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Short Communication

Effect of amino acid substitution of CAPRICE on cell-to-cell movement ability in *Arabidopsis* root epidermis

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ABSTRACT

An R3-type MYB transcription factor, CAPRICE (CPC), is known to promote root hair cell differentiation in *Arabidopsis* root epidermis. The CPC protein moves from non-hair cells to the neighboring cells, and acts as an inducer of root hair formation. In contrast, we previously showed that the CPC homolog, ENHANCER OF TRY AND CPC1 (ETC1), does not move between the root epidermal cells. To clarify the critical difference in the cell-to-cell movement ability of CPC and ETC1 proteins, we generated five different chimeras of CPC and ETC1. As expected, four of the five chimeric proteins with substitution of CPC amino acids with those of ETC1 induced many root hair and no-trichome phenotype, like CPC. These chimeric proteins essentially maintained the cell-to-cell movement ability of CPC. However, one chimeric protein in which ETC1 was sandwiched between the CPC-specific movement motifs of S1 and S2 did not induce ectopic root hair formation. This chimeric protein did not move between the cells. These results indicate that the maintenance of not only the S1 and S2 motifs but also the precise structure of CPC protein might be necessary for the cell-to-cell movement of CPC. Our results should help in further unraveling of the roles of these MYB transcription factors in root hair formation.

1. Introduction

Intercellular communication is important for plant development. The cell-to-cell movement of transcription factors, through plasmodesmata, has been demonstrated to be essential for plant cell differentiation (Ruiz-Medrano et al., 2004; Zambryski, 2004). For instance, the homeodomain transcription factor, KNOTTED1 (KN1), moves between cells in the maize shoot apical meristem (SAM) and functions in the initiation and maintenance of the SAM (Kitagawa and Jackson, 2017). The KNOX homeodomain sequence is necessary for KN1 trafficking (Kim et al., 2005). The mechanism of intercellular movement of KN1 depends on type II chaperonin complex CCT8 (Heinlein, 2015). KN1 also interacts with the microtubule-associated protein, MPB2C, which negatively regulates plasmodesmata targeting and transport of KN1 protein (Heinlein, 2015). Another homeodomain transcription factor, WUSCHEL (WUS), also moves between cells in the SAM of *Arabidopsis* to maintain the stem cell pool (Kitagawa and Jackson, 2017). The mobility of WUS is mediated by multiple domains (Daum et al., 2014). The fate of root endodermis cells is determined by SHORT-ROOT (SHR), a GRAS family transcription factor, which moves from the stele to the endodermis in *Arabidopsis* roots (Han et al., 2014). GRAS and VHIID domains are required for cell-to-cell movement of SHR (Gallagher and Benfey, 2009). The movement of SHR is facilitated by its interaction with the endosome-associated protein, SHR-INTERACTING EMBRYONIC LETHAL (SIEL) (Koizumi et al., 2011).

Previously, we reported that an R3-type MYB transcription factor protein, CAPRICE (CPC), moves between the root epidermal cells in *Arabidopsis* (Wada et al., 2002). This movement ability of CPC, which depends on S1 (MFRSDKAEK) and S2 (WxM) motifs, was proposed to be necessary for root hair differentiation (Kurata et al., 2005). *CPC* forms the *CPC-like MYB* gene family, which includes *ENHANCER OF TRY AND CPC1* (*ETC1*) (Esch et al., 2004; Kirik et al., 2004a), *ENHANCER OF TRY AND CPC2* (*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), *TRYPTICHON* (*TRY*) (Schellmann et al., 2002; Schnittger et al., 1999), *TRICHOMELESS1* (*TCL1*) (Wang et al., 2007), and *TRICHOMELESS2/CPC-LIKE MYB4* (*TCL2/CPL4*) (Gan et al., 2011; Tominaga-Wada and Nukumizu, 2012). The members of this gene family are involved in the induction of root hair differentiation and in the reduction of trichome formation in *Arabidopsis* (Wang and Chen, 2014a).

Recently, we found that the homologs of CPC, namely ETC1 and CPL3, do not move from non-hair cells to root hair cells (Tominaga-Wada et al., 2017a, 2017b; Tominaga-Wada and Wada, 2016). Both

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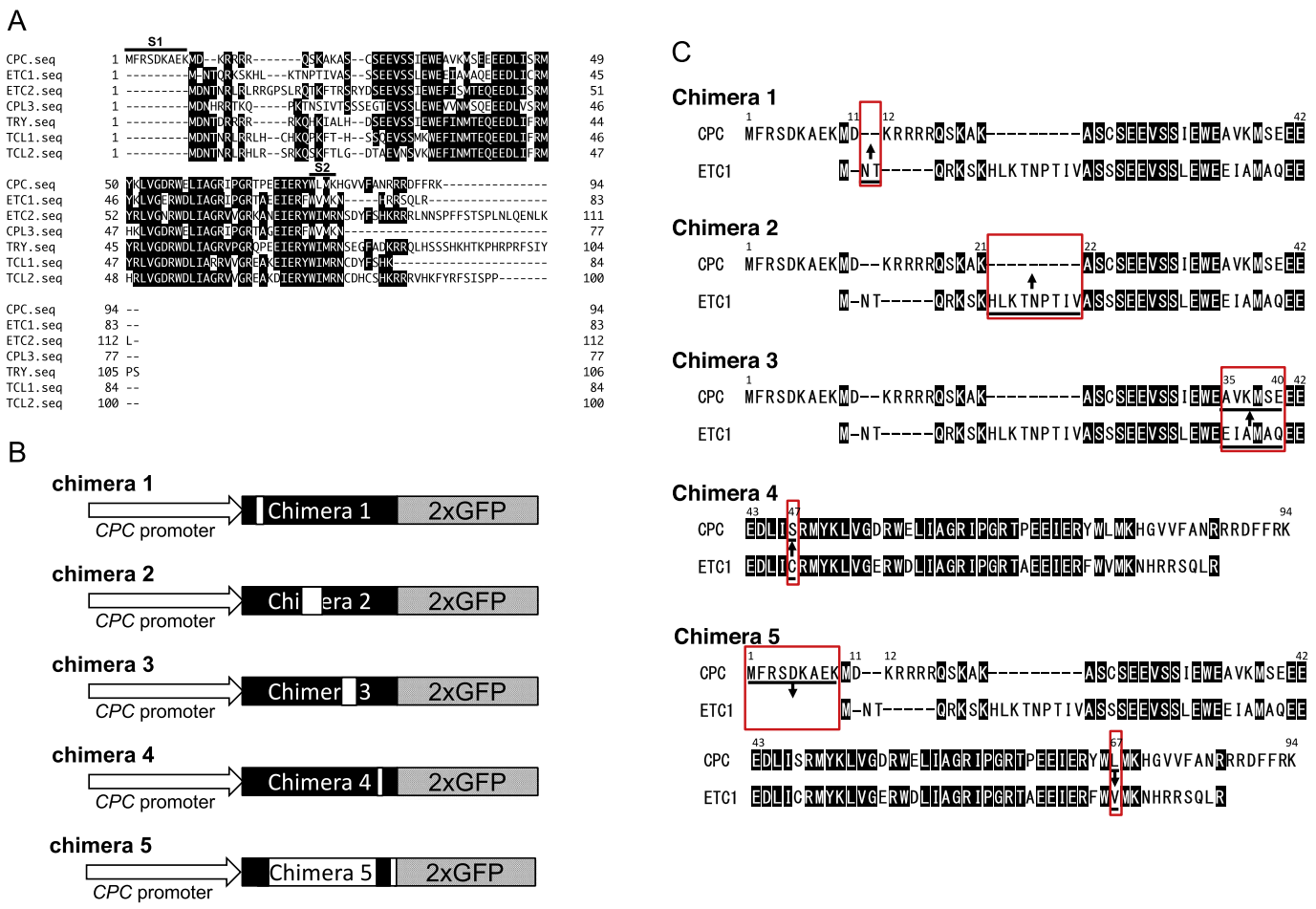

Fig. 1. Amino acid sequences of CPC family and CPC–ETC1 chimeric proteins. (A) Sequence alignment of CPC-homologs of R3-MYB proteins (CPC, ETC1, ETC2, CPL3, TRY, TCL1, and TCL2). Letters shaded in black indicate identical amino acids. The amino acids comprising the S1 and S2 domains are indicated by black lines. (B) Schematic representation of the construct for CPC–ETC1 chimeric proteins (Chimera 1, Chimera 2, Chimera 3, Chimera 4, and Chimera 5). The constructs consisting of the *CPC* promoter, *CPC–ETC1* chimera, and *2xGFP* are shown. Black and white boxes indicate the *CPC* and *ETC1* regions, respectively. (C) Alignment of amino acid sequences of CPC and ETC1. Identical amino acids are shaded in black. The positions of insertion or substitution of amino acids, are indicated with arrows and boxes outlined in red. Numbers above the sequence indicate the position from the N-terminal residue of CPC.

ETC1 and CPL3 lack the S1 motif, but have the S2 motif. We made a construct in which S1 sequence was fused to the ETC1 sequence to produce a chimeric S1:ETC1 protein (Tominaga-Wada et al., 2017b). However, S1:ETC1, with a structure resembling that of CPC, did not move between the root epidermal cells (Tominaga-Wada et al., 2017bc). We, therefore, concluded that S1 and S2 motifs, which are defined as the "movement motifs", are not sufficient for ETC1 to acquire the ability for cell-to-cell movement.

Results obtained in our previous study implied that, in addition to S1 and S2 motifs, CPC might contain amino acid(s) that are critical for cell-to-cell movement (Tominaga-Wada et al., 2017b). Therefore, we generated five constructs to produce CPC–ETC1 chimeric proteins (Chimera 1–5). Each ETC1 specific region was introduced into an equivalent position in CPC. We examined the inhibitory effect of specific ETC1 residue(s) on the movement of CPC–ETC1 chimera.

2. Materials and methods

2.1. Plant material and growth conditions

The *Arabidopsis thaliana* (L.) Heynh ecotype Columbia (Col-0) was used as the wild type plant in this study. Seeds were surface-sterilized and sown on 1.5% agar plates, as described previously (Okada and Shimura, 1990). After sowing, the plates were kept at 4 °C for 2 d and then incubated at 22 °C under constant white light (50–100 μmol m^{-2} s^{-1}). For each transgenic line, ten 5-day-old seedlings were analyzed for the root hair number, and five 2-week-old third leaves were analyzed for the trichome number.

2.2. Gene constructs

All the constructs for chimeric proteins were generated in the *CPCp:CPC:2xGFP* backbone (Wada et al., 2002) by TaKaRa (TaKaRa, Japan). To create Chimera 1 construct, *ETC1*-specific DNA sequence corresponding to NT amino acids was inserted into the *CPC* coding region of *CPCp:CPC:2xGFP* (Fig. 1B and C). To create Chimera 2 construct, *ETC1*-specific DNA sequence corresponding to HLKTNPTIV amino acids was inserted into the *CPC* coding region of *CPCp:CPC:2xGFP* (Fig. 1B and C). To create Chimera 3 construct, AVKMSE of CPC was substituted with EIAMAQ of ETC1 in *CPCp:CPC:2xGFP* (Fig. 1B and C). To create Chimera 4 construct, amino acid S of CPC was substituted with amino acid C of ETC1 in *CPCp:CPC:2xGFP* (Fig. 1B and C). To create Chimera 5 construct, amino acid V of ETC1 was substituted with amino acid L of CPC in *CPCp:S1:ETC1:2xGFP* (Fig. 1B and C) (Tominaga-Wada et al., 2017b).

2.3. Transgenic plants

Plant transformation was performed using the floral dip method (Clough and Bent, 1998), and the transformants were selected on 0.5×