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Note

The *CAPRICE-LIKE MYB* gene family cooperatively controls trichome branching and clustering in *Arabidopsis*

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Abstract The *CAPRICE-LIKE MYB* gene family, including *CAPRICE* (*CPC*), *TRIPTYCHON* (*TRY*), *ENHANCER OF TRY AND CPC1* and *2* (*ETC1* and *ETC2*), *CPC-LIKE MYB3*/*ENHANCER OF TRY AND CPC3* (*CPL3*/*ETC3*), encodes a small protein with an R3-type MYB motif that regulates leaf trichome differentiation in *Arabidopsis thaliana*. To understand the role of *CPC-LIKE MYB* genes in trichome development, we focused on the effect of these genes on trichome branching and clustering. Trichomes of the *try etc1* double mutant consistently had more branches than wild-type and the *try* single mutant, suggesting that the *TRY* and *ETC1* genes cooperatively regulate trichome branch development. The *ETC2* gene has little to no involvement in trichome branching. *TRY* and *CPC* are known to have strong effects on trichome cluster formation. Double and triple mutant analyses revealed that the *ETC1*, *ETC2* and *CPL3* genes have some degree of functional redundancy with *TRY* in trichome cluster formation.

Key words: *Arabidopsis,* trichome, MYB, transcription factor, CPC

The trichomes that differentiate from leaf and stem surfaces have diverse functions, including defense against herbivores, protection from UV irradiation, and limiting transpiration (Myers and Bazely 1991; Wagner et al. 2004). *Arabidopsis* trichomes are large single cells with three characteristic branches that are thought to protect the plant by serving as a physical barrier (Glover and Martin 2000). Wild-type *Arabidopsis* trichomes initiate in the epidermis of developing leaf primordia and are regularly spaced (Hülskamp et al. 1994; Larkin et al. 1996; Schwab et al. 2000). Several genes have been identified that play an important role in *Arabidopsis* trichome development (Hülskamp et al. 1999). *GLABRA1* (*GL1*) (Oppenheimer et al. 1991), *MYB23* (Kirik et al. 2005), *GLABRA3* (*GL3*) (Payne et al. 2000), *ENHANCER OF GLABRA3* (*EGL3*) (Zhang et al. 2003), *TRANSPARENT TESTA GLABRA1* (*TTG1*) (Galway et al. 1994; Walker et al. 1999), and *GLABRA2* (*GL2*) (Masucci et al. 1996; Rerie et al. 1994) are positive regulators of *Arabidopsis* trichome formation. The *CPC-LIKE MYB* family genes, *CPC* (Wada et al. 2002, 1997), *TRY* (Schellmann et al. 2002; Schnittger et al. 1999), *ETC1* (Esch et al. 2004; Kirik et al. 2004a), *ETC2* (Kirik et al. 2004b), and *ETC3*/*CPL3* (Simon et al. 2007; Tominaga et al. 2008; Wang et al. 2008), all encoding proteins containing a single R3-type MYB motif, are negative regulators of *Arabidopsis* trichome formation. Notably, the *try* mutant of *Arabidopsis* develops trichomes in clusters (Schnittger et al. 1999). Leaves of the *cpc try etc1 cpl3* quadruple mutant are entirely covered with trichomes of various sizes and branch numbers (Tominaga et al. 2008). Trichome formation includes three developmental parameters: trichome number, branching and clustering. Trichome numbers of single-, double- or triple mutants of the *CPC-LIKE MYB* gene family were already analyzed (Kirik et al. 2004a, 2004b; Tominaga et al. 2008). In this study, we focused on trichome branching and clustering to clarify the exact roles of members of the *CPC-LIKE MYB* gene family. To date, trichome branching has not been analyzed precisely except for *try* (Hülskamp et al. 1994), and *cpl3* (Tominaga et al. 2008). The effects of *try etc1* or *try etc2* on trichome clustering were previously reported using mutants in several different genetic backgrounds (*etc1-1* in Col-0, *etc2-1* in Ws, *try-JC* in Col-0, and *try-82* in Ler) (Kirik et al. 2004a, 2004b). In contrast, all of the mutants used in this study are in the Col-0 background. Quadruple or triple mutants of the *CPC-LIKE MYB* gene family, including the *cpc try* mutations, have extreme phenotypes with trichome clustering and large variation in the size and branch number of individual trichomes. Individual trichomes are difficult to distinguish and accurately analyze in seedlings containing both the *cpc* and *try* mutations simultaneously (Kirik et al. 2004b; Tominaga et al. 2008). Thus, we did not include this pair of mutations in our study.

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Table 1. Trichome branch numbers. Data, including s.d., were obtained from at least 10 two-week-old third leaves from each line.

Genotype	Branches (br)/trichome (%)					
	1 br	2 br	3 br	4 br	5 br	6 br
Col-0	1±0.7	12±2	85±3	2±1	0	0
try	0	1±0.3	53±3	34±2	11±2	1±0.3
try etc1	0	1±0.5	38±4	44±2	16±2	1±0.5
try etc2	1±0.6	4±1	52±4	32±3	10±1	1±0.3
try etc1 etc2	0	3±1	40±2	42±3	14±1	1±0.5
try etc1 cpl3	0	2±0.7	35±2	43±2	18±2	2±0.9

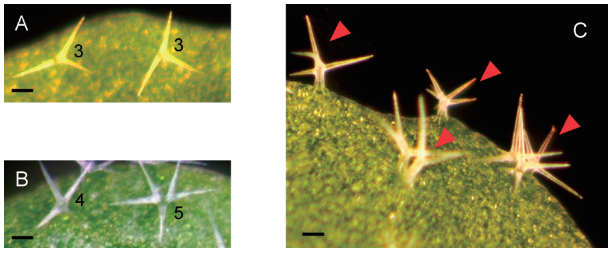

Figure 1. Leaf trichome phenotypes of *Arabidopsis* Col-0 and the *try etc1* mutant. Trichome phenotypes of wild-type Col-0 (A) and the *try etc1* double mutant (B, C) from the third leaves of 2-week-old *Arabidopsis* seedlings. The numbers shown in A, B indicate branch numbers. Arrowheads in C indicate trichome sites. Scale bars: 100 μm in A–C.

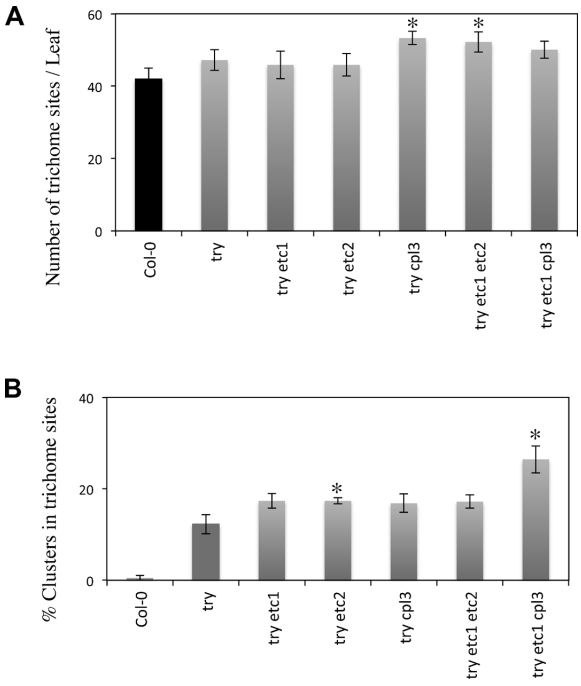

Figure 2. Trichome sites and trichome cluster formation on *Arabidopsis* leaves. (A) The number of trichome sites on the third leaves of wild-type Col-0, *try*, *try etc1*, *try etc2*, *try cpl3*, *try etc1 etc2*, and *try etc1 cpl3*. A trichome site is defined by the presence of a single trichome or a trichome cluster. The number of trichome sites per leaf was determined by counting at least 10 third leaves of 2-week-old seedlings from each mutant line. Error bars indicate the standard error. Bars marked with asterisks indicate a significant difference between the wild-type (Col-0) and the mutant lines in a Student' *t*-test ($p<0.050$). (B) The rate of cluster formation per trichome sites (%) on the third leaves of wild-type Col-0, *try*, *try etc1*, *try etc2*, *try cpl3*, *try etc1 etc2*, and *try etc1 cpl3*. The number of clusters per leaf was determined by counting at least 10 third leaves from 2-week-old seedlings of each mutant line. Error bars indicate the standard error. Bars marked with asterisks indicate a significant difference between the *try* mutant and the double or triple mutant lines in a Student' *t*-test ($p<0.050$).

The *Arabidopsis* Col-0 ecotype was used as the wild-type in this study. Construction of the *try* single mutant, *try etc1*, *try etc2* and *try cpl3* double mutants, and *try etc1 etc2* and *try etc1 cpl3* triple mutants was described previously (Tominaga et al. 2008). Seeds were surface sterilized and sown on 1.5% agar plates as described previously (Okada and Shimura 1990) and grown out for observation of seedling phenotypes. Seeded plates were kept at 4°C for 2 days and then incubated at 22°C under constant white light (50–100 μmol m^{-2} s^{-1}). For each mutant line, the trichomes on at least 10 third leaves of 2-week-old seedlings were recorded with a VC4500 3D digital fine microscope (Omron, Kyoto, Japan) or a digital microscope (VH-8000; Keyence, Osaka, Japan).

Consistent with the observations of previous studies (Hülskamp et al. 1994; Tominaga et al. 2008), trichomes of the *try* mutant had more branches than wild-type (Col-0) (Table 1). Trichomes of the *try etc1* double mutant consistently had more branches than wild-type or the *try* single mutant, with 44% and 16% of *try etc1* trichomes having four and five branches, respectively (Table 1; Figure 1A, B). This result suggests that the *TRY* and *ETC1* genes cooperatively regulate trichome branching. Trichomes of the *try etc2* double mutant had a branching phenotype similar to that of the *try* single mutant (Table 1). Furthermore, the branching phenotype of the *try etc1 etc2* triple mutant trichomes were similar to that of the *try etc1* double mutant (Table 1). These results suggest that the *ETC2* gene has little to no involvement in trichome branching. The *try etc1 cpl3* triple mutant trichomes also showed a similar branching phenotype to that of the *try etc1* double mutant (Table 1). Previously, we reported that the *cpl3* mutant trichomes consistently have fewer branches than wild-type, with approximately half of the *cpl3* trichomes having two branches (Tominaga et al. 2008). However, the *cpl3 try* double mutant showed a trichome branching phenotype

similar to that of the *try* single mutant (Tominaga et al. 2008), and the trichome branching phenotype of the *try etc1 cpl3* triple mutant was similar to that of the *try etc1* double mutant (Table 1). These results suggest that the *TRY* gene function in trichome branching is epistatic to the *CPL3* gene. Thus, the *cpl3* mutant phenotype with a reduced number of trichome branches may be masked by the *try* mutant phenotype with an increased number of trichome branches in the *try etc1 cpl3* triple mutant (Table 1). Previously, Szymanski and Marks reported that the *try* mutant induces increased endoreduplication in trichomes and reduced endoreduplication in the epidermis (Szymanski and Marks 1998). Another research group proposed that *TRY* is expressed in trichomes, thereby repressing endoreduplication in trichomes, followed by diffusion into neighboring cells to mediate lateral inhibition (Schellmann et al. 2002). Previously, we reported that the *cpl3* mutant had an increased level of endoreduplication in the epidermis and suggested that a decrease in trichome branching might be the result of reduced endoreduplication in trichomes (Tominaga et al. 2008). Thus, we proposed a model in which *CPL3* is expressed in young leaf epidermal cells and represses endoreduplication, after which *CPL3* affects neighboring trichome cells by slightly promoting endoreduplication (Tominaga et al. 2008). As was the case for *TRY*, the *ETC1* gene is expressed in trichomes (Tominaga et al. 2008) and may cooperatively act with *TRY* as a repressor of endoreduplication in trichomes (Table 1). Taken together, these findings suggested that *TRY* and *ETC1* were cooperative inhibitors, whereas *CPL3* was an enhancer and *ETC2* had no effect on trichome branching.

It was difficult to distinguish and accurately count individual trichomes in clusters, so we counted the number of trichome sites (Figure 1C). The number of trichome sites for the *try cpl3* double mutant, and *try etc1 etc2* triple mutant were significantly greater than wild-type (Col-0) (Figure 2A). The percentage of clusters in trichome sites were greater for the *try etc2* double mutant and the *try etc1 cpl3* triple mutant compared with the *try* single mutant (Figure 2B). The *try etc1 cpl3* triple mutant had significantly more clusters in trichome sites compared with the *try etc1* and *try cpl3* double mutants (Figure 2B). These results suggest that *ETC1*, *ETC2* and *CPL3* each promote the cluster formation of *TRY* (Figure 2B).

Among members of the *CPC-LIKE MYB* family, it is well-documented that *CPC* mainly acts in root hair differentiation, *TRY* mainly acts in trichome differentiation, and *ETC1*, *ETC2* and *CPL3* enhance *CPC* and *TRY* function in a redundant manner. In this study, we revealed that *ETC1* also enhances *TRY* function in the regulation of trichome branch number, and *ETC1*, *ETC2* and *CPL3* enhance *TRY* function in the regulation of trichome clustering. Members of the *CPC-LIKE MYB* gene family are thought to have evolved by gene duplication. Gene family members may have not completely diverged and, thus, retain some functional redundancy.

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References

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

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

Glover BJ, Martin C (2000) Specification of epidermal cell morphology. *Adv Bot Res* 31: 193–217

Hülskamp M, Misŕa S, Jürgens G (1994) Genetic dissection of trichome cell development in *Arabidopsis*. *Cell* 76: 555–566

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

Kirik V, Lee MM, Wester K, Herrmann U, Zheng Z, Oppenheimer D, Schiefelbein J, Hülskamp M (2005) Functional diversification of MYB23 and GL1 genes in trichome morphogenesis and initiation. *Development* 132: 1477–1485

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, 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

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

Masucci JD, Rerie WG, Foreman DR, Zhang M, Galway ME, Marks MD, Schiefelbein JW (1996) The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana*. *Development* 122: 1253–1260

Myers J, Bazely D (1991) Thorns, spines, prickles, and hairs: are they stimulated by herbivory and do they deter herbivores. In *Phytochemical induction by herbivores.* Edited by Tallamy DW, R.M. pp. 325–344. Wiley, New York.

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
Rerie WG, Feldmann KA, Marks MD (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, Hülskamp 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, Jürgens G, Hülskamp M (1999) Generation of a spacing pattern: the role of triptychon in trichome patterning in *Arabidopsis*. *Plant Cell* 11: 1105–1116
Schwab B, Folkers U, Ilgenfritz H, Hülskamp M (2000) Trichome morphogenesis in *Arabidopsis*. *Philos Trans R Soc Lond B Biol Sci* 355: 879–883
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
Szymanski DB, Marks MD (1998) GLABROUS1 overexpression and TRIPTYCHON alter the cell cycle and trichome cell fate in *Arabidopsis*. *Plant Cell* 10: 2047–2062
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
Wada T, Kurata T, Tominaga R, Koshino-Kimura Y, Tachibana T, Goto K, Marks MD, Shimura Y, Okada K (2002) Role of a positive regulator of root hair development, CAPRICE, in *Arabidopsis* root epidermal cell differentiation. *Development* 129: 5409–5419
Wada T, Tachibana T, Shimura Y, Okada K (1997) Epidermal cell differentiation in *Arabidopsis* determined by a Myb homolog, CPC. *Science* 277: 1113–1116
Wagner GJ, Wang E, Shepherd RW (2004) New approaches for studying and exploiting an old protuberance, the plant trichome. *Ann Bot* (Lond) 93: 3–11
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
Wang S, Hubbard L, Chang Y, Guo J, Schiefelbein J, Chen JG (2008) Comprehensive analysis of single-repeat R3 MYB proteins in epidermal cell patterning and their transcriptional regulation in *Arabidopsis*. *BMC Plant Biol* 8: 81
Zhang F, Gonzalez A, Zhao M, Payne CT, Lloyd A (2003) A network of redundant bHLH proteins functions in all TTG1-dependent pathways of *Arabidopsis*. *Development* 130: 4859–4869