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Short communication

Localization of ENHANCER OF TRY AND CPC1 protein in *Arabidopsis* root epidermis

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ABSTRACT

CAPRICE (CPC) is a R3-type MYB transcription factor, which induces root-hair cell differentiation in *Arabidopsis thaliana*. The *CPC* homologous gene *ENHANCER TRY AND CPC1* (*ETC1*) has a similar function to *CPC,* and acts in concert with *CPC*. The CPC protein moves between root epidermal cells, from hairless cells to the neighboring cells, and promotes root-hair differentiation. Therefore, ETC1 is predicted to have movement ability similar to that of CPC. In this study, we generated *ETC1:ETC1:GFP* and *CPC:ETC1:GFP* transgenic plants to clarify whether ETC1 exhibits cell-to-cell movement. Transgenic plants showed many-root-haired and trichome-less phenotypes, similar to those observed in *CPC:CPC:GFP* plants, suggesting a similar function of ETC1 and CPC. However, the ETC1:GFP fusion protein located exclusively to the hairless cells in both *ETC1:ETC1:GFP* and *CPC:ETC1:GFP* transgenic plants. These results indicate that, unexpectedly, the ETC1 protein cannot move in the root epidermis from hairless cells to the neighboring cells.

1. Introduction

Root hair and trichrome development represent well-studied model systems for cell-fate specification in *Arabidopsis thaliana*. Some transcription factors have been shown to contribute to this regulatory network (Tominaga-Wada et al., 2011). *CAPRICE* (*CPC*) encodes a single repeat R3-type MYB transcription factor, which regulates the differentiation of root hair cells (Wada et al., 1997). Six additional *CPC*-like MYB genes have been identified in the *Arabidopsis* genome. The CPC family includes *ENHANCER OF TRY AND CPC1* and *2* (*ETC1* and *ETC2*) (Esch et al., 2004; Kirik et al., 2004a), *CPC-LIKE MYB3/ENHANCER OF TRY AND CPC3* (*CPL3/ETC3*) (Simon et al., 2007; Tominaga et al., 2008; Wang et al., 2008), *TRYPTICHON* (*TRY*) (Schellmann et al., 2002; Schnittger et al., 1999), *TRICHOMELESS1* (*TCL1*) (Wang et al., 2007), and *CPC-LIKE MYB4/TRICHOMELESS2* (*CPL4/TCL2*) (Gan et al., 2011; Tominaga-Wada and Nukumizu, 2012). As the names suggest, ETC1 and ETC2 act to enhance the activity of TRY and CPC (Esch et al., 2004; Kirik et al., 2004a, 2004b).

Previously, we have demonstrated that the CPC protein moves from the hairless cells, where it is expressed, to neighboring root hair cells in the *Arabidopsis* root epidermis (Kurata et al., 2005; Wada et al., 2002). The ability of CPC to move from hairless cells to neighboring cells might be important for the identity of root hair cells (Kurata et al., 2005). Therefore, CPC and other CPC-like MYBs are expected to move from the hairless cells to the root hair cells (Schiefelbein et al., 2014; Wang and Schiefelbein, 2014). However, recently we demonstrated that one of the CPC-like MYB proteins, CPL3, cannot move from hairless cells to root hair cells (Tominaga-Wada and Wada, 2016). Normally, *CPL3* is not expressed in roots, and is only expressed in leaves (Tominaga et al., 2008). We introduced *CPL3:GFP* into *Arabidopsis* using the *CPC* promoter to drive the expression of *CPL3* in roots. The CPL3:GFP fusion protein localized entirely to the hairless cells of *CPC:CPL3:GFP* transgenic plants, suggesting that CPL3 cannot move between the cells (Tominaga-Wada and Wada, 2016).

Further analyses were performed to investigate CPC-like MYBs in the root epidermis. Because the *ETC2*, *TCL1,* and *TCL2* genes are not expressed in roots, and the primary function of TRY relates to trichome differentiation (Gan et al., 2011; Hulskamp et al., 1994; Kirik et al., 2004b; Tominaga-Wada and Nukumizu, 2012; Wang et al., 2007), we excluded these genes from further analyses. In this study, we focused on the ability of ETC1 to move from cell-to-cell. We compared *ETC1:ETC1:GFP* and *CPC:ETC1:GFP* transgenic plants with *CPC:CPC:GFP* transgenic plants.

Abbreviations: CPC, CAPRICE; ETC1, ENHANCER OF TRY AND CPC1; ETC2, ENHANCER OF TRY AND CPC2; CPL3, CPC LIKE MYB3; TCL1, TRICHOMELESS1; TCL2, TRICHOMELESS2; TRY, TRYPTICHON

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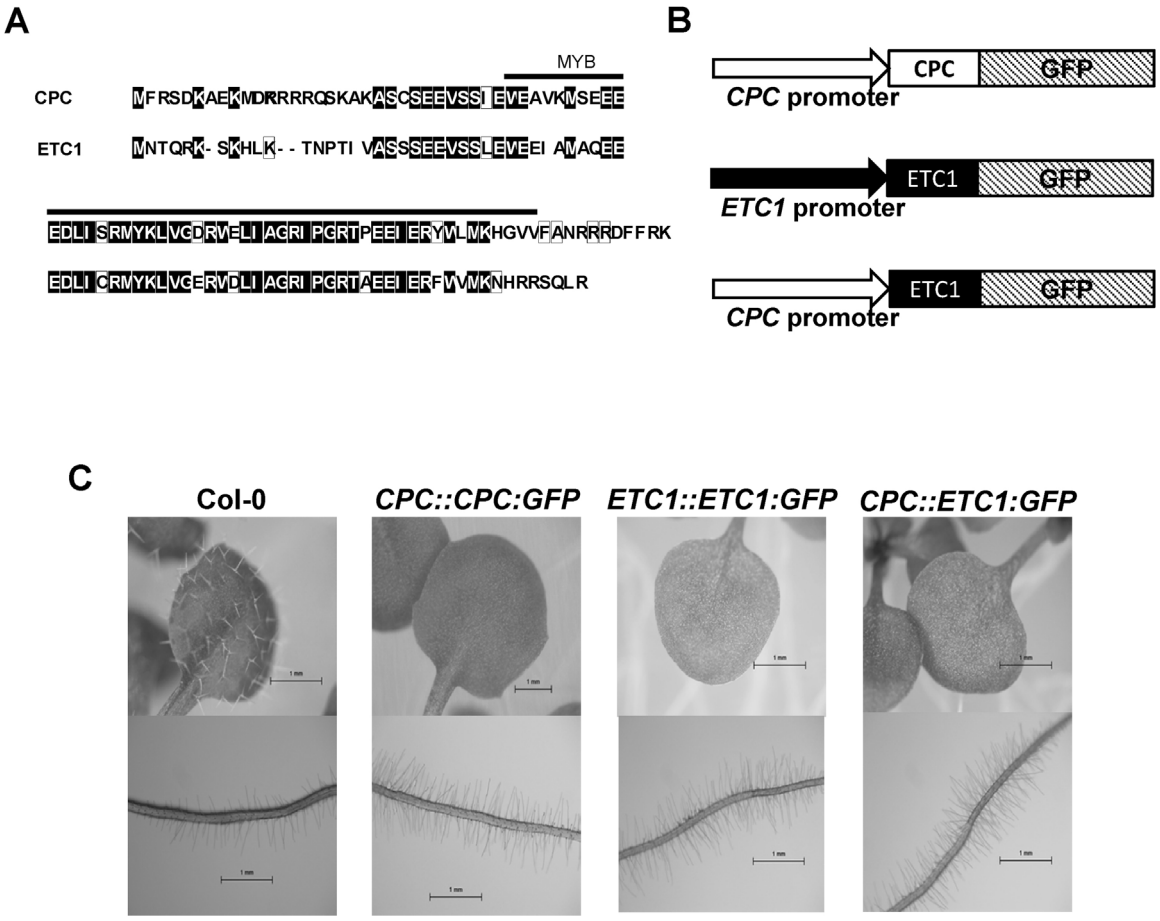

Fig. 1. Phenotypes of ETC1:GFP transgenic plants.(A) Alignment of CPC and ETC1 amino acid sequences. The line at the top indicates the R3 MYB domain. Letters shaded in black indicate identical amino acids. (B) Schematic illustration of the *CPC:CPC:GFP*, *ETC1:ETC1:GFP*, and *CPC:ETC1:GFP* chimeric constructs. At the top, the construct consisting of the *CPC* promoter, *CPC*, and GFP is shown. The *ETC1* promoter, *ETC1*, and GFP constructs are also shown. At the bottom, the chimeric construct containing the *CPC* promoter, *ETC1*, and GFP is shown. (C) Two-week-old third leaves of wild-type Col-0, *CPC:CPC:GFP*, *ETC1:ETC1:GFP*, and *CPC:ETC1:GFP* plants. Five-day-old seedling roots of wild-type Col-0, *CPC:CPC:GFP*, *ETC1:ETC1:GFP*, and *CPC:ETC1:GFP*. Scale Bars: 1 mm.

Table 1
Leaf trichome and root hair numbers in wild type Col-0, *CPC:CPC:GFP*, *ETC1:ETC1:GFP* and *CPC:ETC1:GFP Arabidopsis* plants.

	Trichomes per leaf	Root hairs per mm
Col-0	44.0 ± 3.7	40.8 ± 0.8
CPC:CPC:GFP	0 ± 0	79.0 ± 4.5
ETC1:ETC1:GFP#1	0 ± 0	81.1 ± 3.8
ETC1:ETC1:GFP#2	0 ± 0	89.3 ± 4.0
CPC:ETC1:GFP#1	0 ± 0	79.6 ± 4.0
CPC:ETC1:GFP#2	0 ± 0	91.5 ± 3.6

Data are presented as means ± SD of at least five plants per experiment.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana (L.) Heynh. ecotype Columbia (Col-0) was used as the wild type. The *CPC:CPC:GFP* transgenic line has been previously described (Wada et al., 2002). Seeds were sown on 1.5% agar plates using a previously described method (Okada and Shimura, 1990). The number of root hairs per millimeter was determined using ten 5-day-old seedlings from each line. The number of trichomes per leaf was determined using five 2-week-old third leaves from each line.

2.2. Gene constructs and transgenic plants

Primer sequences used to amplify the genomic fragments are listed in Table S1. To create *ETC1:ETC1:GFP*, a PCR-amplified 2.3-kb *ETC1* genome fragment (primer pairs RT67/RT68) was ligated into *pBS-GFP*. To create *CPC:ETC1:GFP*, a 1.9-kb PCR-amplified fragment (primer pairs RT296/CF2_NOSterSma) was inserted into the *pBS-CPC* promoter. Subsequently, sequenced fragments were ligated into *pJHA212K* (Yoo et al., 2005) using appropriate restriction sites. The floral dip method was used to *transform Arabidopsis* (Clough and Bent, 1998).

2.3. Real time RT-PCR

Total RNA was prepared, and real-time RT-PCR was performed as described previously (Wada and Tominaga-Wada, 2015) using the primer pairs listed in Table S1.

2.4. Microscopy

Root and leaf images were observed using a Leica MZ16FA stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany). GFP fluorescence was observed using a Zeiss LSM-510 Meta confocal laser scanning microscope (CLSM).