Expression and protein localization analyses of *Arabidopsis GLABRA3 (GL3)* in tomato (*Solanum lycopersicum*) root epidermis

Rumi Tominaga-Wada[1]*, Kyosuke Ota[2], Naoto Hayashi[2], Koh Yamada[2], Ryosuke Sano[3], Takuji Wada[1]

[1]Graduate School of Biosphere Sciences, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan; [2]School of Applied Biological Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan; [3]Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan
*E-mail: rtomi@hiroshima-u.ac.jp　Tel & Fax: +81-82-424-7966

Abstract　The arrangement of root hair and non-hair cells in the root epidermis provides a useful model for understanding the cell fate determination system in plants. A network of related transcription factors, including GLABRA3 (GL3), influences the patterning of cell types in *Arabidopsis*. *GL3* is expressed primarily in root hair cells and encodes a bHLH transcription factor, which inhibits root hair differentiation in *Arabidopsis* root epidermis. By transforming the *GL3 promoter::GFP* into tomato, we demonstrated that the *Arabidopsis GL3* promoter can function in tomato root epidermis. GFP fluorescence was observed in almost all root epidermal cells in the *GL3::GFP* transgenic tomato plants, indicating that all root epidermal cells of tomato possess root hair cell identity similar to that of *Arabidopsis* root hair cells. This is consistent with the phenotype of the tomato root, in which all epidermal cells produce root hairs. Moreover, we observed the localization of a GL3:GFP fusion protein in *GL3::GL3:GFP* transgenic tomato; although GL3 is known to exclusively localize in non-hair cell nuclei in *Arabidopsis* root epidermis, GL3:GFP fluorescence was detected not in the nuclei but in the cytoplasm of transgenic tomato epidermal cells. These results suggest that the nuclear localization mechanism differs between tomato and *Arabidopsis*.

Key words:　Arabidopsis, epidermis, *GL3*, root hair, tomato.

Control of root hair and non-hair cell fate determination is a critical issue in plant developmental biology. The difference between root hair cell and non-hair cell identities is evident prior to root hair initiation (Brena-Medina et al. 2014). Three types of root hair patterns are defined (Dolan and Costa 2001). Tomato exhibits a type I root hair pattern, in which root hairs develop in a random pattern; all of the epidermal cells have potential to produce root hairs. *Arabidopsis*, in contrast, shows a type III pattern, in which root hairs are arranged in cell files that are interspersed with non-hair cell files (Pemberton et al. 2001). In *Arabidopsis*, root hair or non-hair cell identity is precisely controlled by several transcription factors, including GLABRA3 (GL3) (Payne et al. 2000). The *GL3* gene encodes a bHLH transcription factor, which is involved in the inhibition of root hair differentiation in *Arabidopsis* root epidermis. Previously, we identified a *GL3* homologous gene from the tomato (*Solanum lycopersicum*) genome and named it *SlGL3* (Tominaga-Wada et al. 2013). To

elucidate the functions of *SlGL3*, we introduced *SlGL3* into *Arabidopsis*. However, we could not detect any remarkable effect of *SlGL3* on the root hair phenotype in *Arabidopsis* (Tominaga-Wada et al. 2013). Tomato has root hairs in all epidermal cells. Thus, we wondered whether *GL3* could repress root hair formation in the tomato. In this study, we adopted the reverse approach and examined the promoter activity and protein behavior of the *Arabidopsis GL3* gene in tomato. We introduced *GL3::GFP* into tomato to clarify *GL3*'s promoter activity and examined GL3:GFP fluorescence in *GL3::GL3:GFP* transgenic tomato plants to elucidate the protein localization and movement ability of GL3 in tomato root epidermal cells.

Tomato (*Solanum lycopersicum* L. 'Micro-Tom') seeds were surface-sterilized and incubated as previously described (Tominaga-Wada et al. 2013). The generation of *GL3::GL3:GFP* transgenic tomato plants has been described previously (Wada et al. 2014). To create the *GL3::GFP* construct, a 3.2-kb promoter region of the *GL3*
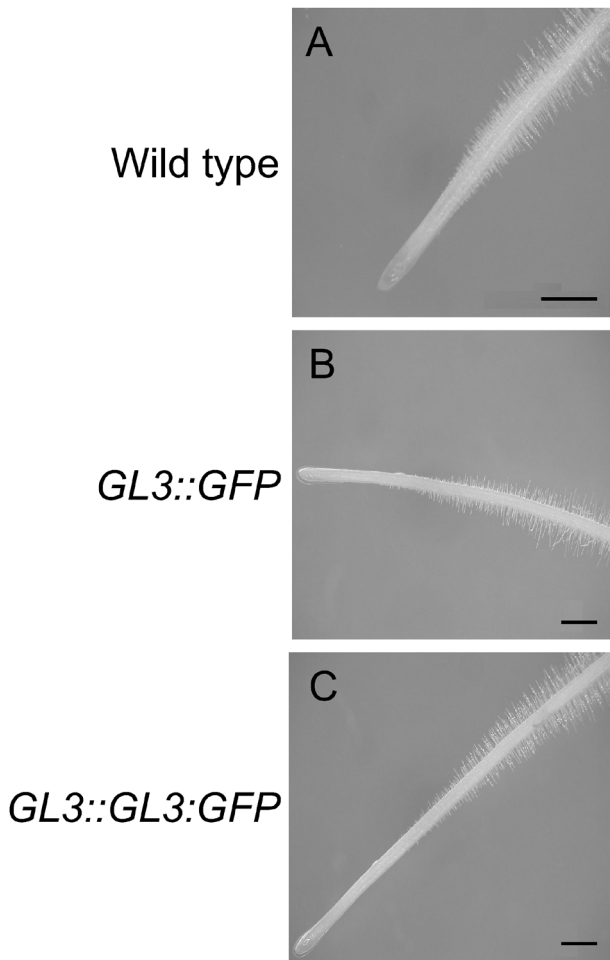
Figure 1.   Root epidermal phenotypes of transgenic tomato plants. (A) Five-day-old seedling roots of wild-type plant. (B) Five-day-old seedling roots of *GL3::GFP* transgenic plant. (C) Five-day-old seedling roots of *GL3::GL3:GFP* transgenic plant. Scale bars: 1 mm.
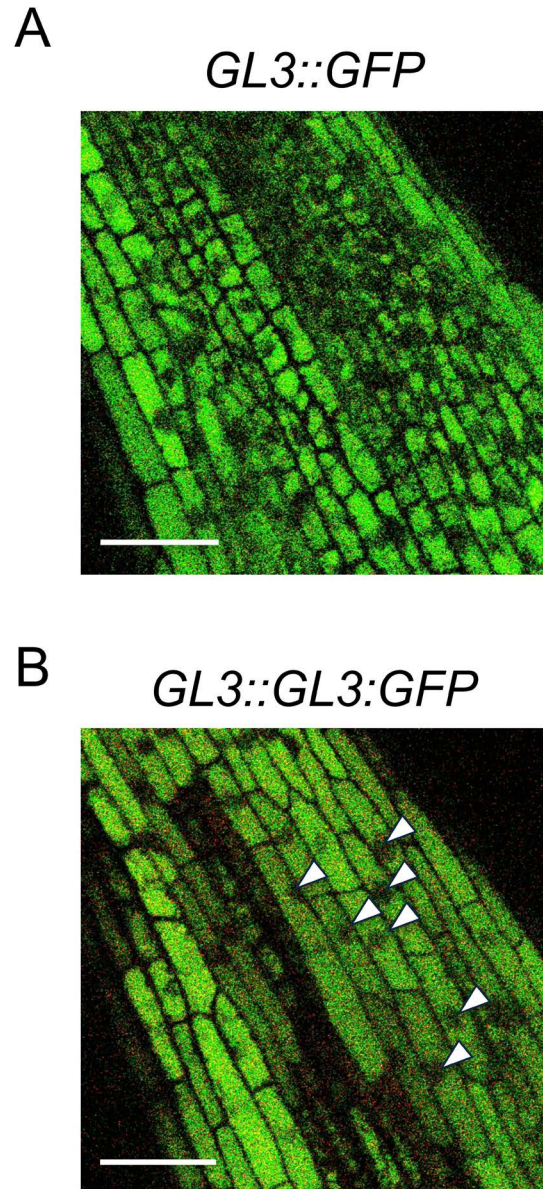

Figure 2.   Distribution of GFP fluorescence in transgenic tomato plants. (A) CLSM images showing GFP fluorescence in the root epidermis of a *GL3::GFP* transgenic tomato plant. (B) CLSM images showing GFP fluorescence in the root epidermis of a *GL3::GL3:GFP* transgenic tomato plant. Arrowheads indicate the nucleus. Scale bars: 50 μm.

gene from the *Arabidopsis* genome was PCR-amplified, fused to GFP, and then sub-cloned into a modified pPZP212 binary vector. The *GL3::GFP* construct was introduced into tomato according to a highly efficient transformation protocol for Micro-Tom using *Agrobacterium* (Sun et al. 2006). Homozygous transgenic lines were selected based on kanamycin resistance.

Root 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).

The *GL3::GFP* and *GL3::GL3:GFP* transgenic tomato plants were phenotypically similar to the wild-type plants. We did not detect any notable differences in root hair phenotype between *GL3::GFP* or *GL3::GL3:GFP* transgenic tomato plants and the wild-type tomato plant (Figure 1). *GL3* is known to inhibit root hair formation in *Arabidopsis* (Bernhardt et al. 2003); however, as previously reported, the *GL3::GL3:GFP* transgenic tomato plants did not show the expected hairless

phenotype (Supplemental Figure 1) (Wada et al. 2014). In addition, we did not detect any notable differences in trichome formation between *GL3::GL3:GFP* transgenic tomato and wild-type tomato plants (Wada et al. 2014)

The *GL3* gene is preferentially expressed in root hair cells in the *Arabidopsis* root epidermis (Bernhardt et al. 2005). Transgenic tomato plants carrying the *GL3::GFP* construct, in which the expression of GFP was driven by the *GL3* promoter, showed GFP fluorescence in almost all epidermal cells (Figure 2A). This result indicates that the *GL3* promoter sequence from *Arabidopsis* is evidently also functional in tomato (Figure 2A). Because all root

epidermal cells produce root hairs in tomato (Figure 1), they must possess the identity of root hair cells. Thus, the *GL3* promoter, which is specific to root hair cells in *Arabidopsis*, should be active in all root epidermal cells in tomato (Figure 2A). We confirmed that GFP was not detectable without the epidermis in the root on CSLM observation (Supplemental Figure 2).

The *GL3* gene encodes a bHLH transcription factor, and therefore it is expected that the GL3 protein is transported into and functions in the nucleus. In fact, GL3:YFP protein has been observed in the nuclei of *Arabidopsis* root epidermal cells (Bernhardt et al. 2005). However, in the present study, we did not detect GL3:GFP fluorescence in the nuclei of tomato epidermal cells, although fluorescence was observed in most parts of the cytoplasm (Figure 2B).

In this study, we demonstrated that the *Arabidopsis GL3* promoter is also functional in tomato. This result was shown to be useful in a plant engineering experiment, in which specific gene expression in tomato root epidermis was achieved by using the *GL3* promoter. We also confirmed that no GFP fluorescence was present in the root epidermis of wild-type tomato plants, indicating that the GFP fluorescence detected in this study was not due to the influence of auto-fluorescence of tomato tissue (Supplemental Figure 3).

The *SlGL3* gene was identified to be homologous to *GL3* in tomato (Tominaga-Wada et al. 2013). Although the precise functions of the tomato *SlGL3* gene have not been determined, it is hypothesized that the SlGL3 has a function similar to that of GL3 in the tomato root epidermis owing to their amino acid sequence similarity. Therefore, GL3 was expected to function in tomato root epidermis as an inhibitor of root hair formation, as observed in *Arabidopsis* root epidermis. However, the introduction of the *GL3* gene into tomato had no effect on the root hair phenotype of tomato (Figure 1). This might be due to the inability of the GL3 protein to translocate into the nucleus (Figure 2B), perhaps because it is missing a specific signal sequence or binding of another component that guides the protein to the nucleus. This inability of GL3 to move to and function in the nucleus indicates that it probably does not function as a transcription factor. The *GL3* promoter functioned in the epidermal cells of both in *Arabidopsis* and tomato.

However, the GL3 protein was non-functional in tomato because GL3 did not move from the cytoplasm to the nucleus. It is therefore hypothesized that the function of GL3 in tomato is entirely different from that in *Arabidopsis*. Our study suggests that the cell differentiation system of tomato is different from that of *Arabidopsis* at present and that the tomato evolved to lose its bHLH transcription factor-mediated root-hair inhibitory mechanism. SlGL3 has the same origin as GL3 but might have evolved with a different function in tomato.

Acknowledgements

## References

Bernhardt C, Lee MM, Gonzalez A, Zhang F, Lloyd A, Schiefelbein J (2003) The bHLH genes *GLABRA3 (GL3)* and *ENHANCER OF GLABRA3 (EGL3)* specify epidermal cell fate in the *Arabidopsis* root. *Development* 130: 6431–6439

Bernhardt C, Zhao M, Gonzalez A, Lloyd A, Schiefelbein J (2005) The bHLH genes *GL3* and *EGL3* participate in an intercellular regulatory circuit that controls cell patterning in the *Arabidopsis* root epidermis. *Development* 132: 291–298

Brena-Medina V, Champneys AR, Grierson C, Ward MJ (2014) Mathematical modeling of plant root hair initiation: Dynamics of localized patches. *SIAM J Appl Dyn Syst* 13: 210–248

Dolan L, Costa S (2001) Evolution and genetics of root hair stripes in the root epidermis. *J Exp Bot* 52(suppl 1): 413–417

Payne CT, Zhang F, Lloyd AM (2000) *GL3* encodes a bHLH protein that regulates trichome development in arabidopsis through interaction with GL1 and TTG1. *Genetics* 156: 1349–1362

Pemberton LMS, Tsai SL, Lovell PH, Harris PJ (2001) Epidermal patterning in seedling roots of eudicotyledons. *Ann Bot (Lond)* 87: 649–654

Sun HJ, Uchii S, Watanabe S, Ezura H (2006) A highly efficient transformation protocol for Micro-Tom, a model cultivar for tomato functional genomics. *Plant Cell Physiol* 47: 426–431

Tominaga-Wada R, Nukumizu Y, Sato S, Wada T (2013) Control of plant trichome and root-hair development by a tomato (*Solanum lycopersicum*) R3 MYB transcription factor. *PLoS One* 8: e54019

Wada T, Kunihiro A, Tominaga-Wada R (2014) Arabidopsis CAPRICE (MYB) and GLABRA3 (bHLH) control tomato (*Solanum lycopersicum*) anthocyanin biosynthesis. *PLoS One* 9: e109093

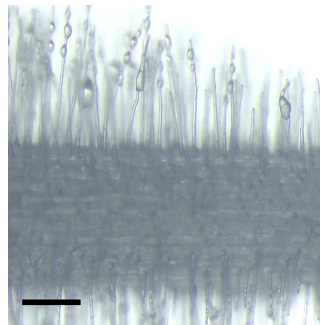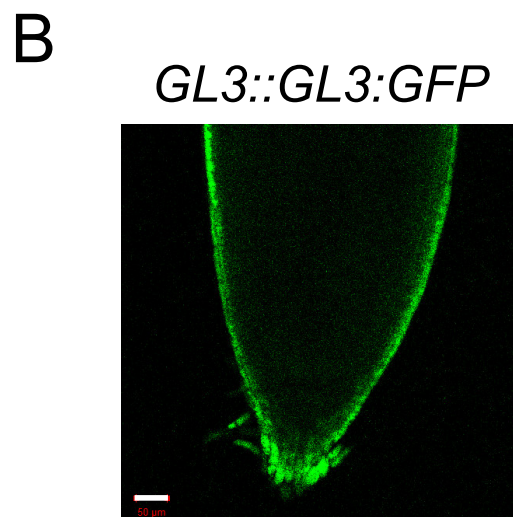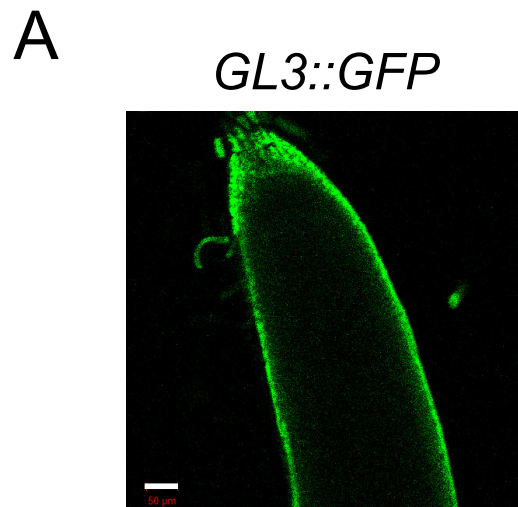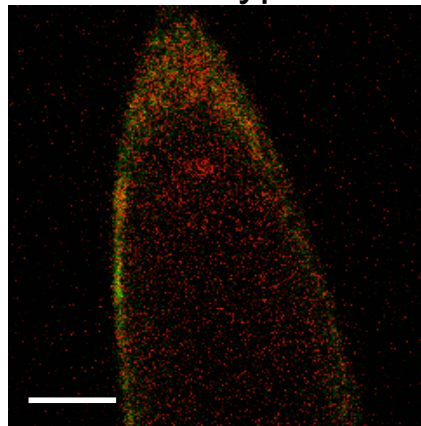**Wild type**
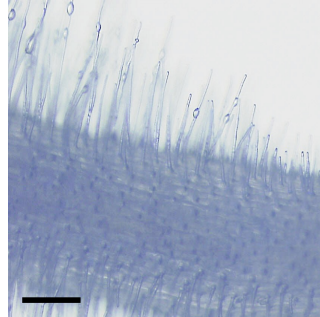
GL3::GFP
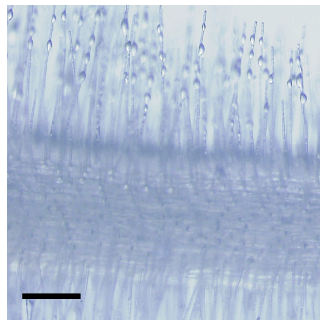
GL3::GL3:GFP

Supplemental Figure 1. Root hair phenotypes of transgenic tomato plants. Five-day-old seedling roots of wild type, *GL3::GFP*, and *GL3::GL3:GFP* plants. Scale bars: 200 μm.

A

GL3::GFP

B

GL3::GL3:GFP

Supplemental Figure 2. Distribution of GFP fluorescence in transgenic tomato plants. (A) CLSM image showing GFP fluorescence in the root epidermis of GL3::GFP transgenic tomato plant. (B) CLSM image showing GFP fluorescence in the root epidermis of GL3::GL3:GFP transgenic tomato plant. Scale bars: 50 μm.

Wild type

Supplemental Figure 3. Distribution of GFP fluorescence in wild type tomato plant. CLSM images showing GFP fluorescence in the root epidermis of wild type tomato plant. Scale bar: 50 μm.