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Note

The *Arabidopsis* CAPRICE protein fused to the VP16 transcriptional activation domain alters root hair and trichome development

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Abstract *CAPRICE* (*CPC*) is a key regulator of epidermal cell fate determination, including root hair and trichome formation, in *Arabidopsis thaliana*. *CPC* encodes an R3 MYB transcription factor and is known to be involved in the transcriptional repression of the downstream gene *GLABRA2* (*GL2*). We examined transgenic plants harboring CPC fused to a virus-derived transcriptional activator domain (CPC:VP16). Plants carrying *35S::CPC:VP16* showed increased root hair formation, similar to *35S::CPC* plants, compared to the wild-type plants. However, transgenic plants harboring *CPC:VP16* under the control of *CPC* promoter (*CPC::CPC:VP16*) showed similar root hair phenotype to that of the wild-type plants, suggesting an inherent cell-to-cell movement ability of CPC:VP16. In this study, all transgenic plants harboring CPC:VP16 possessed a reduced number of trichomes, nearly identical to that of *35S::CPC* plants, compared to the wild type. Furthermore, we observed some unusual tissues with ectopic trichome clusters in all transgenic plants harboring CPC:VP16. These results indicate that VP16 generally does not confer the transcriptional activation ability to CPC.

Key words: Arabidopsis, CAPRICE, root hair, trichome, VP16.

In *Arabidopsis thaliana*, the MYB (myeloblastosis) family is one of the largest groups of transcription factors with multiple functions in plant morphogenesis (Kranz et al. 1998; Stracke et al. 2001). Single-repeat R3 MYB transcription factors are a small subfamily of MYB transcription factors (Dubos et al. 2010). R3 MYB proteins consist of about 100 amino acids and are well known for their regulatory roles in epidermal cell fate determination, including root hair and trichome formation, in *Arabidopsis* (Tominaga-Wada et al. 2011). The *CPC* (*CAPRICE*) and *CPC*-like genes, including *TRIPTYCHON (TRY), ENHANCER OF TRY AND CPC1 (ETC1), ENHANCER OF TRY AND CPC2 (ETC2), ENHANCER OF TRY AND CPC3/CPC LIKE MYB3 (ETC3/CPL3), TRICHOMELESS1 (TCL1)*, and *TRICHOMELESS2/CPC LIKE MYB4 (TCL2/CPL4)* encode single-repeat R3 MYBs and positively regulate root hair formation and negatively regulate trichome formation in *Arabidopsis* (Esch et al. 2004; Gan et al. 2011; Kirik et al. 2004a, b; Schellmann et al. 2002; Simon et al. 2007; Tominaga et al. 2008; Tominaga-Wada and Nukumizu 2012; Wang et al. 2007). Moreover, the CPC protein moves from non-hair cell to root-hair cell, and its movement correlates with the formation of root hairs (Kurata et al. 2005). Wang and Chen proposed the cell-to-cell movement model of CPC family proteins in trichome development (Wang and Chen 2014).

In contrast to *CPC*, *WEREWOLF* (*WER*) encodes an R2R3 type MYB transcription factor and promotes differentiation of the non-hair cell fate (Lee and Schiefelbein 1999). Previously, we suggested that CPC evolved from WER as a result of truncation of the activation domain and loss of DNA binding ability (Tominaga et al. 2007). The *GLABRA2* (*GL2*) gene encodes a homeodomain-leucine zipper protein and is thought to act farthest downstream in the root hair and trichome regulatory cascade (Bernhardt et al. 2005; Galway et al. 1994; Lee and Schiefelbein 1999; Rerie et al. 1994; Wada et al. 1997). WER, GLABRA3 (GL3), ENHANCER OF GLABRA3 (EGL3), and TRANSPARENT TESTA GLABRA1 (TTG1) transcription factors form a complex and promote *GL2* expression (Koshino-Kimura et al. 2005). The CPC protein binds to this transcriptional complex and inhibits WER binding, resulting in the repression of *GL2* expression (Koshino-Kimura et al. 2005; Tominaga et al. 2007; Wada et al. 2002). In addition, we demonstrated that at least two amino acids are sufficient to convert WER to a CPC function, from an activator to a repressor of *Arabidopsis* non-hair cell development (Tominaga-Wada et al. 2012). In the present study, we examined the function of CPC fused to a virus-derived transcriptional

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activator domain (CPC:VP16). VP16 has been reported to change the suppressor function of GL2 to the activator function (Lin et al. 2015). Our results contribute to the molecular understanding of how CPC acts as a transcriptional regulator.

Wild-type plants of *Arabidopsis thaliana* ecotype Columbia (Col-0) and the *cpc-2* mutant (Col-0 background) (Kurata et al. 2005) were used in the present study. Seeds were surface sterilized and sown on the surface of 1.5% agar plates according to a previously described method (Okada and Shimura 1990) and the seedling phenotypes were subsequently observed. Seeded plates were incubated at 4°C for 2 days and then at 22°C for 5 days under continuous white light (50–100 μmol m^{-2} s^{-1}). For each transgenic line, 5-day-old seedlings were assayed for root hair formation, and 2-week-old third leaves were observed for trichome formation.

The *35S::CPC:VP16* construct was introduced into wild-type and *cpc-2* mutant plants, and the *CPC::CPC:VP16* construct was introduced into wild-type plants by using the floral dip method (Clough and Bent 1998). To produce *35S::CPC:VP16*, *CPC* cDNA and the *VP16* activation domains were cloned into pBI121 (Clontech, Mountain View, CA, USA). To produce *CPC::CPC:VP16*, the 1.3-kb *CPC* promoter region, *CPC* cDNA, and *VP16* activation domain were cloned into pBI101 (Clontech).

The *35S::CPC:VP16* construct in wild-type, the *35S::CPC:VP16* construct in *cpc-2* mutant, and the *CPC::CPC:VP16* construct in wild-type transgenic plants were observed using a Leica MZ16FA stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany). Images were recorded using a high-sensitivity CCD color camera system (Keyence VB 7010, Osaka, Japan). To observe the phenotype of trichomes, we used a scanning electron microscope (model JSM5610-LV; JEOL, Akishima, Japan).

Compared to the wild type, *35S::CPC:VP16* transgenic plants showed a phenotype with greater number of root hairs (Figure 1A and C). This result is similar to that found in *35S::CPC* plants, suggesting a similar function of CPC:VP16 and CPC (Wada et al. 1997). Plants carrying *35S::CPC:VP16* possessed fewer trichomes than did wild-type plants (Figure 1B and D), whereas *35S::CPC* plants show no trichomes on the leaves (Wada et al. 1997). Although *35S::CPC:VP16* plants produced trichomes, especially on the leaf margin, the phenotype with less trichomes was also nearly identical to that of *35S::CPC* plants (Figure 1D). The *35S::CPC:VP16* construct in *cpc-2* transgenic plants also showed phenotypes with many root hairs and less trichomes compared to the wild type (Figure 1A, B, F, and G). Some trichomes were observed on the leaf margin (Figure 1G). Taken together, *35S::CPC:VP16* in the wild type and in *cpc-2* showed similarity in phenotypes in terms of root hair and leaf trichome.

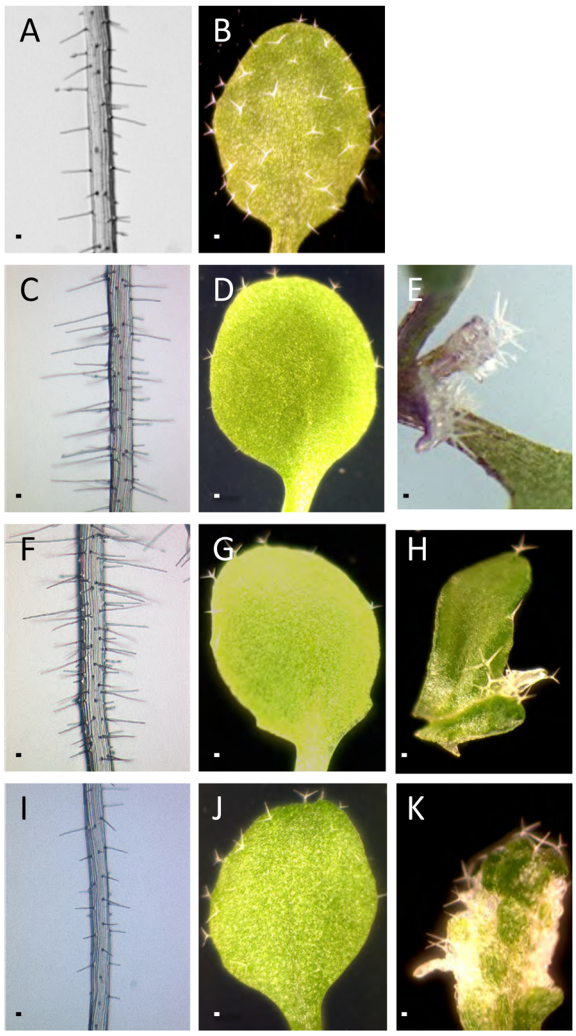

Figure 1. Root and leaf phenotypes of transgenic *Arabidopsis*. Root and leaf epidermal phenotypes of *Arabidopsis* expressing *35S::CPC:VP16* and *CPC::CPC:VP16*. Root hair formation in 5-day-old *Arabidopsis* seedlings of wild-type Col-0 (A), *35S::CPC:VP16* (C), *35S::CPC:VP16* in *cpc-2* (F), and *CPC::CPC:VP16* (I). Trichome formation on 2-week-old *Arabidopsis* third leaves in wild-type Col-0 (B), *35S::CPC:VP16* (D), *35S::CPC:VP16* in *cpc-2* (G), and *CPC::CPC:VP16* (J). Ectopic trichome formation on 2-week-old *Arabidopsis* true leaves in *35S::CPC:VP16* (E), *35S::CPC:VP16* in *cpc-2* (H), and *CPC::CPC:VP16* (K). Scale bars: 500 μm.

In contrast, *CPC::CPC:VP16* plants did not show any obvious difference in root hair formation compared to the wild type (Figure 1A and I). However, trichome formation was reduced and few trichomes were observed at the leaf margins of *CPC::CPC:VP16* plants, similar to *35S::CPC:VP16* plants (Figure 1J).

Surprisingly, all transgenic lines carrying *CPC:VP16* transgenes sometimes produced unusual tissues with ectopic trichome clusters (Figure 1E, H, and K). SEM images of the adaxial surface of the true leaf showed that trichomes of *35S::CPC:VP16* plants had greater number

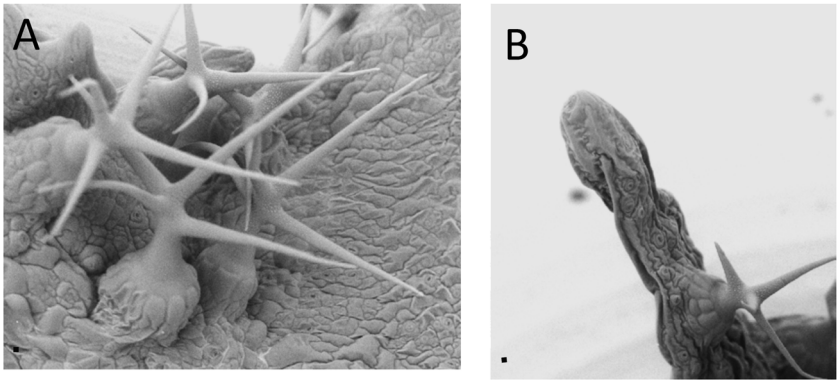

Figure 2. SEM observation of transgenic *Arabidopsis* leaves. SEM observation of epidermal phenotypes of true leaves expressing *35S::CPC:VP16* and *CPC::CPC:VP16*. Adaxial surface of true leaves of *35S::CPC:VP16* in *cpc-2* (A) and *CPC::CPC:VP16* (B) plants. Scale bars: 100 μm.

of branches than the wild type (Figure 2A). In addition, protruding tissues covered by pavement cells, guard cells, and trichomes were observed in *CPC::CPC:VP16* plants (Figure 2B).

The activity of the CPC:VP16 fusion protein is similar to that of the CPC protein in terms of root hair formation. The root hair phenotype of *35S::CPC:VP16* plants is similar to that of *35S::CPC* plants (Wada et al. 1997). This result indicated that, by using the *35S* promoter, CPC protein, or CPC:VP16 protein is localized in all epidermal cells where CPC repressed *GL2* expression and induced the initiation of root hair formation (Tominaga-Wada et al. 2011). However, the root hair phenotype of *CPC::CPC:VP16* is similar to that of the wild type, suggesting the movement of CPC:VP16 protein from non-hair cell to root-hair cell probably because the CPC promoter is active only in hairless cells (Kurata et al. 2005; Wada et al. 2002). These results suggest that CPC:VP16 maintains the CPC function of cell-to-cell movement in root epidermal cells and is able to establish the non-hair and root-hair cell files in the *Arabidopsis* root epidermis.

For trichome formation, *35S::CPC:VP16*, *35S::CPC:VP16* in *cpc-2*, and *CPC::CPC:VP16* reduced the number of trichomes, which is nearly identical to the phenotype of *35S::CPC* (Figure 1D, G, and J) (Wada et al. 1997). Occasionally, these transgenic plants showed strange trichome phenotypes, demonstrated by the clustering trichomes and protruding trichomes (Figure 1E, H, and K). It is unclear whether these ectopic trichome phenotypes reflect the direct function of CPC:VP16 or are caused by a secondary effect. Nonetheless, CPC:VP16 influences trichome phenotypes directly or indirectly and might disturb the formation of appropriate trichome number and branching number and induce clustering. In contrast to the root epidermis, trichome phenotype in *CPC::CPC:VP16* plants was different from that of the wild type (Figure 1B, J, and K). CPC:VP16 might at least partially lose the ability of movement from a trichome precursor cell to its neighboring cell.

We concluded that CPC:VP16 has a similar function to that of CPC, but it occasionally promotes the development of ectopic trichomes. The root hair phenotype of the transgenic plants expressing *CPC:VP16* under the control of the *CPC* promoter (*CPC::CPC:VP16*) was similar to that of the wild-type plants, suggesting the cell-to-cell movement of CPC:VP16 from non-hair cells to root-hair cells. In contrast, *CPC::CPC:VP16* phenotype showed reduced number of trichomes, suggesting at least partial loss of the cell-to-cell movement ability in leaf epidermal cells.

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