

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

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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.

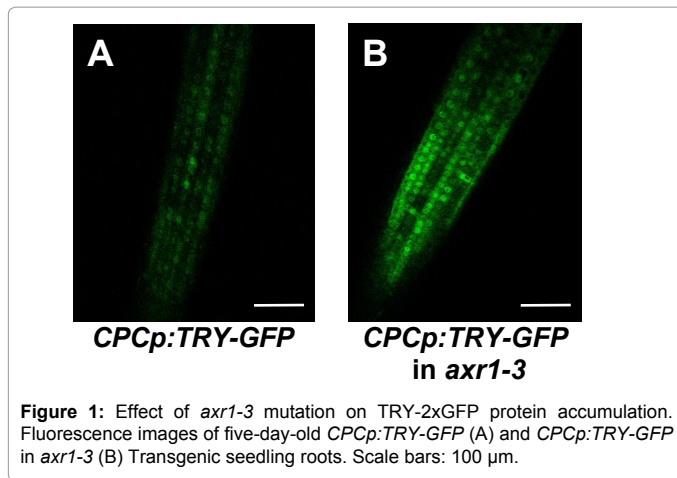
Previously, we demonstrated that TRY was degraded due to the properties of its extended C-terminal region [3]. In addition, and based solely on proteasome inhibitor assay results using MG132 and MG115, we concluded that TRY was degraded through the ubiquitin/26S proteasome mechanism [3]. Our current 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-

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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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References

1. Wada T, Tachibana T, Shimura Y, Okada K (1997) Epidermal cell differentiation in Arabidopsis determined by a Myb homolog. *CPC Science* 277: 1113-1116.
2. Tominaga-Wada R, Ishida T, Wada T (2011) New insights into the mechanism of development of Arabidopsis root hairs and trichomes. *Int Rev Cell Mol Biol* 286: 67-106.
3. Tominaga-Wada R, Wada T (2017) Extended C termini of CPC-LIKE MYB proteins confer functional diversity in Arabidopsis epidermal cell differentiation. *Development* 144: 2375-2380.
4. Kirik V, Simon M, Huelskamp M, Schiefelbein J (2004) The enhancer of TRY and CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis. *Dev Biol* 268: 506-513.
5. Tominaga R, Iwata M, Sano R, Inoue K, Okada K, et al. (2008) Arabidopsis caprice-like MYB 3 (CPL3) controls endoreduplication and flowering development in addition to trichome and root hair formation. *Development* 135: 1335-1345.
6. Santner A, Estelle M (2010) The ubiquitin-proteasome system regulates plant hormone signaling. *Plant J* 61: 1029-1040.
7. Vierstra RD (2009) The ubiquitin-26S proteasome system at the nexus of plant biology. *Nat Rev Mol Cell Biol* 10: 385-397.
8. Leyser HMO, Lincoln CA, Timpte C, Lammer D, Turner J, et al. (1993) Arabidopsis Auxin-Resistance Gene-Axr1 Encodes a Protein Related to Ubiquitin-Activating Enzyme-E1. *Nature* 364: 161-164.
9. Okada K, Shimura Y (1990) Reversible Root Tip Rotation in Arabidopsis Seedlings Induced by Obstacle-Touching Stimulus. *Science* 250: 274-276.