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TRIPTYCHON, not CAPRICE, participates in feedback regulation of *SCM* expression in the *Arabidopsis* root epidermis

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The *Arabidopsis* root epidermal cells decide their fates (root-hair cell and non-hair cell) according to their position. SCRAMBLED (SCM), an atypical leucine-rich repeat receptor-like kinase (LRR RLK) mediates the positional information to the epidermal cells enabling them to adopt the proper fate. Via feedback regulation, the SCM protein accumulates preferentially in cells adopting the root-hair cell fate. In this study, we determine that TRY, but not the related factor CPC, is responsible for this preferential SCM accumulation. We observed severe reduction of *SCM::GUS* expression in the *try-82* mutant root, but not in the *cpc-1* mutant. Furthermore, the overexpression of *TRY* by *CaMV35S* promoter caused an increase in the expression of *SCM::GUS* in the root epidermis. Intriguingly, the overexpression of *CPC* by *CaMV35S* promoter repressed the expression of *SCM::GUS*. Together, these results suggest that TRY plays a unique role in generating the appropriate spatial expression of *SCM*.

Lateral inhibition is used to specify 2 cell fates in the *Arabidopsis* root epidermis: root-hair cells and non-hair cells.[1] The non-hair-cell fate is promoted by an activator complex that includes WEREWOLF (WER), a R2R3 MYB transcription factor, GLABRA3 (GL3), a bHLH MYC transcription factor, and TRANSPARENT TESTA GLABRA1 (TTG1), a WD40-repeat protein, which together stimulates the transcription of the *GLABRA2* (*GL2*) gene.[2,3] GL2 represses the expression of *ROOT HAIR DEFECTIVE6* (*RHD6*) that is required for the transcription of downstream root-hair genes, and thereby directs cells to differentiate into non-hair cells.[4] The non-hair-cell fate activator complex also induces the transcription of the root-hair-cell fate activators, *CAPRICE* (*CPC*) and *TRIPTYCHON* (*TRY*). CPC and TRY are single R3 MYB transcription factors, and they are small enough to diffuse freely into neighboring cells via plasmodesmata.[5-8] Like WER, CPC and TRY are able to bind to GL3 which disrupts the WER/GL3/TTG1 non-hair-cell fate activator complex.[9,10] It has been experimentally shown that CPC and WER compete for GL3 binding quantitatively.[3] Therefore, an epidermal cell with a higher WER/CPC ratio acquires functional activator complex, whereas an epidermal cell with a lower WER/CPC ratio fails to form a functional activator complex, and adopts the root-hair-cell fate. Without the guidance of a positional cue, a similar lateral inhibition mechanism appears to occur stochastically during trichome patterning in the epidermis of *Arabidopsis* leaves.[1] In the root epidermis, the WER/CPC ratio is determined in a position-dependent manner. A root epidermal cell adjacent to 2 underlying cortical cells (H cell) has a lower WER/CPC ratio and differentiates into root-hair cells, on the other hand, the WER/CPC ratio is higher in a root epidermal cell in contact with one cortical cell (N cell) and, so adopts the non-hair-cell fate.[11-14] Accordingly, the CPC and WER proteins were found predominantly in the nuclei of the H cells and N cells, respectively.[8,15,16] SCRAMBLED (SCM), an atypical leucine-rich repeat receptor-like kinase (LRR RLK), mediates the positional information by reducing the expression of *WER* in the H cells.[17,18] Moreover, the SCM proteins exhibit preferential accumulation in the H cells than the N cells, due to a feedback regulation.[19]

In our previous study, we found that *SCM* expression in the root epidermis is negatively regulated by WER and GL3, and positively regulated by TRY.[19] However, we were unable to define the possible effect of CPC in this study, due to the use of the unusual *cpc-3* mutant allele, which does not affect root epidermal patterning. The *cpc-3* allele does bear a missense mutation ($Glu^{26}Lys$) in the *CPC* gene, but it alters root hair formation only in the *cpc-3 try-82* double mutant.[19] Therefore, to accurately assess the possible role of CPC in SCM feedback regulation, we sought to examine the effect of *cpc-1*, the strong allele.[5]

In the root epidermis of *try-82* mutant plants, *SCM::GUS* expression was reduced severely, relative to the wild-type (**Fig. 1A and B**). However, *SCM::GUS* expression was not detectably altered in the *cpc-1* mutants (**Fig. 1C**). To confirm that TRY is involved in increasing *SCM* expression, we examined the effect of overexpressing *TRY* on *SCM* expression. Following the introduction of the *SCM::GUS* reporter transgene into the *35S::TRY*

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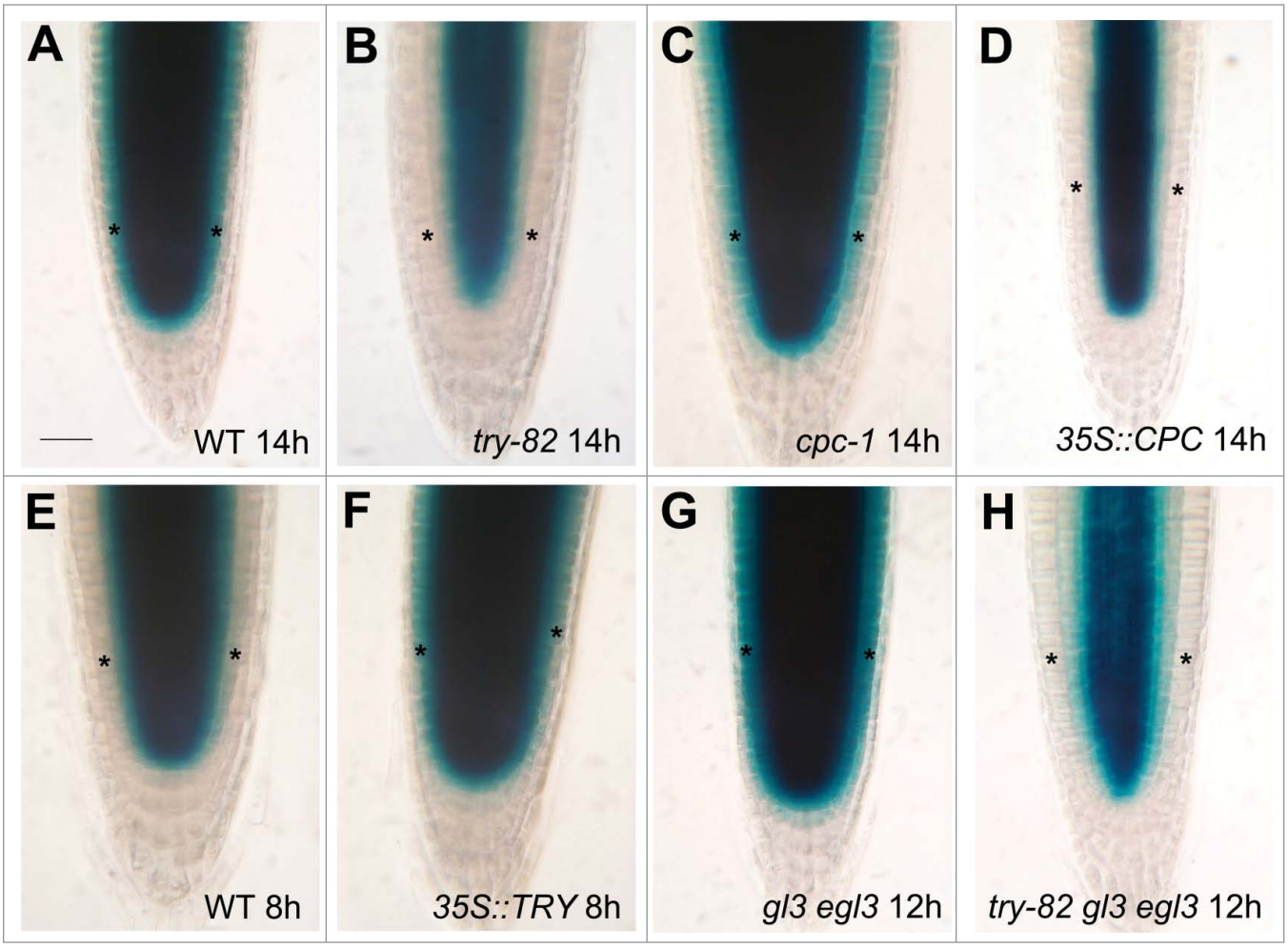

Figure 1. Expression of *SCM::GUS* reporter gene in the root of wild-type and various epidermal cell fate mutants. *Arabidopsis* seeds were sterilized and germinated.[21] Four-day-old roots harboring *SCM::GUS* reporter gene were stained with GUS staining solution at 37°C for indicated time as described in previous work.[19] (**A**) The wild-type root stained for GUS for 14 hours, (**B**) the *try-82* root stained for GUS for 14 hours, (**C**) the *cpc-1* root stained for GUS for 14 hours, (**D**) the *35S::CPC* root stained for GUS for 14 hours, (**E**) the wild-type root stained for GUS for 8 hours, (**F**) the *35S::TRY* root stained for GUS for 8 hours, (**G**) the *gl3 egl3* root stained for GUS for 12 hours and (**H**) the *try-82 gl3 egl3* root stained for GUS for 12 hours. Asterisks indicate the epidermis of the roots. The scale bar represents 50 μm.

line by crossing, we observed that overexpression of *TRY* (using *CaMV35S* promoter) increased *SCM::GUS* expression in the root epidermis (**Fig. 1E and F**). Thus, we conclude that TRY, but not CPC, acts as a positive regulator of *SCM* expression in the epidermis of *Arabidopsis* roots.

CPC and TRY both repress *GL2* expression by inhibiting the activity/formation of the WER/GL3/TTG1 complex, consistent with the observation that the *wer* mutant is epistatic to the *cpc* mutant with respect to *GL2* expression.[20] However, we discovered that, with respect to *SCM* expression, the *try-82* mutant is epistatic to the *gl3 egl3* mutant (**Fig. 1H**) because the *try-82 gl3 egl3* triple mutant exhibited severely reduced *SCM::GUS* expression in the root epidermis, similar to the *try-82* single mutant (**Fig. 1H**), whereas *SCM::GUS* expression in the epidermis of the *gl3 egl* mutant was up-regulated as described in previous study (**Fig. 1G**).[19] Because the effect of *try-82* was epistatic to *gl3 egl3* rather than additive, it may be that the WER/GL3/TTG1 negatively regulates *SCM* expression by inhibiting TRY function. We further analyzed the effect of overexpression of *CPC* by the *CaMV35S* promoter and we found that it repressed *SCM::GUS* expression in the epidermis (**Fig. 1D**). This confirms that CPC is not the positive regulator of *SCM* expression. The ability of overexpressed CPC proteins to alter the *SCM::GUS* expression may be due to inhibition of TRY as competitive analogs of TRY.

It is notable that our results uncover a distinction in the action of TRY and the WER/GL3/TTG1 complex on different part of this gene regulatory network. Specifically, it appears that WER/GL3/TTG1 complex and TRY regulate *SCM* expression in a different way from *GL2* expression. Considering that TRY is presumed to be a transcriptional repressor, a novel *SCM* expression inhibitor may exist in the epidermis whose expression is suppressed by TRY and induced by the WER/GL3/TTG1 complex.

In this study, we describe a TRY-dependent, but CPC-independent feedback regulation of *SCM* expression in the *Arabidopsis* root epidermis. Although CPC and TRY are closely related proteins, some differences in their functions have been previously identified. For example, the leaves of the *cpc-1* mutant develop more trichomes over the entire epidermal surface; whereas, the *try-82* mutant leaves produce increased trichomes in clusters.[6] EGL3, a redundant homolog of GL3, also has different characteristics than its closely related GL3. The GL3 proteins move from the H cells to neighboring N cells via plasmodesmata, but the EGL3 proteins do not move between the root epidermal cells, and they appear to trap the CPC proteins in the nuclei of the H cells.[8,22] Thus, the TRY-specific SCM feedback regulation reported here can be considered as an example of a gene duplication leading to the acquisition of novel functions during plant evolution.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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