Suppression of root apical meristem activity in ribosomal protein mutant *rps6a-2* in *Arabidopsis thaliana*

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Abstract. The ribosome, a protein-manufacturing machine, is completed by the assembly of a large number of proteins. In plants, many genes encoding ribosomal proteins are duplicated, and the growth of mutants of one of these genes is frequently inhibited. In this study, I focused on the growth inhibition of a mutant of the ribosomal protein gene, *rps6a-2*. As cell cycle activity is a factor in growth inhibition, we examined the color reaction of *CYCB1;1::GUS*, a marker of the cell cycle. The results showed that the length of the root meristematic zone was significantly decreased in *rps6a-2* than in the wild type. Based on these results, the mechanism of growth inhibition in ribosomal protein gene mutants is discussed.

1. Introduction

Eukaryotic ribosomes are composed of large and small subunits, both of which together contain about 80 different ribosomal proteins [1, 2]. In plants, many of these genes are duplicated and a mutant of one of them is often viable. However, many of these mutants commonly exhibit a various growth defects, including delayed germination, inhibited root elongation, abnormal vascular bundle development, and abnormal leaf morphology [3, 4]. For example, in Arabidopsis, the small subunit Ribosomal Protein S6 (RPS6) is encoded by two genes, *RPS6A* and *RPS6B*, and mutants of *RPS6A* show growth defects and abnormal leaf morphology [5].

Plant roots elongate by supplying cells through cell division in the root apical meristem and elongating cells in the transition and elongation zones located above the meristem [6]. In root meristematic tissues, cell cycle progression is controlled by cyclin-dependent kinases (CDKs), which associate with cyclins (CYCs) to confer substrate specificity [7]. Different CDK/CYC complexes act throughout the cell cycle. The CDKA/CYCD complex triggers the G1-S phase transition. After DNA replication in the G2 phase, CDKA and CDKB combine with A-type and B-type CYC to induce the G2/M phase transition, whereas in the late M phase, CYCA and CYCB are degraded by the APC/C complex and cell cycle is completed [8]. Among them, *CYCB1;1::GUS* has been used as a useful marker for cells corresponding to the G2/M phase of the cell cycle [9].

In this study, we investigated the suppression of root elongation in *rps6a-2*, an *RPS6A* mutant. We further examined the mechanism underlying the *rps6a-2* phenotype by quantifying the root apical meristem of *rps6a-2* using the cell cycle marker *CYCB1;1::GUS*.

2. Results and Discussion

Since ribosomal protein mutants often exhibit growth defects, we quantified the primary root length to test whether the case was similar for *CYCB1;1::GUS* in *rps6a-2*, which was obtained by crossing *CYCB1;1::GUS* with *rps6a-2*. Comparison at 7-day of seedling showed that *CYCB1;1::GUS* in *rps6a-2* had a significantly shorter primary root length than the *CYCB1;1::GUS* in wild type (Figure 1A). This result is similar to those observes in the other ribosomal protein mutants [3, 4]. The *rps6a-2* mutant used here had almost no expression of *RPS6A* gene and had no effect on the expression level of its paralog gene *RPS6B* [5]. Since only the RPS6B protein is expressed in the *rps6a-2* mutant, the suppression of growth in the mutant could be due to a lack of RPS6 proten in quantity, the absence of RPS6A in quality, or other causes. Possible causes of these growth defects in primary root elongation include differences in the timing of germination, cell length, and cell cycle activity. In this study, differences in cell cycle activity were verified using GUS staining for the G2/M phase cell cycle marker *CYCB1;1::GUS*. Quantification showed that the length of the GUS signal observed in the root apical meristem was significantly shorter in the *rps6a-2* mutant than in the wild type (Figure 1B). Furthermore, there were several roots in which no signal was detected in the *rps6a-2* mutant, suggesting that the *rps6a-2* mutant delayed the establishment of root apical meristem organization compared to the wild type. These results indicate that the reason for the short main root length observed in the *rps6a-2* mutant was the delayed establishment of the root apical meristem and weak cell division activity in the root apical meristem. The next question is what molecular mechanisms are responsible for the abnormal establishment of the root meristem and cell cycle in *rps6a-2* mutant. To explore this, it is necessary to use *rps6a-2* mutant to examine changes in ribosome abundance and its effect on mRNA selection during translation. Alternatively, the abnormality in the mutant may also be related to the ribosomal stress response, a stress response which occurs when ribosome biosynthesis is impaired [4, 10].

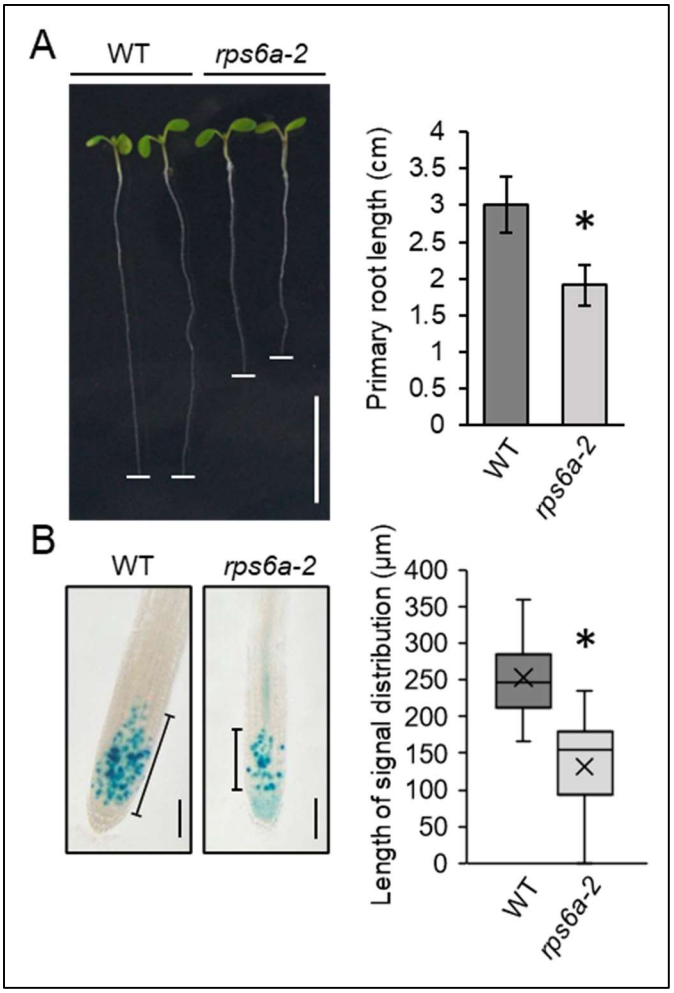

Figure 1

A. Representative photograph (left panel) and quantification (right panel) of primary root length of 7-days seedlings. The horizontal lines were placed at the tip of the primary root. Scale bar: 1 cm. Error bars indicate S.D. (n = 30).

B. Representative photograph (left panel) and quantification (right panel) of *CYCB1;1::GUS* expression at primary root tips of 7-days seedlings. Scale bar: 100 µm. The graph shows a box plot of the signal-distribution length (n = 30). Crosses indicate mean values.

"WT" and "*rps6a-2*" indicate *CYCB1;1::GUS* in wild type and in *rps6a-2*, respectively. *Asterisks* indicate statistical significance between the *rps6a-2* and WT using Student's *t*-test ($p < 0.05$).

3. Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana Col-0 mutant *rps6a-2* [5] seeds were kindly provided by Dr. Gorou Horiguchi (Rikkyo Univ.). Seeds of *CYCB1;1::GUS* in Col-0 were also provided by Dr. Horiguchi and used as the "WT." *CYCB1;1::GUS* in *rps6a-2* was produced by artificial pollination and used as the "*rps6a-2*." The seeds were sterilized and sown on solid plates containing half-strength Murashige and Skoog medium supplemented with 1% sucrose and 0.4% gerangum. After a 2-day cold (4 °C) treatment, the plates were placed at 23 °C under continuous light conditions (approximately 70 μmol m^{-2} s^{-1}) for 7-day growth.

Histochemical GUS Staining

GUS staining was performed according to a standard protocol [11] with minor modifications. Plant seedlings (n = 30) were incubated in 5 ml 80% acetone for 15 min at 4 °C and then washed twice with 5 ml wash buffer [50 mM sodium phosphate buffer (pH7.0), 2 mM potassium ferricyanide, 2 mM potassium ferrocyanide, 10 mM EDTA (pH8.0) and 0.1% Triton X-100]. Thereafter, the samples were submerged in 2 ml GUS staining buffer [wash buffer with 100 mM 5-bromo-4-chloro-3-indolyl β-D-glucuronide]. The staining reaction was performed at 37 °C in the dark for 4 h, and the samples were then washed twice with 5 ml 70% ethanol to stop the reaction. Samples were examined under a optical microscope and photographed using a smartphone (iPhoneSE, Apple). The vertical length of the meristemic zone was quantified using the ImageJ software (https://imagej.net/ij/).

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