1-1 cpc-1	0.2±0.2	0.5±0.4
ttg1-1 cpl3-1	0±0	46.5±3.2
ttg1-1 35S::CPL3	0±0	99.7±4.4

Data represent the mean±S.D. of at least 5 leaves or 10 roots per experiment. Student's *t*-test, * $p<0.05$, ** $p<0.02$ *vs. ttg1-10*.

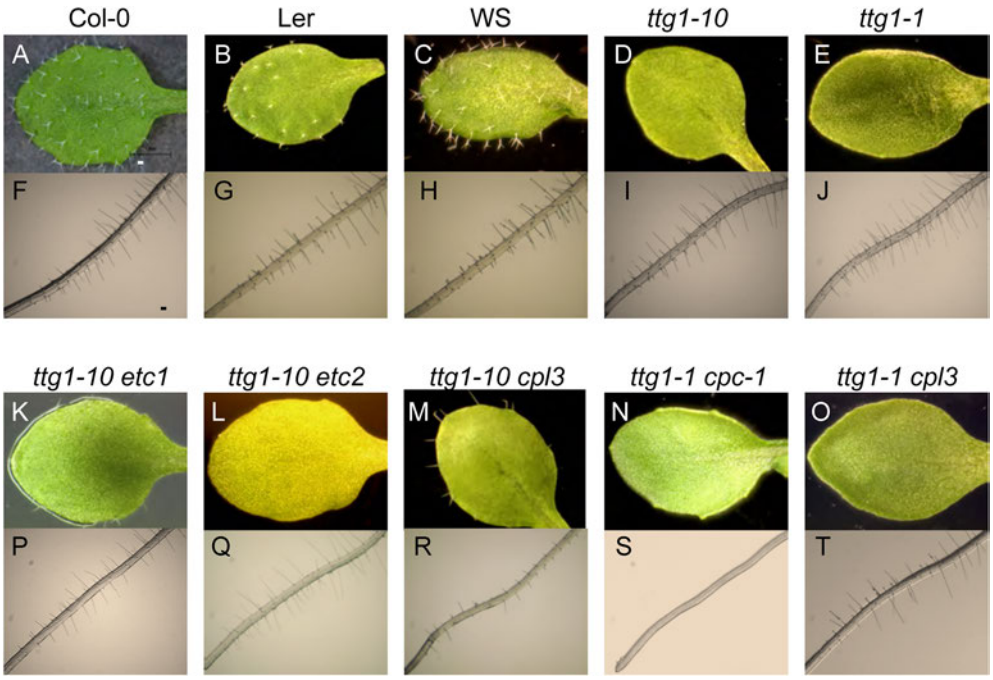

Figure 1. Leaf and root phenotypes of *Arabidopsis* mutants. Trichome formation on the third leaves of two-week-old *Arabidopsis* seedlings of Col-0 (A), Ler (B), WS (C), *ttg1-10* (D), *ttg1-1* (E), *ttg1-10 etc1-1* (K), *ttg1-10 etc2-2* (L), *ttg1-10 cpl3-1* (M), *ttg1-1 cpc-1* (N), and *ttg1-1 cpl3-1* (O). Root hair formation in five-day-old *Arabidopsis* seedlings of Col-0 (F), Ler (G), WS (H), *ttg1-10* (I), *ttg1-1* (J), *ttg1-10 etc1-1* (P), *ttg1-10 etc2-2* (Q), *ttg1-10 cpl3-1* (R), *ttg1-1 cpc-1* (S), and *ttg1-1 cpl3-1* (T). Scale bars: 1 mm in (A)–(E) and in (K)–(O): 200 μm in (F)–(J) and in (P)–(T).

mutants produced significantly more trichomes (Figure 1, Table 1). Although most parts of the leaves of double mutants were glabrous, *ttg1-10 etc1-1* and the *ttg1-10 cpl3-1* double mutants produced several trichomes, especially at the leaf margin (Figure 1K, M). In addition, the *ttg1-10 etc1-1* and *ttg1-10 cpl3-1* double mutants had significantly reduced numbers of root hairs compared to that of the *ttg1-10* single mutant (Figure 1, Table 1). These results suggest that the mutation in *ETC1* and *CPL3* have opposing effects to TTG1 on *Arabidopsis* root and leaf epidermal cell development. On the other hand, the *ttg1-10 etc2-2* double mutant showed similar trichome and root hair phenotypes to those of the *ttg1-10* single mutant (Figure 1, Table 1). These observations indicate that the *etc2* mutation did not have a noticeable effect on the trichome and root hair development in a *ttg1-10* mutant background. As shown in Figure 2J, *ETC2* was not expressed in roots. This result strongly indicates that *ETC2* is not involved in root hair formation. Although the *ETC1*, *ETC2*, and *CPL3* genes encode functionally equivalent R3 MYB proteins (Kirik et al. 2004a, 2004b; Tominaga et al. 2008), mutations in these genes showed different effects on the *ttg1-10* mutant, suggesting their functions may not be redundant.

The *ttg1-1 cpc-1* double mutant had a dramatically reduced number of trichomes and root hairs compared to that measured in wild type plants (Figure 1, Table 1). The *ttg1-1 cpc-1* double mutant leaves were nearly lacking all trichomes and resembled that of the *ttg1-1* single mutant leaves, suggesting epistasis of *ttg1-1* to *cpc-1* in leaf trichome formation. The non-hair phenotype of the *ttg1-1 cpc-1* double mutant roots resembled that of the *cpc-1* single mutant roots, suggesting epistasis of *cpc-1* to *ttg1-1* on root hair formation. The *ttg1-1 cpl3-1* double mutant had a glabrous leaf phenotype resembling that of the *ttg1-1* single mutant, and possessed an intermediate number of root hairs (46.5±3.2) compared to *ttg1-1* (61.1±3.7) and *cpl3-1* (31.3±2.0) single mutants (Figure 1, Table 1). These results suggest that the *ttg1-1* mutant is epistatic to the *cpc-1* and *cpl3-1* mutants regarding to trichome formation, whereas, the *cpc-1* mutant is epistatic to the *ttg1-1* mutant during root hair differentiation. However, because of genetic background differences, it is difficult to simply compare *ttg1-1 cpl3-1* and *ttg1-10 cpl3-1*. Consistent with the *ttg1* single mutant phenotypes, a relatively stronger effect of *ttg1-1* than *ttg1-10* was found even in double mutants with *cpl3-1* (Table 1). Although *ttg1-1 cpl3-1* had a no-trichome *ttg1-1*-like phenotype, *ttg1-10 cpl3-1* had a slightly increased number of trichomes compared with *ttg1-10*. The double mutant *ttg1-1 cpl3-1* possessed an intermediate number of root hairs compared to *ttg1-1* and *cpl3-1*; however, *ttg1-10 cpl3-1* produced a comparable number of root hairs to *cpl3-1*. Therefore, the *ttg1* mutation is epistatic to the *cpl3* mutation for trichome formation, whereas the *cpl3* mutation may be epistatic to the *ttg1* mutation for root hair formation in both *ttg1-1 cpl3-1* and *ttg1-10 cpl3-1* mutants.

When expressed in *ttg1-1* transgenic plants, the *35S::CPL3* construct produced an increased number of root hairs compared to untransformed parental lines, *35S::CPL3* or *ttg1-1* mutant. These results suggest a synergistic effect between *CPL3* overexpression and the *ttg1-1* mutation on root hair formation (Table 1).

To explore the relationship between *TTG1* and *CPC-like MYBs* further, we introduced *ETC1::GUS*,

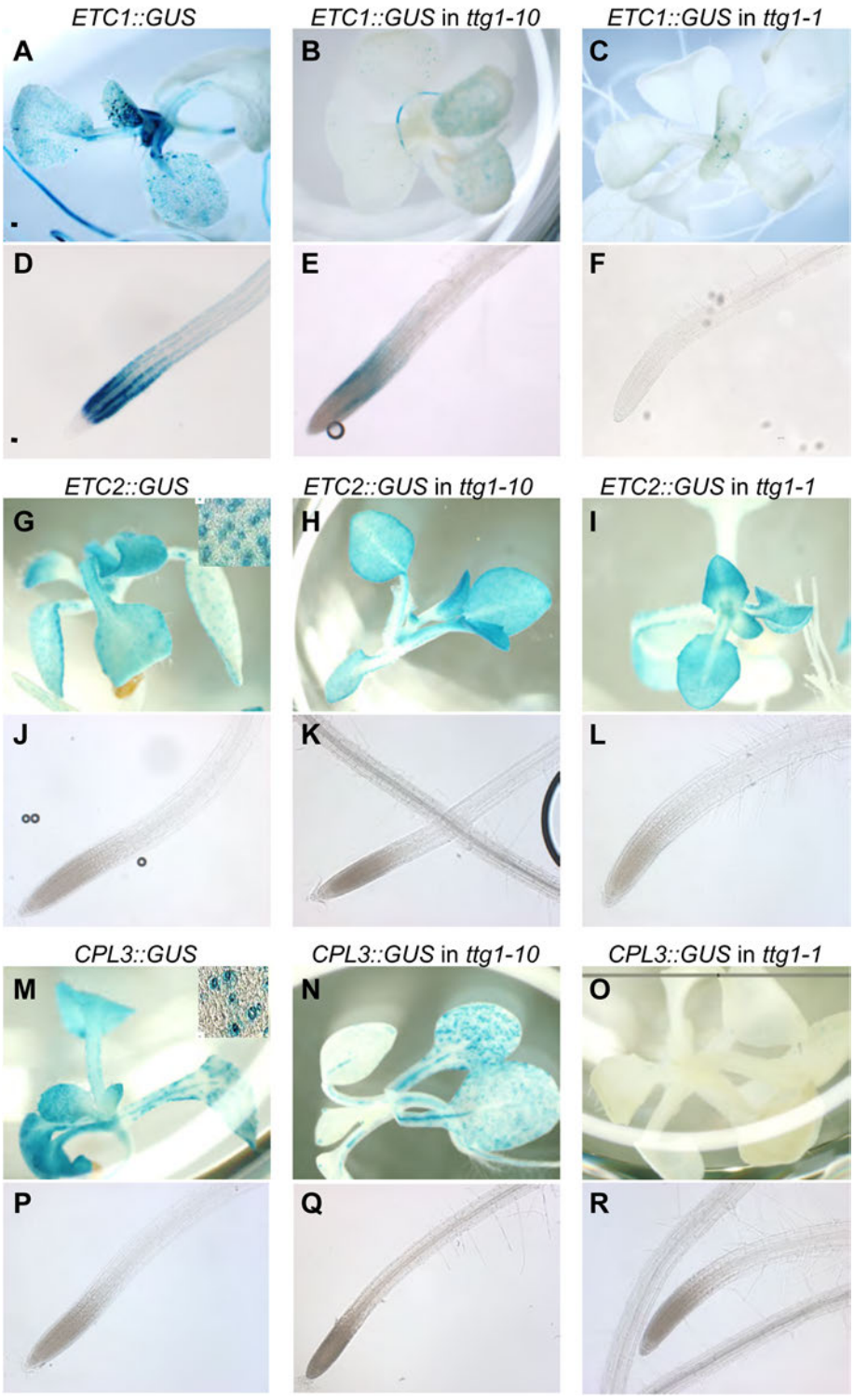

Figure 2. Histochemical staining of GUS activity in transgenic plants. Expression of the *ETC1::GUS* (A–F), *ETC2::GUS* (G–L), and *CPL3::GUS* (M–R) reporters in two-week-old leaves or in roots of five-day-old wild type Col-0 (A, D, G, J, M, and P), *ttg1-10* (B, E, H, K, N, and Q), and *ttg1-1* (C, F, I, L, O, and R) seedlings. Whole plants were stained with X-Gluc. Inset in G and M show magnified leaf epidermal cells (×10). Scale bars: 1 mm in (A, B, C, G, H, I, M, N, and O): 200 μm in (D, E, F, J, K, L, P, Q, and R).

ETC2::GUS, and *CPL3::GUS* constructs into the two *ttg1* mutant alleles, *ttg1-10* and *ttg1-1*. Consistent with previous studies, *ETC1::GUS* was expressed primarily in trichomes and non-hair cell files in wild type *Arabidopsis* roots (Figure 2A, D); and *ETC2::GUS* and *CPL3::GUS* were expressed in wild type *Arabidopsis* young leaves, especially in guard cells (Figure 2G, M, insets) (Tominaga et al. 2008). *ETC1::GUS* expression was repressed in the *ttg1-10* mutant leaves and roots comparing with that of wild type (Figure 2B, E). *ETC1::GUS* expression was also strongly repressed in *ttg1-1* trichomes and abolished in *ttg1-1* roots (Figure 2C, F). However, the *ETC2::GUS* expression level did not change in the *ttg1-10* and *ttg1-1* mutant background when compared to its expression in wild type plants (Figure 2H, I). *ETC2::GUS* was expressed in young leaves of *ttg1-10* and *ttg1-1* with approximately the same intensity as that measured in wild type leaves (Figure 2H, I). *ETC2::GUS* expression was not observed in wild type, *ttg1-10*, and *ttg1-1* roots (Figure 2J–L). Similar to *ETC1::GUS* expression, the *CPL3::GUS* signal was strongly reduced in *ttg1-10* mutant leaves compared to that in wild type leaves (Figure 2N). Moreover, the *CPL3::GUS* expression was not detected in the *ttg1-1* mutant leaves (Figure 2O). *CPL3::GUS* was not expressed in root epidermal cells of wild type, *ttg1-10*, and *ttg1-1* (Figure 2P–R). These results suggest that the *TTG1* gene is necessary for the native expression of the *ETC1* and *CPL3* genes in *Arabidopsis*. In contrast, the expression level of *ETC2* was not affected by the loss of the *TTG1* gene.

It is remarkable that the *cpl3-1* single mutant and the *ttg1-10 cpl3-1* double mutant exhibited a root hair phenotype, even though *CPL3:GUS* expression was not detected in the root (Figure 2P). To clarify this apparent contradiction, we performed a semi-quantitative PCR analysis to examine expression of selected genes. As expected, strong expression of *CPC* and *ETC1* in roots and rosette leaves was found using 25 cycles of amplification (Figure 3). *CPC* was also strongly expressed in siliques (Figure 3). However, even after 35 cycles of amplification, distinct *ETC2* expression could be detected only in rosette leaves (Figure 3). Relatively strong *CPL3* expression was detected in rosette leaves (Figure 3). After 35 cycles of amplification, a low level of *CPL3* expression was detected in roots (Figure 3). *CPC3* expression was detected in all tissues examined in this experiment after

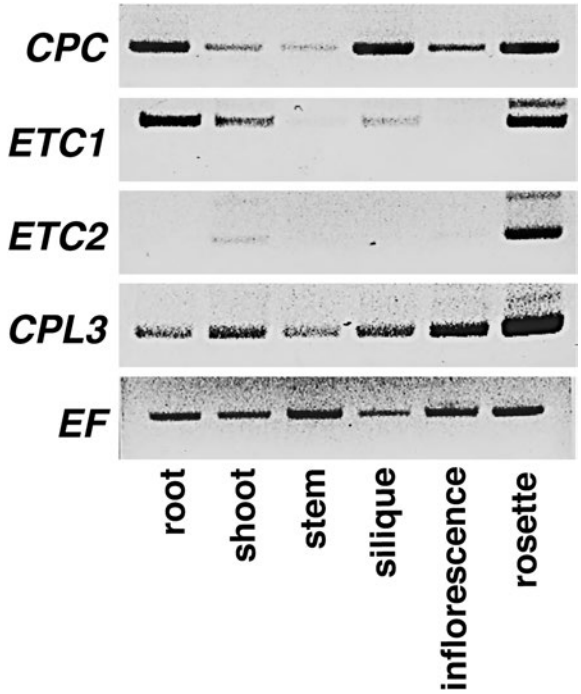

Figure 3. Semi-quantitative RT-PCR analysis of the expression of *CPC* family genes. Expression of *CPC*, *ETC1*, *ETC2*, and *CPL3* in different organs of *Arabidopsis*. Wild-type Col-0 was grown for 5 days. Total RNA was prepared from the root, shoot, stem, silique, inflorescence, and rosette leaf and subjected to semi-quantitative RT-PCR. Twenty-five amplification cycles were used for *CPC*, *ETC1*, and *EF*. Thirty-five amplification cycles were used for *ETC2* and *CPL3*. The expression of *EF* was used as a control.

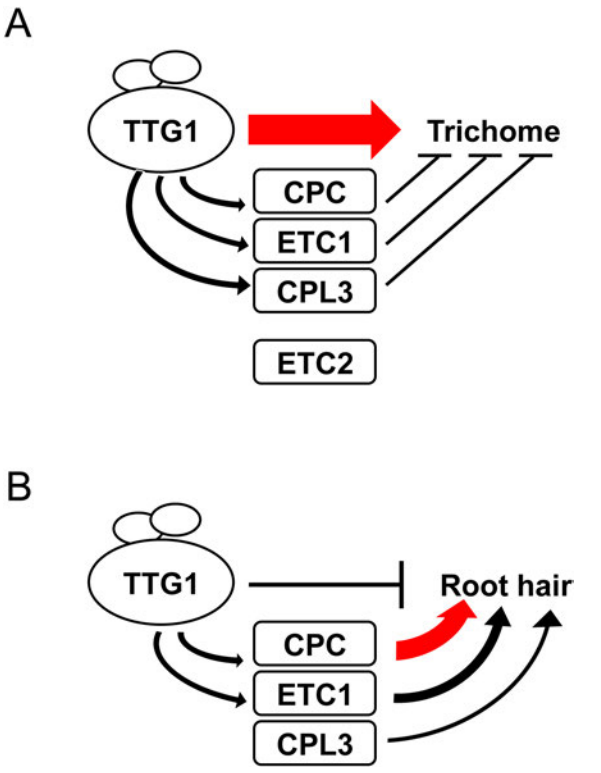

Figure 4. Model of root epidermal cell specification showing the regulation and proposed role of TTG1, CPC, ETC1, ETC2, and CPL3. (A) Trichome formation is strongly induced by a transcriptional complex that includes TTG1. This TTG1 complex induces *CPC*, *ETC1*, and *CPL3* expression in leaves. CPC, ETC1, and CPL3 participate in inhibition of trichome formation. (B) The TTG1 complex inhibits root hair formation, but CPC counteracts its effect and strongly promotes root hair formation. TTG1 complex-induced *CPC* and *ETC1* expression in roots. ETC1 and CPL3 also enhance root hair formation. Arrows indicate positive effects. Red arrows indicate strongly positive effects. Blunt lines indicate negative effects.

35 cycles of amplification. These results suggest that a low level of expression of *CPL3*, which was not detected by the *CPL3::GUS* analysis, might occur in roots and contribute to root-hair formation and therefore explain the root hair phenotype of *cpl3-1* and *ttg1-10 cpl3-1* mutants.

In this study, we showed that *TTG1* is necessary for the expression of *ETC1* and *CPL3*, but does not for *ETC2* expression in *Arabidopsis* leaf and root epidermal cells (Figure 4A). Mutations in the *TTG1* gene are epistatic to that of the *CPC-like MYB* genes during trichome cell differentiation; however, the *cpc* mutation has a stronger effect on root epidermal cell differentiation than do *ttg1* mutant alleles (Figure 4B). The *ttg1-1 cpc-1* double mutant showed a totally glabrous phenotype both in leaves and roots. We concluded that *ETC1* and *CPL3* were regulated by a transcriptional complex, including TTG1, as in the case for *CPC* (Figure 4). By contrast, *ETC2* was not regulated by this TTG1 complex and did not contribute to trichome or root hair formation (Figure 4). Our findings also indicate that subtle *CPL3* expression in roots may contribute to root hair formation against the effect of *TTG1*.

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References

Bernhardt C, Lee MM, Gonzalez A, Zhang F, Lloyd A, 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

Esch JJ, Chen MA, Hillestad M, Marks MD (2004) Comparison of TRY and the closely related At1g01380 gene in controlling Arabidopsis trichome patterning. *Plant J* 40: 860–869

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

Galway ME, Masucci JD, Lloyd AM, Walbot V, Davis RW, Schiefelbein JW (1994) The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root. *Dev Biol* 166: 740–754

Hung CY, Lin Y, Zhang M, Pollock S, Marks MD, Schiefelbein J (1998) A common position-dependent mechanism controls cell-type patterning and GLABRA2 regulation in the root and hypocotyl epidermis of Arabidopsis. *Plant Physiol* 117: 73–84

Kirik V, Simon M, Huelskamp M, 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, 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* 55: 389–398

Koornneef M, Dellaert LW, van der Veen JH (1982) EMS- and radiation-induced mutation frequencies at individual loci in *Arabidopsis thaliana* (L.) Heynh. *Mutat Res* 93: 109–123

Kurata T, Kawabata-Awai C, Sakuradani E, Shimizu S, Okada K, Wada T (2003) The YORE-YORE gene regulates multiple aspects of epidermal cell differentiation in Arabidopsis. *Plant J* 36: 55–66

Larkin JC, Oppenheimer DG, Lloyd AM, Paparozzi ET, Marks MD (1994) Roles of the GLABROUS1 and TRANSPARENT TESTA GLABRA Genes in Arabidopsis Trichome Development. *Plant Cell* 6: 1065–1076

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

Lee MM, Schiefelbein J (2002) Cell pattern in the Arabidopsis root epidermis determined by lateral inhibition with feedback. *Plant Cell* 14: 611–618

Okada K, Shimura Y (1990) Reversible Root Tip Rotation in Arabidopsis Seedlings Induced by Obstacle-Touching Stimulus. *Science* 250: 274–276

Oppenheimer DG, Herman PL, Sivakumaran S, Esch J, Marks MD (1991) A myb gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. *Cell* 67: 483–493

Payne CT, Zhang F, Lloyd AM (2000) GL3 encodes a bHLH protein that regulates trichome development in arabidopsis through interaction with GL1 and TTG1. *Genetics* 156: 1349–1362

Schiefelbein J, Lee MM (2006) A novel regulatory circuit specifies cell fate in the Arabidopsis root epidermis. *Physiol Plant* 126: 503–510

Simon M, Lee MM, Lin Y, Gish L, Schiefelbein J (2007) Distinct and overlapping roles of single-repeat MYB genes in root epidermal patterning. *Dev Biol* 311: 566–578

Tominaga R, Iwata M, Sano R, Inoue K, Okada K, 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

Tominaga-Wada R, Nukumizu Y (2012) Expression Analysis of an R3-Type MYB Transcription Factor CPC-LIKE MYB4 (TRICHOMELESS2) and CPL4-Related Transcripts in Arabidopsis. *Int J Mol Sci* 13: 3478–3491

Tominaga-Wada R, Nukumizu Y, Wada T (2013) Flowering is delayed by mutations in homologous genes CAPRICE and TRYPTICHON in the early flowering Arabidopsis cpl3 mutant. *J Plant Physiol* 170: 1466–1468

Tominaga-Wada R, Wada T (2014) Regulation of root hair cell differentiation by R3 MYB transcription factors in tomato and Arabidopsis. *Front Plant Sci* 5: 91

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

Walker AR, Davison PA, Bolognesi-Winfield AC, James CM, Srinivasan N, Blundell TL, Esch JJ, Marks MD, Gray JC (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

Zhang F, Gonzalez A, Zhao MZ, Payne CT, Lloyd A (2003) A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis. *Development* 130: 4859–4869