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Short communication

# The ectopic localization of CAPRICE LIKE MYB3 protein in *Arabidopsis* root epidermis

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ABSTRACT

Cell fate determination is a critical step of plant morphogenesis. Root hair and trichome formation is a good model for studying cell fate determination. The gene *CAPRICE* (*CPC*) encodes an R3 type MYB transcription factor, promotes root hair formation, and inhibits trichome formation in *Arabidopsis thaliana*. The *CPC* homologous gene *CPC LIKE MYB3* (*CPL3*) encoded 66% similar amino acid sequence to CPC, and it also possessed a cell-to-cell movement WxM motif. CPC protein moves from non-hair cells to neighboring root hair forming cells and induces root hair formation in *Arabidopsis* root epidermal cells. In this study, to investigate the function and cell-to-cell movement ability of CPL3, we generated *CPC:CPL3:GFP* transgenic plants to compare against *CPL3:CPL3:GFP* transgenic plants. *CPC:CPL3:GFP* transgenic plants showed no-trichome and many root-hair phenotypes, confirming similar function of CPL3 to CPC in root hair and trichome cell fate determination. However, CPL3:GFP fusion protein localized exclusively in non-hair cells in *CPC:CPL3:GFP* transgenic plants. Collectively, our results suggest that the CPL3 protein does not have cell-to-cell movement ability. Our findings indicate that the CPC family includes a movement protein and a protein that does not move. We believe our results provide new insight into the regulatory mechanism that mediates epidermal cell fate determination.



## 1. Introduction

*Arabidopsis* (*Arabidopsis thaliana*) epidermal cell differentiation, including trichome and root hair formation, provides a model system for understanding cell fate determination. Previous molecular genetic studies have identified several transcription factors, which are involved in this regulatory network (Tominaga-Wada et al., 2011). The gene *CAPRICE* (*CPC*), which encodes an R3 type MYB (myeloblastosis) transcription factor, is known as an important regulator of root hair differentiation in *Arabidopsis* (Wada et al., 1997). Additional *CPC* homologous genes have been identified, including *TRYPTICHON* (*TRY*) (Schellmann et al., 2002; Schnittger et al., 1999), *ENHANCER OF TRY AND CPC1* (*ETC1*) (Esch et al., 2004; Kirik et al., 2004a), *ENHANCER OF TRY AND CPC 2* (*ETC2*) (Kirik et al., 2004b), *ENHANCER OF TRY AND CPC3/CPC-LIKE MYB3* (*ETC3/CPL3*) (Simon et al., 2007; Tominaga et al., 2008; Wang et al., 2008), *TRICHOMELESS1* (*TCL1*) (Wang et al., 2007), and *TRICHOMELESS2/CPC-LIKE MYB4* (*TCL2/CPL4*) (Gan et al., 2011; Tominaga-Wada and Nukumizu, 2012). The genes *GLABRA3* (*GL3*) (Payne et al., 2000) and *ENHANCER OF GLABRA3* (*EGL3*) (Bernhardt et al., 2003) encode homologous basic helix-loop-helix (bHLH) transcription factors and function in trichome and non-hair cell differentiation in *Arabidopsis*. *GLABRA2* (*GL2*), which encodes a homeodomain leucine zipper protein, is thought to act as a crucial regulatory factor farthest downstream of the epidermal cell fate regulatory pathway to promote trichome and non-hair cell differentiation in *Arabidopsis* (Bernhardt et al., 2005; Galway et al., 1994; Lee and Schiefelbein, 1999; Rerie et al., 1994; Wada et al., 1997). Transcription of *GL2* is enhanced by the WEREWOLF (WER)-GL3/EGL3-TRANSPARENT TESTA GLABRA1 (TTG1) (WER-GL3/EGL3-TTG1) transcriptional protein complex (Koshino-Kimura et al., 2005). *WER* encodes an R2R3 type MYB transcription factor and positively regulates non-hair cell differentiation in the *Arabidopsis* root epidermis (Lee and Schiefelbein, 1999). *TTG1*, which encodes a WD-40 protein, also promotes non-hair cell differentiation (Galway et al., 1994). The GL3/EGL3 proteins are reported to interact with WER (Bernhardt et al., 2003) and TTG1 in yeast two-hybrid systems (Esch et al., 2003; Payne et al., 2000; Zhang et al., 2003). *GLABRA1* (*GL1*), which encodes a WER homologous MYB transcription factor, also forms a transcriptional complex with GL3/EGL3 and TTG1 to induce *GL2* expression mainly in trichomes (Lee and Schiefelbein, 2001; Payne et al., 2000; Szymanski et al., 1998; Zhang et al., 2003). The CPC, TRY, ETC1, ETC2, and CPL3 proteins are shown to interact with GL3/EGL3 in yeast cells (Tominaga

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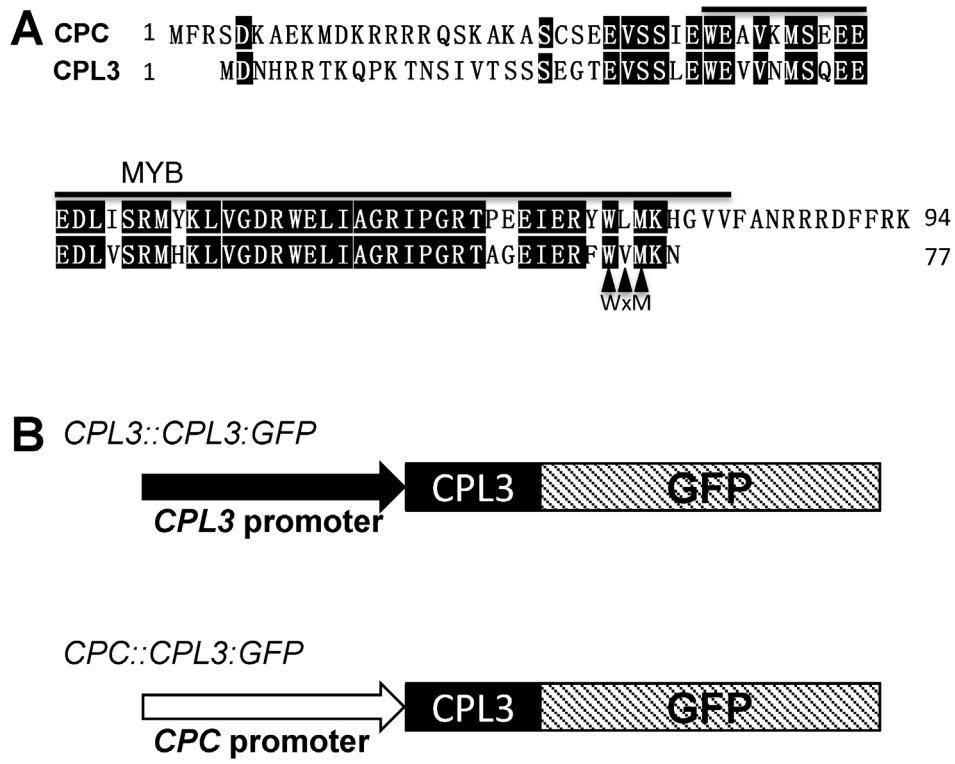


**Fig. 1.** CPC and CPL3 amino acid sequences and constructs containing *CPC* and *CPL3*.
(A) Amino acid sequence alignment of *CPC* and *CPL3*. Identical amino acids are shaded in black. The amino acids comprising the R3 MYB domain are indicated by a black line. The WxM sequence, which is required for cell-to-cell movement, is indicated by arrowheads on the bottom of the amino acids. (B) Schematic representation of the *CPL3:CPL3:GFP* and *CPC:CPL3:GFP* chimera constructs. At the top, the construct consisting of the *CPL3* promoter, *CPL3*, and 2xGFP is shown. At the bottom, the chimera construct containing the *CPC* promoter, *CPL3*, and 2xGFP is shown.

et al., 2008). The CPC protein competitively inhibits WER binding to GL3/EGL3 and generates the CPC-GL3/EGL3-TTG1 complex leading to inhibition of *GL2* expression (Lee and Schiefelbein, 2002; Tominaga et al., 2008).

Previously, we have shown that *CPC* is expressed exclusively in non-hair cells and the translated CPC protein moves from non-hair to root-hair cells in the *Arabidopsis* root epidermis (Kurata et al., 2005; Wada et al., 2002). Cell-to-cell movement of CPC is thought to be crucial for root hair formation (Kurata et al., 2005). In addition, the cell-to-cell movement motif, WxM, is conserved across all CPC family MYB proteins (Wang and Schiefelbein, 2014). Thus, CPC-like MYB proteins, specifically CPC/TRY/ETC1, are assumed to move from non-hair to root-hair cells in the *Arabidopsis* root epidermis (Schiefelbein et al., 2014; Wang and Schiefelbein, 2014).

In this study, we explored the cell-to-cell movement ability of the CPL3 protein in *Arabidopsis* by comparing *CPC:CPL3:GFP* to *CPL3:CPL3:GFP* transgenic plants.

## 2. Materials and methods

### 2.1. Plant materials and growth conditions

*Arabidopsis thaliana* (L.) Heynh. Col-0 ecotype was used as the wild type. The *CPL3:CPL3:GFP* transgenic plants used in this study were described previously (Tominaga et al., 2008). Seeds were surface-sterilized and sown on the surface of 1.5% agar plates as described previously (Okada and Shimura, 1990). Seeded plates were incubated at 4 °C for 2 d, then at 22 °C under constant white light (50–100 $\mu$mol $m^{-2}s^{-1}$) for 5 d for root hair and GFP observations, and for 2 weeks for trichome observations. For each transgenic line, a minimum of five two-week-old third leaves were assayed for trichome numbers, and a minimum of ten individual five-day-old seedlings were assayed for root epidermis changes.

### 2.2. Gene construct

A 1.9 kb PCR-amplified linear *CPL3:2xGFP* fragment (primers ATATGAGCTCATGGATAACCATCGCAGGAC-TAAG/TATACCCGGGCTGCAGGATTCTCA), using *pBS-CPL3:2xGFP* as the template (Tominaga et al., 2008), was digested with *SacI*. This fragment was ligated into the *SacI* and *EcoRV* sites of the *pBS-CPCpromoter* (Kurata et al., 2005) to create *pBS-CPC:CPL3:GFP*. *pBS-CPC:CPL3:GFP* was digested with *SalI* and the fragment was ligated into the *SalI* site of the binary vector, *pJHA212K* (Yoo et al., 2005) to create *CPC:CPL3:GFP*.

### 2.3. Transgenic plant

Plant transformation with *CPC:CPL3:GFP* was performed using a floral dip method (Clough and Bent, 1998), and transformants were selected on 0.5 × MS (minimal salt) agar plates containing 50 mg/L kanamycin. Homozygous transgenic lines were selected for kanamycin resistance.

### 2.4. Microscopy

To observe trichomes, at least five two-week-old third leaves in individual transgenic lines were analyzed for trichome number by light microscopy using a Leica MZ16FA stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany). The images were recorded with a VC4500 3D digital fine microscope (Omron, Kyoto, Japan) or a digital microscope (VH-700; Keyence, Osaka, Japan). For each transgenic line, a minimum of ten individual