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Triptychon (TRY) Protein Accumulation in the Roots of Mutant *Auxin-resistant 1 (axr1) Arabidopsis thaliana*

Rumi Tominaga-Wada* and Takuji Wada

Graduate School of Biosphere Sciences, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, 739-8528, Japan

Abstract

Plant root hairs play an essential role in water and nutrient uptake. CAPRICE (CPC) family transcription factors act to induce root hairs in *Arabidopsis thaliana*. Previously, using a proteasome inhibitor assay, we proposed that the CPC family protein TRIPTYCHON (TRY) was degraded through the ubiquitin/26S proteasome pathway. The *Auxin resistant 1* (*AXR1*) gene encodes the ubiquitin-activating enzyme E1. In this study, to further investigate the mechanism of TRY degradation, we introduced a *CPCp:TRY-GFP* construct into *axr1-3 A. thaliana* mutants. The *CPCp:TRY-GFP* transgenic plant showed weak TRY-GFP fluorescence while the *CPCp:TRY-GFP* in *axr1-3* transgenic plant showed strong TRY-GFP fluorescence. These results support the suggestion that TRY is degraded by the ubiquitin/26S proteasome mechanism.

Keywords: *Arabidopsis*; AXR1; Degradation; Root hair; TRY

Introduction

Plant root hair is an important organ for water and nutrient absorption from the soil. In, *Arabidopsis thaliana (L.)* Heynh., *CAPRICE* (*CPC*) family genes, which encode the R3-type MYB transcription factor, serve as positive regulators of root hair formation [1,2]. Previously, we reported that the CPC family protein Triptychon (TRY) might be degraded through the ubiquitin/26S proteasome-mediated pathway [3]. We showed that TRY was unstable and had a longer C-terminal region (about 20 amino acids) than other CPC family proteins including CPC enhancer of TRY and CPC1 (ETC1) and CPC-LIKE MYB3 (CPL3) [3-5]. Deletion of the extended C-terminal region of TRY enhanced its stability [3]. Treatment with MG132 or MG115, proteasome inhibitors led to the accumulation of TRY, indicating that TRY proteolysis is mediated by the proteasome-dependent pathway [3]. However, the precise mechanism of TRY degradation is still unclear.

About 5% of the *A. thaliana* proteome seem to be directly involved in the ubiquitin/26S proteasome system [6]. This system influences almost every aspect of plant growth and development, including hormone signaling, morphogenesis, and environmental and pathogen responses [7]. In the ubiquitin/26S proteasome system, ubiquitin is attached to a target protein and polyubiquitinated target proteins are degraded by the 26S proteasome. Ubiquitination is accomplished through the sequential action of E1, E2, and E3 enzymes [6]. Ubiquitin is first activated by an E1 ubiquitin-activating enzyme and then transferred to the E2 ubiquitin-conjugating enzyme; helped by E3 ligase, the ubiquitin is finally conjugated to the target protein [6].

The *Auxin resistant 1* (*AXR1*) gene encodes a protein related to ubiquitin-activating enzyme E1 in *A. thaliana* [8]. To assess if TRY degradation is mediated by the AXR1 involved in the ubiquitin/26S proteasome system, we produced TRY-GFP in *axr1* transgenic plants and examined its fluorescence in these mutant plants.

Materials and Methods

Plant materials and growth condition

Arabidopsis thaliana ecotype Columbia (Col-0) was used as the wild type background. The *CPCp:TRY-GFP* transgenic line used in the present study has been previously described [3]. The *axr1-3* mutant was obtained from the ABRC, as described in Leyser et al. [8]. The *CPCp:TRY-GFP* construct was introduced into the *axr1-3* mutant by conventional crosses. Seeds were sown on 1.5% agar plates using a previously described method [9].

Microscopy

Images of GFP-fusion in five-day-old *CPCp:TRY-GFP* and *CPCp:TRY-GFP* in *axr1-3* transgenic plant roots were obtained with a Zeiss LSM-510 Meta confocal laser scanning microscope.

Results and Discussion

To further examine if the rapid degradation of TRY-GFP protein in *A. thaliana* root epidermis is mediated by the ubiquitin/26S proteasome system, we introduced *CPCp:TRY-GFP* into *axr1-3* mutants (Figure 1). In accordance with our previous report on wild type plants [3], only weak TRY-GFP fluorescence was observed in the *CPCp:TRY-GFP* transgenic line (Figure 1A). By contrast, relatively strong TRY-GFP fluorescence was observed in *CPCp:TRY-GFP* in the *axr1-3* transgenic line (Figure 1B). In addition, TRY-GFP proteins were detected in the nuclei of root epidermal cells, as in ETC1-GFP or CPL3-GFP transgenic lines, and these do not degrade GFP fusion proteins in root epidermal cells (Figure 1B) [3]. Thus, these results suggest that TRY degradation is related to AXR1 activity.

Previously, we demonstrated that TRY was degraded due to the properties of its extended C-terminal region [3]. In addition, and based solely on proteasome inhibitor assay results using MG132 and MG115, we concluded that TRY was degraded through the ubiquitin/26S proteasome mechanism [3]. Our current data confirmed and reinforced this conclusion. Because AXR1 is expected to participate in the sequential actions defining ubiquitination, as it is part of E1, the non-

***Corresponding author:** Rumi Tominaga-Wada, Graduate School of Biosphere Sciences, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, 739-8528, Japan, Tel: +81-82-424-796; E-mail: rtomi@hiroshima-u.ac.jp

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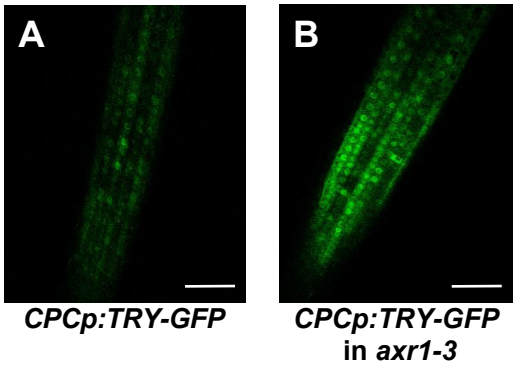

Figure 1: Effect of *axr1-3* mutation on TRY-2xGFP protein accumulation. Fluorescence images of five-day-old *CPCp:TRY-GFP* (A) and *CPCp:TRY-GFP* in *axr1-3* (B) Transgenic seedling roots. Scale bars: 100 μm.

degradation of TRY in *axr1-3* mutants (Figure 1B) was predictable. This result also supports TRY degradation by the ubiquitin/26S proteasome mechanism. Further investigations will reveal the overall mechanism of root hair formation controlled by the CPC family.

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