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The ribosomal protein RPS6A modulates auxin signaling and root development in *Arabidopsis*

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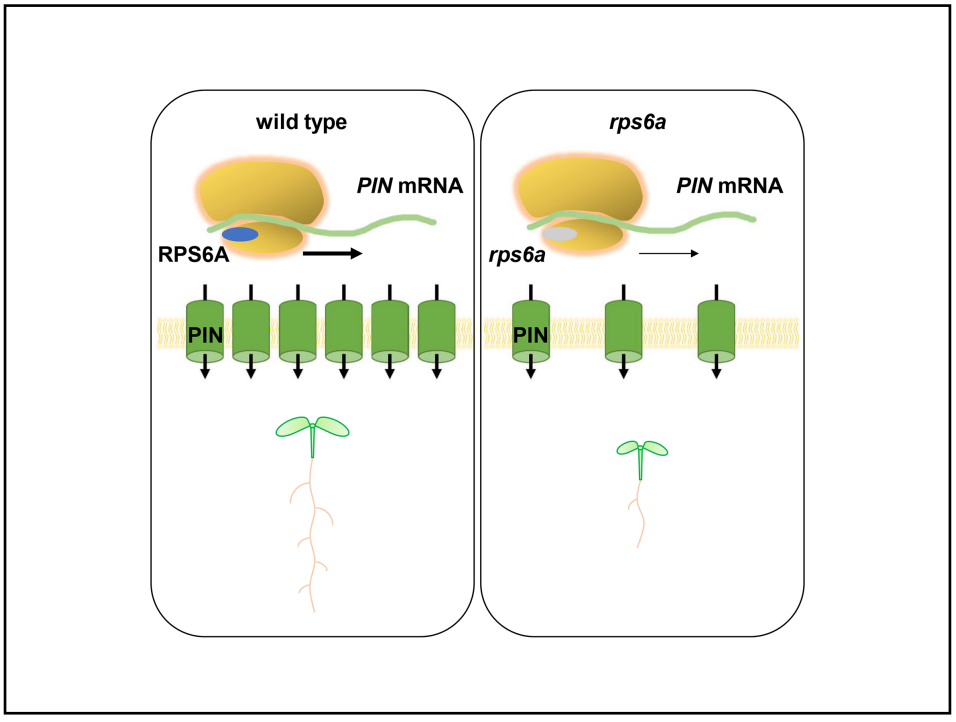
Graphical abstract



Loss of RPS6A function reduces PIN protein translational efficiency, leading to defects in auxin signaling and root development.

Public summary

- The *rps6a* mutant presented a series of auxin-related defects, including shortened primary roots, reduced lateral root numbers and gravitropic defects.
- The RNA-seq results revealed that the expression levels of auxin-related genes and root development-related genes changed in *rps6a*, indicating that RPS6A influences the auxin signaling pathway and root growth.
- The abundance of PIN1, PIN3, and PIN7 auxin transporters decreased in *rps6a*, suggesting that RPS6A possibly affects auxin transport.
- The *rps6a* mutant presented a shortened root meristem and fewer meristematic cells, and the quiescent center (QC) was impaired in the *rps6a* mutant.

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SI Supporting Information

Abstract: Protein biosynthesis by the ribosome is a fundamental biological process in living systems. Recent studies suggest that ribosomal subunits also play essential roles in cell growth and differentiation beyond their roles in protein translation. The ribosomal subunit RPS6 has been studied for more than 50 years in various organisms, but little is known about its specific roles in certain signaling pathways. In this study, we focused on the functions of *Arabidopsis* RPS6A in auxin-related root growth and development. The *rps6a* mutant presented a series of auxin-deficient phenotypes, such as shortened primary roots, reduced lateral root numbers, and defective vasculatures. Treatment of the *rps6a* mutant with various concentrations of auxin and its analogs did not restore the root defect phenotypes, suggesting a defect in the auxin signaling pathway. Further cell biological and global transcriptome analyses revealed that auxin signaling was weakened in the *rps6a* mutant and that there was a reduced abundance of PIN-FORMED (PIN) auxin transporters. Our work provides insights into the role of the protein biosynthesis pathway involved in auxin signaling.

Keywords: ribosome; RPS6A; auxin; PIN; *Arabidopsis*

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1 Introduction

Protein biosynthesis is a fundamental biological process in all living systems. The ribosome is an organelle composed of two subunits (40S subunits and 60S subunits), each composed of multiple components, and its central function in protein biosynthesis is highly conserved in all living lineages. The major components of ribosomes are ribosomal proteins (RPs) and ribosomal RNAs (rRNAs). The eukaryotic ribosome consists of a small 40S subunit, which plays a key role in translation initiation and contains an 18S rRNA chain and 33 proteins, and a large 60S subunit, which contains three rRNA molecules (5S, 5.8S, and 25S) and 46 protein subunits[1,2]. The ribosome not only plays an important role in protein translation but also has other functions, such as regulating the cell cycle, apoptosis, DNA repair and transcription[3,4].

In the model plant *Arabidopsis thaliana*, ribosomal protein S6 (RPS6) is a component of the 40S small ribosomal subunit and contains two members, RPS6A (AT4G31700) and RPS6B (AT5G10360), which are functionally redundant and interchangeable[5]. RPS6 is an evolutionarily conserved protein that is widely expressed in yeast, plants, invertebrates, and vertebrates but not in *E. coli* or archaebacteria[6]. RPS6 consists of an N-terminal-ribosome-bound domain (1–82 aa), a central domain (84–177 aa) and a C-terminal α-helix (178–249 aa)[7]. RPS6 can directly cross-link to mRNAs, tRNAs and initiation factors, determining its location in regions involved in the initiation of translation[8]. There are seven phosphorylation sites of RPS6 in *Arabidopsis*, namely, Ser229, Ser231, Ser237, Ser240, Ser241, Ser247, and Tyr249, in the N-terminus, which are exposed on the surface of the 40S small subunit; thus, RPS6 can be phosphorylated by 40S ribosomal protein S6 kinase (S6K)[9]. Studies in yeast and animals suggest that S6K-mediated phosphorylation of RPS6 is critical for its biological functions in multiple processes.

Target of Rapamycin (TOR) kinase is a key developmental regulator that integrates environmental and nutrient signals to regulate multiple developmental processes from embryogenesis to senescence in plants[10]. S6K is a conserved phosphorylation target of TOR and regulates seedling growth by affecting the brassinosteroid (BR) signaling pathway[10,11]. RPS6 is downstream of the TOR kinase pathway and can be phosphorylated by S6K, which plays an important role in cellular metabolism and protein synthesis[11]. During active ribosome biogenesis, RPS6 is phosphorylated in the nucleolus, whereas in the cytoplasm, RPS6 is phosphorylated to promote the selective translation of a subset of transcripts[8]. Therefore, phosphorylation is essential for RPS6 function.

In animals, the phosphorylation of RPS6 is involved in glucose metabolism, cell growth[12], and cell size regulation[13]. In *Arabidopsis*, RPS6 participates in light signaling and the auxin pathway through a TOR-dependent phosphorylation cascade, thereby regulating light-triggered translational enhancement in deetiolated *Arabidopsis* seedlings[14]. The phosphorylation of RPS6 varies with photoperiod, suggesting that differential phosphorylation of RPS6A/B may contribute to

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the modulation of diurnal protein synthesis in plants[15,16]. The *rps6a* phospho-deficient mutant has a mild pointed-leaf phenotype and a shortened primary root length, which might be due to misexpression of cell cycle-related mRNAs and ribosome biogenesis proteins[17]. Additionally, ribosomal proteins have been reported to control developmental programs through translational regulation of the auxin signaling component Auxin Response Factors (ARFs) in *Arabidopsis*[18].

Previous studies have shown that in *rps6* mutants, the sizes of the two cotyledons are always different. The expression of many photosynthesis genes is defective, and the quantum efficiency of photosystem II is reduced in *rps6*, leading to a pale green color in the seedlings and rosette-stage plants. These effects are exacerbated at cooler temperatures[17]. In this study, we focused on the biological functions of the ribosomal protein RPS6A in root growth and development. Phenotypic analysis, cell biological experiments, and transcriptome sequencing revealed that loss of *RPS6A* function interferes with the abundance of the auxin transporter PIN and regulates auxin distribution in *Arabidopsis*. Therefore, in addition to its fundamental role in ribosomal function, RPS6A can also participate in auxin-regulated root growth and development in *Arabidopsis*.

2 Materials and methods

2.1 Plant materials and growth conditions

The *Arabidopsis thaliana* (L.) mutants and transgenic lines were all of the Columbia-0 (Col-0) ecotype background. The mutant *rps6a-2* (SALK_048825C, short as *rps6a*) in the Columbia-0 background was reported previously[19] and was obtained from the Nottingham Arabidopsis Stock Centre (NASC). The *rps6b* mutant was generated via CRISPR in the Col-0 background. The marker lines *DR5rev::GFP*[20], *pPIN1::PIN1-GFP*[21], *pPIN3::PIN3-GFP*[22], and *pPIN7::PIN7-GFP*[23] were published previously (Table S1 in Supporting information). The strains were introduced into the *rps6a-2* mutant background by crossing. For phenotyping of seedlings or pharmacological experiments, surface-sterilized seeds were sown on solid Murashige and Skoog (MS) media (0.5× MS media supplemented with 1% (w/v) sucrose, 0.8% (w/v) phytoagar, MES buffer, pH 5.9), stratified at 4 °C for 2 d, and then grown vertically in a growth chamber at 21 °C with a 16-h light/8-h dark photoperiod.

2.2 Phenotype observation

For the root gravitropism assay, four-day-old Col-0 and *rps6a* seedlings were arranged on MS media and rotated 90°. The plants were subsequently grown in a growth chamber and observed every 2 h. For vasculature pattern analysis, seven-day-old seedlings were first incubated in 100% ethanol for 30 min to remove the chlorophylls and then gradually exchanged with 75% (v/v) ethanol, 50% (v/v) ethanol and H_2O; each mixture was incubated for 30 min. The samples were mounted with a clearing solution (30 mL H_2O, 10 mL glycerol, 80 g chloral hydrate, to a total of 100 mL), incubated for at least 1 h, and finally imaged via microscopy (Nikon P-DSL32). For the dark treatment, Col-0 and *rps6a* seeds were sown on vertical plates with MS media. After 2 d of stratification, the plates were transferred to a growth chamber for 1 d and then wrapped in tin foil for 3 d. Then, the hypocotyl length was measured.

2.3 CRISPR–Cas9 method for generating the *rps6b* mutant

The sgRNAs 5'-GCAAAAGACGGACACGCCC-3' were designed to target *RPS6B*. The sgRNA was subsequently cloned and inserted into the pHY07 vector (kindly provided by Prof. Shisong Ma, University of Science and Technology of China[24]). Two PCRs were performed using pUC57_U6 as the template, with the primer pairs pUC57_P1F/pUC57_P2 and pUC57_P3/pUC57_P4. The resulting PCR fragments were purified and recombined to generate the intermediate plasmid pUC57_sgRNA containing a single sgRNA expression cassette (Carb resistance in DH5α). The sgRNA expression cassette from the recombinant pUC57_sgRNA vector was PCR amplified via the pHY07-Fu-F and pHY07-Fu-R primers and recombined into the pHY07 vector at the HindIII site (Kan resistance in DH5α). This construct was subsequently introduced into *Agrobacterium tumefaciens* strain GV3101. The floral dip method[25] was used for *Arabidopsis* transformation.

2.4 Pharmacological treatments

For auxin and its analog treatment assays, *Arabidopsis* seeds were sown on vertical plates with MS media. After 2 d of stratification, the plants were transferred to a growth chamber as described in the "plant materials and growth conditions" section for 4 or 7 d. Then, the seedlings were placed on MS media supplemented with the indicated chemicals, including indole 3-acetic acid (Sigma, I2886), 2,4-dichlorophenoxyacetic acid (Solarbio, D8100), *α*-naphthaleneacetic acid (Solarbio, N8010) and dimethyl sulfoxide (DMSO, Sigma, D4540), which served as mock controls. The root length and number of lateral roots were measured after an additional 3 d of growth.

2.5 Imaging by confocal laser scanning microscopy (CLSM)

Fluorescence imaging was performed via a Zeiss LSM980 confocal laser scanning microscope with a GaAsP detector (Zeiss, Germany). The manufacturer's default settings (smart mode) were used for imaging proteins tagged with GFP (excitation, 488 nm; emission, 495–545 nm) or FM4-64 (excitation, 543 nm; emission, 600–700 nm). All of the images were obtained at 8 bit depth with 2× line averaging. The images were analyzed and visualized with the Fiji program.

For the IAA treatment assays used to observe the DR5rev::GFP signal, 3-day-old seedlings were transferred to MS media supplemented with 3-acetic acid or DMSO as the mock control and observed after 24 h.

To image FM4-64-stained cells, 4-day-old seedlings were incubated in FM4-64 [N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl)hexatrienyl)pyridinium dibromide, Invitrogen, T13320] (1 μg/mL in liquid MS medium) for 15 min and rinsed three times in liquid MS medium before observation.

2.6 Imaging and morphological analysis

For observation of the seedling root phenotype, photographs

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were taken via a camera (Sony A6000 with a macrolens), and then, the primary root length or root tip angles were analyzed with ImageJ[26]. Lateral root numbers were counted directly. The veins of the cotyledons were observed via a Nikon SMZ18.

To observe the fluorescence in the roots, all the images were obtained via CLSM, and the fluorescence intensity was measured via ImageJ.

2.7 Protein extraction and Western blot

Col-0, *rps6a*, *pPIN1::PIN1-GFP*, *rps6a pPIN1::PIN1-GFP*, *pPIN3::PIN3-GFP* and *rps6a pPIN3::PIN3-GFP* seedlings (7 d) were used for total protein extraction. After harvesting, the samples were ground in liquid nitrogen, resuspended in 10% TCA acetone solution and stored at −20 °C for 3 h. The mixture was subsequently centrifuged, and the precipitate was resuspended in acetone. This process was repeated five times to completely clean the precipitate. Finally, the precipitate was resuspended in protein extraction buffer (1×PBS, 9 M urea, 10 mM DTT) and spun down to discard the cell debris[26]. The protein samples were then analyzed via western blotting. PIN1-GFP and PIN3-GFP were detected with an anti-GFP antibody (JL8, Clontech, 1∶2000). HRP activity was detected by HRP-labeled goat anti-mouse IgG (H+L) (Beyotime) and imaged with AMERSHAM ImageQuant 800.

2.8 RNA-seq analysis

For RNA-seq analysis, 7-day-old Col-0 and *rps6a* seedlings were treated with 1 μM IAA or DMSO for 3 h. Every group had three independent 0.1 g samples for RNA extraction, and 1 μg of each RNA sample was used for library preparation. Library preparation and RNA-seq were performed via BGI via the BGISEQ platform, resulting in approximately 70 million reads per sample. The quality of the raw RNA-seq reads was checked and trimmed to remove adaptor sequences, contaminants, and low-quality reads. The trimmed reads of each sample were aligned to the publicly available reference genome of Arabidopsis (TAIR10, https://support.illumina.com/sequencing/sequencing_software/igenome.html) via HISAT2 version 2.0.4 with default parameters. Data analyses were performed with the Dr. Tom platform of BGI. The RNA-seq data have been deposited at NCBI (SAR: SUB14957736) and are publicly available as of the date of publication. The accession numbers are listed in Table S2. Lists of DEGs are shown in the supplemental data 1 to 4 in Supporting information. A list of auxin- and root-related genes is provided in the supplemental data 5 and 6 in Supporting information.

2.9 Quantitative PCR with reverse transcription (RT–qPCR)

RT–qPCR was used to examine the transcripts of genes in *rps6a* mutants, and Col-0 *ACTIN7* (*AT5G09810*) was used as an internal reference. Total RNA was extracted with a FastPure Universal Plant Total RNA Isolation Kit (Vazyme). Then, 1 μg of the RNA sample was reverse transcribed with 5×PeimeScript RT Master Mix. The cDNA of the corresponding genes and *ACTIN7* was analyzed via SYBR Premix Ex Taq (Takara) with a Bio-Rad CFX Connect Real-Time System. The relative transcript levels of the examined genes were normalized to the expression of *ACTIN7* and were calculated by setting the wild-type (WT) or a certain tissue as 1, and the data are presented as the means ± SDs from three biological replicates. The primers used are shown in Table S3.

2.10 Quantification and statistical analysis

Most experiments were repeated at least three times independently, with similar results obtained. For measurements of primary root length and root tip angles, photographs or scans were analyzed with the ImageJ program (https://imagej.nih.gov/ij/download.html). The fluorescence intensity of the CLSM images was quantified with Fiji (https://fiji.sc/)[27]. Data visualization and statistical analysis were mostly performed with GraphPad Prism 8. For the bending curves of the root tips, polar graphs were generated via Origin 2023, and the *n* and *p* values are indicated in the figures or legends.

3 Results

3.1 Loss-of-function mutants of *Arabidopsis rps6a* and *rps6b* exhibit auxin-related defects

To explore the biological function of the RPS6 protein, we generated a transfer DNA (T-DNA) insertion mutant with a loss of function in *rps6a-2* (short as *rps6a*, Fig. S1a, b in Supporting information). In addition, we generated a r*ps6b* mutant with the CRISPR-Cas9 system in which a C-base deletion resulted in a subsequent frameshift change and premature termination of protein translation (Fig. S1c). Compared with *Arabidopsis* Col-0 WT plants, *rps6a* and *rps6b* mutant plants presented considerable decreases in primary root length, lateral root number and lateral root density under normal conditions (Fig. 1a–e, Fig. S2e–h). Since both are functionally redundant and interchangeable[5], we used *rps6a* for physiological analysis in our study. In addition, the results of the root gravitropism assay revealed that, as expected, the roots of Col-0 bent toward gravity (Fig. 1f, g). In contrast, the rate of root bending toward gravity was lower in the *rps6a* mutant than in the WT, indicating an impaired gravitropic response (Fig. 1f, g). These phenotypic results suggest that these effects of *rps6a* mutation on root development are reminiscent of the dysfunction of auxin signaling, a phytohormone that can be transported in response to gravity stimulation[20].

Auxin plays an essential role in regulating root growth and development[28]. Therefore, we examined the effects of exogenously applied auxin on root growth. When grown on Murashige and Skoog (MS) media supplemented with different concentrations of auxin (indole-3-acetic acid, IAA), the auxin analogs 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) (0, 50, 100, and 200 nM)[29,30], compared with untreated seedlings, Col-0 seedlings presented shortened primary roots (Fig. 2a–f) and an increased number of lateral roots (Fig. 2g–l). Notably, the *rps6a* mutant presented fewer lateral roots than did Col-0 at the seedling stage (Fig. 1c, d). However, the changes in primary root length and the number of lateral roots with or without chemical compounds were significantly weaker in *rps6a* than in the WT, especially in terms of the number of lateral roots (Fig. 2). Additionally, when WT and *rps6a* mutant seedlings were grown on MS media in the dark, they grew upright, whereas

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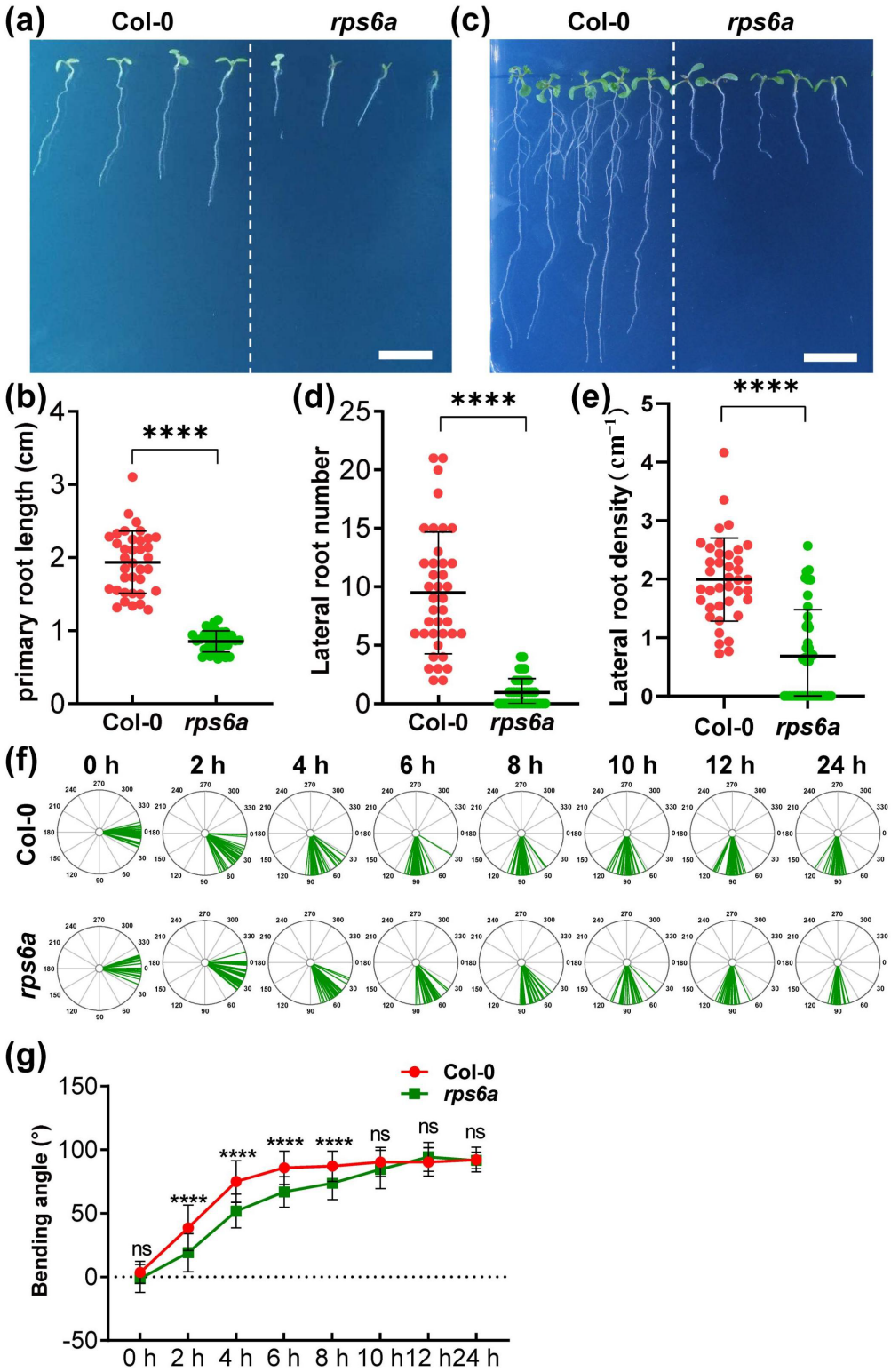

Fig. 1. *rps6a* results in an auxin-deficient phenotype. (a) The *rps6a* mutant has a shorter primary root. Seven-day-old seedlings were subsequently grown on MS media. Scale bar, 1 cm. (b) Quantitation of primary root lengths in figure (a), n=36 and 36. The dots represent individual values, and the lines indicate the means ± SDs. ****p<0.0001 according to Welch's t test. (c) The *rps6a* mutant has fewer lateral roots. Eleven-day-old seedlings were subsequently grown on MS media. Scale bar, 1 cm. (d) Quantification of the number of lateral roots in figure (c), n=39 and 41. The dots represent individual values, and the lines indicate the means ± SDs. ****p<0.0001 according to Welch's t test. (e) Lateral root density in figure (c). n=39 and 41. The dots represent individual values, and the lines indicate the means ± SDs. ****p<0.0001 according to Welch's t test. (f) The *rps6a* mutant has a relatively slow response to gravity. Four-day-old seedlings were rotated 90° and observed for 24 h. (g) Quantitation of the root bending angle. At 2, 4, 6, and 8 h, the root bending angle of the *rps6a* mutant was significantly smaller than that of Col-0. The dots represent average values, and the lines indicate the means ± SDs. ****p<0.0001 according to Welch's t test.

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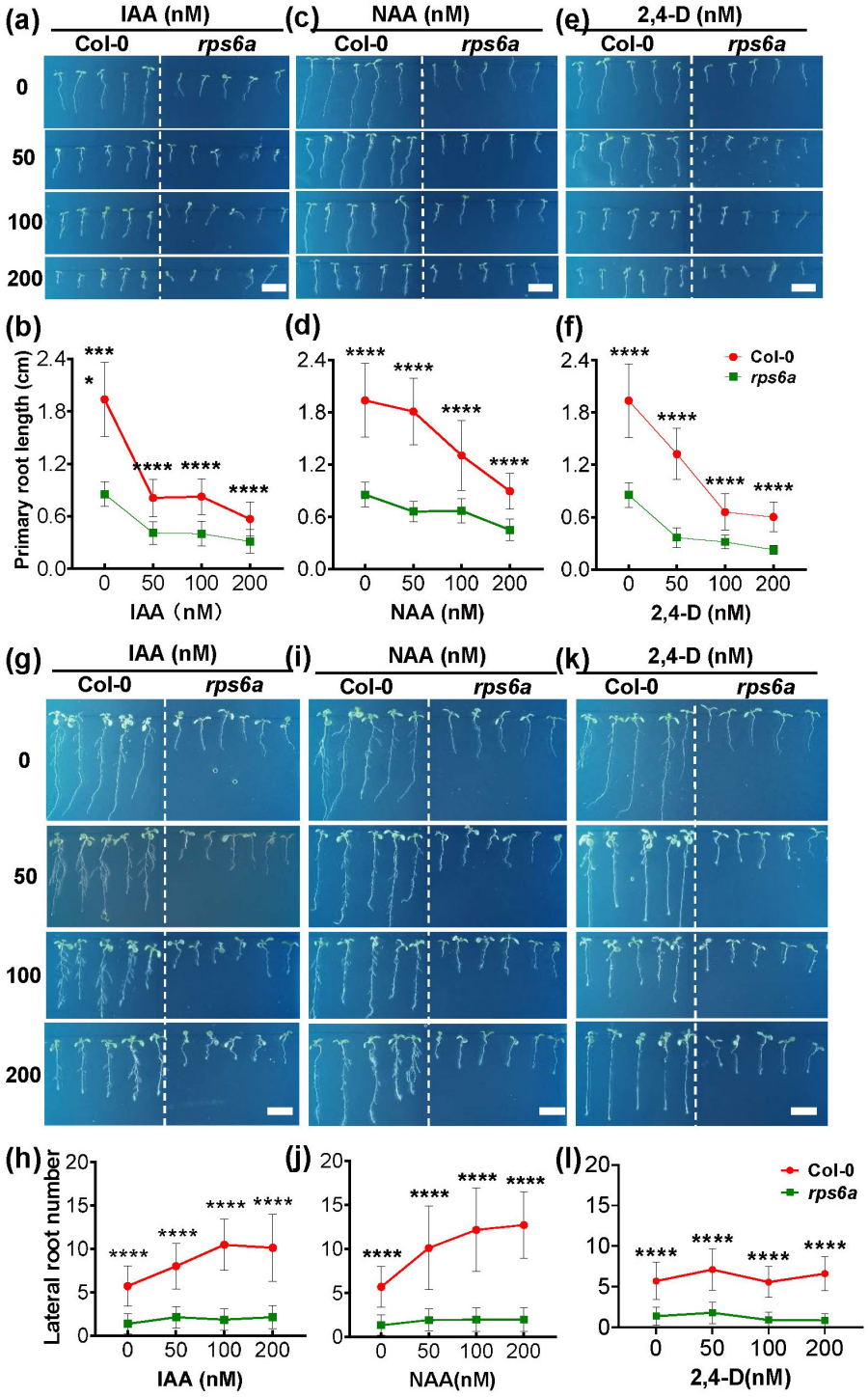

Fig. 2. *rps6a* is less sensitive to auxin than is Col-0. (a, c, e) Representative images revealing that auxin and its analogs decrease primary root length. Four-day-old Col-0 seedlings were transferred to MS media supplemented with gradient concentrations of IAA/NAA/2,4-D for an additional 3 d. DMSO was used as the solvent control. Scale bar, 1 cm. (b, d, f) Quantitation of primary root lengths from the lines in figure (a, c, e). (b) n=36, 18, 18, and 18 replicates for Col-0 seedlings and 36, 18, 18, and 18 replicates for *rps6a* seedlings under gradient IAA treatment. (d) n=36, 18, 18, and 18 replicates for Col-0 seedlings and 36, 18, 18, and 18 replicates for *rps6a* seedlings under gradient NAA treatment. (f) n=36, 18, 18, and 18 replicates for Col-0 seedlings and 36, 18, 19, and 18 replicates for *rps6a* seedlings under gradient 2,4-D treatment. ****p<0.0001 according to Welch's t test. (g, i, k) Auxin and its analogs did not increase the number of lateral roots of *rps6a*. Eight-day-old Col-0 seedlings were transferred to MS media supplemented with gradient concentrations of IAA/NAA/2,4-D for an additional 3 d. DMSO was used as the solvent control. Scale bar, 1 cm. (h, j, l) Quantitation of primary root lengths from the lines in figure (g, i, k). (h) n=36, 18, 17, and 18 replicates for Col-0 seedlings and 36, 18, 18, and 16 replicates for *rps6a* seedlings under gradient IAA treatments. (j) n=36, 18, 18, and 18 replicates for Col-0 seedlings and 36, 18, 18, and 18 replicates for *rps6a* seedlings under gradient NAA treatment. (l) n=36, 18, 18, and 18 replicates for Col-0 seedlings and 36, 18, 18, and 18 replicates for *rps6a* seedlings under gradient NAA treatment. ****p<0.0001 according to Welch's t test.

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the hypocotyls of the *rps6a* mutant were shorter than those of the WT (Fig. S2a, b). Auxin is required for leaf vasculature formation[31], and we found that the vasculature of *rps6a* mutant cotyledons was fragmented and that the leaves were smaller than those of WT cotyledons were (Fig. S2c, d). These results suggest that the loss of *RPS6A* function impairs numerous processes involved in auxin-regulated plant growth and tropic responses.

To further investigate the role of auxin in RPS6A-mediated root growth, we treated Col-0 and *rps6a* plants with different concentrations of the auxin transport inhibitor NPA[32]. The primary root length was significantly inhibited by 1 μM NPA (Fig. S3a, b). The root tip angle of *rps6a* was more dispersed, and the phenotype was more pronounced after NPA treatment (Fig. S3c). The number of lateral roots in Col-0 did not change significantly at lower NPA concentrations and decreased at higher NPA concentrations (Fig. S4). However, the number of LRs associated with *rps6a* was significantly lower in the 0.1 μM NPA treatment group than in the control group, and there were no LRs associated with the 0.2 μM NPA treatment group, indicating that *rps6a* was more sensitive to NPA. NPA treatment aggravated the gravitropic and lateral root defects in *rps6a*, suggesting that the auxin response of *rps6a* is defective.

3.2 Deficiency of RPS6A affects the local auxin distribution

Next, to further test whether RPS6A affects auxin distribution or signaling pathways, we examined the subcellular localization of the auxin-responsive reporter *DR5rev::GFP*[20]. In *Arabidopsis* root tips, auxin accumulates locally via PIN proteins[33,34], which are required for root meristem activity and the gravitropic response[31,35]. Treatment with the auxin transport inhibitor NPA could lead to an enlarged *DR5* expression region and overproliferation of root columellar cells. A similar phenomenon was also observed for genetic mutants defective in auxin transport. For example, our previous study revealed that the *DR5* expression region in the root tip was enlarged in the *pdk1.1 pdk1.2* mutant, which affects PIN protein activity[36,37].

Therefore, we generated a cross between *DR5rev::GFP* and *rps6a* to analyze changes in auxin distribution or signaling. Microscopic analysis revealed that DR5rev::GFP was expressed in vascular tissues in Col-0 seedlings in the absence of IAA, with the highest expression in root columellar cells (Fig. 3). However, DR5rev::GFP in the *rps6a* mutant presented a broader region of columellar cells without IAA treatment (Fig. 3). Since exogenous application of auxin can activate the canonical transcriptional auxin pathway, resulting in increased *DR5rev::GFP* expression, we detected no change in the GFP signal after IAA treatment in *rps6a* seedlings, which is different from the findings in Col-0 (Fig. 3a). The results of the GFP fluorescence signal were consistent with those of the root tips of *rps6a* seedlings, which responded to gravistimulation by 90° reorientation more slowly than did Col-0 (Fig. 1f, g). Therefore, we hypothesized that the loss of *RPS6A* function may affect auxin transport or redistribution in plants.

3.3 RPS6A regulates auxin transport by affecting the abundance of PIN proteins

These physiological phenotypes and cell biology experimental results indicate that RPS6A might affect the distribution or accumulation of auxin. Polarly localized PIN auxin transporters at the plasma membrane play important roles in plant growth and development as auxin efflux carriers that mediate polar auxin transport[38,39]. To explore whether RPS6A affects polar auxin transport, we crossed *rps6a* with GFP-fused PIN marker lines. Confocal laser scanning microscopy (CLSM) revealed that although the fluorescence signals of PIN1-GFP, PIN3-GFP and PIN7-GFP were weakened in *rps6a* roots, there was no change in the polar distribution of PIN1-GFP (Fig. 4a, b), PIN3-GFP (Fig. 4c, d) or PIN7-GFP (Fig. 4e, f). Additionally, the fluorescence intensity and polar localization of PIN2-Venus did not change (Fig. S5). To further verify the CSLM results, we performed Western blot experiments and found that the abundance of PIN1-GFP and PIN3-GFP was decreased in the *rps6a* mutant (Fig. 4g). Overall, we propose that RPS6A regulates polar auxin transport by modulating the abundance but not the polarity of PIN proteins.

RPS6A is a component of the 40S ribosome subunit and is involved in protein translation[14]. To explore whether RPS6A affects the transcription of *PIN* genes, we further analyzed the expression levels of *PIN* genes in *rps6a* and the WT via RT–qPCR. The results revealed that there was no significant difference in the expression levels of *PIN1*, *PIN4*, *PIN6*, and *PIN8* between the *rps6a* mutant and WT (Fig. S6). The expression levels of *PIN2* and *PIN5* decreased, whereas those of *PIN3* and *PIN7* increased (Fig. S6). The results of RT–qPCR and RNA–seq are generally consistent. The differences in the transcription and protein levels of *PIN1*, *PIN3*, and *PIN7* (Fig. 4) in *rps6a* and WT plants suggested that RPS6A regulates plant growth and development by modulating the translation of these PIN auxin transporters.

3.4 RPS6A regulates root meristem size and cell production

To further investigate the molecular mechanism by which RPS6A is involved in auxin-regulated root growth and development, we performed RNA sequencing (RNA-seq) profiling experiments with WT and *rps6a Arabidopsis* seedlings treated with IAA or the solvent control DMSO. Changes in the expression of auxin-related genes were analyzed in WT and *rps6a* plants under IAA treatment compared with those under the control (DMSO) treatment (Fig. S6). Compared with those in the WT, the number of IAA-induced genes associated with *rps6a* significantly increased (Fig. 5a and Fig. S7), suggesting potential defects in the auxin signaling pathway in the *rps6a* mutant. Given the major role of RPS6A in global protein translation, these changes in gene transcription might be due to feedback regulation of these genes. Further GO enrichment analysis of the DEGs in the *rps6a* mutant revealed that most of the DEGs were enriched in defense-related pathways (Fig. S8), suggesting the possible involvement of RPS6A in plant defense responses.

A heatmap of auxin-related genes revealed that RPS6A could affect the auxin signaling pathway (Fig. S9). To determine the effect of RPS6A on auxin signaling, we performed RT–qPCR analysis of several auxin downstream marker genes[40–43]. In the *rps6a* mutant, the expression levels of *ABP1*, *LBD39*, *IAA31*, and *IAA33* decreased, and the

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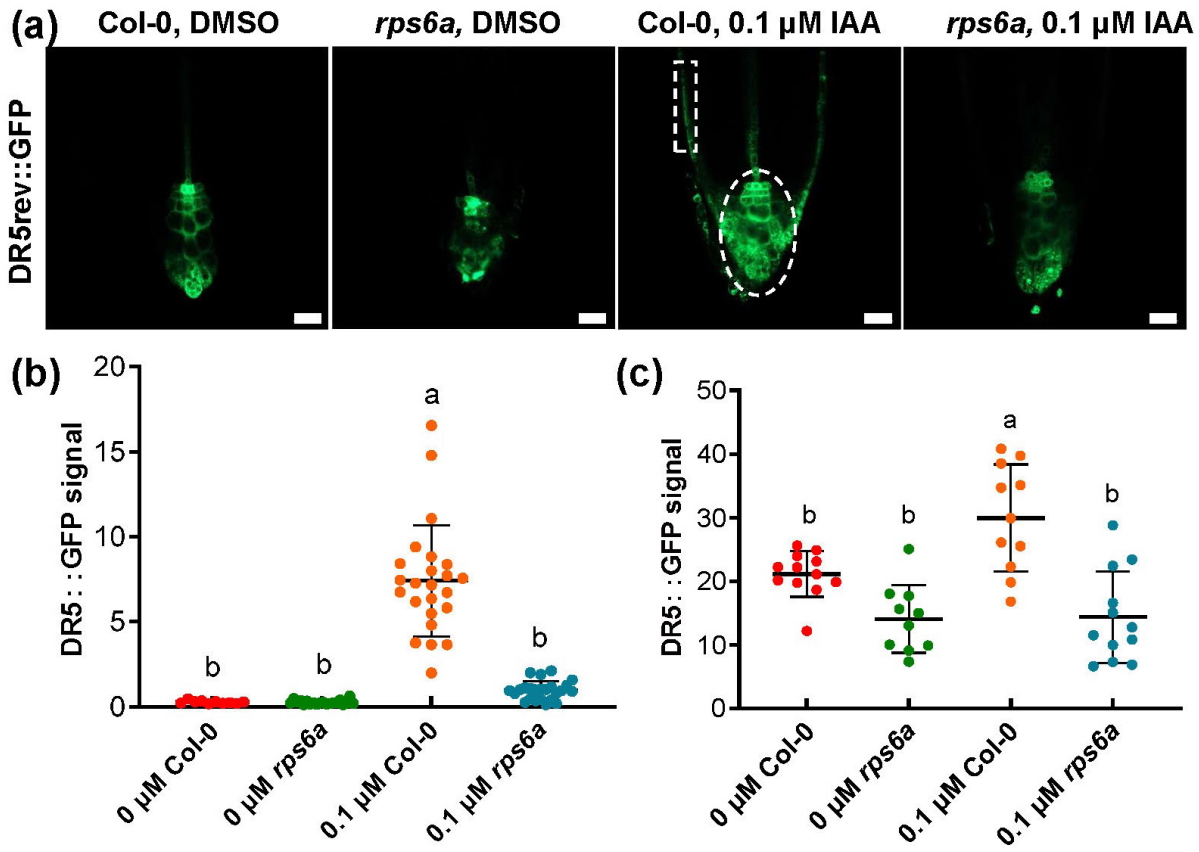

Fig. 3. IAA treatment did not rescue the decrease in the *DR5rev::GFP* signal in the *rps6a* mutant. (a) The DR5rev::GFP signal decreased in the *rps6a* mutant, and the signal did not increase after 24 h of auxin treatment. Three-day-old *DR5rev::GFP* and *rps6a DR5rev::GFP* seedlings were transferred to MS media supplemented with 0.1 μM IAA for an additional 1 d, and DMSO was used as a control. Scale bar, 20 μm. (b) The GFP signal in the epidermis was measured to indicate the fluorescence intensity. n = 24, 20, 24, and 24. The dots represent individual values, and the lines indicate the means ± SDs. ****p<0.0001 according to Welch's t test. (c) The GFP signal in the root tip was measured to indicate the fluorescence intensity. n = 12, 11, 13, and 13. The dots represent individual values, and the lines indicate the means ± SDs. *p<0.05 according to Welch's t test.

expression levels of *AFB5*, *GH3.3*, and *TIR1* increased (Fig. 5b, c). These results were consistent with our RNA-seq results, indicating that RPS6A affects the auxin signaling pathway.

In addition, we further analyzed RNA-seq data and found that RPS6A can affect the expression of root development-related genes in *Arabidopsis* (Fig. S10). The regulatory effect of RPS6A on the growth and development of roots may be related to the effect of RPS6A on the auxin signaling pathway.

Auxin plays a central role in root growth and development by regulating root cell division, growth and differentiation[28,44,45]. In *Arabidopsis*, the stem cell niche and the size of the root meristem are maintained by the quiescent center (QC), stele stem cells, columellar stem cells, and cortex/endodermis initial cells[46,47]. QC cells exhibit low levels of cell division activity, which is essential for root stem cell maintenance. To understand the origin of the root growth defect in *rps6a*, we measured the size of the root meristem of the primary root in WT and *rps6a* seedlings, which was determined by the number of cortex cells between the QC and elongation zones. Compared with those of the WT, the QC zone of *rps6a* was deficient, and the cortex cell number and meristem length were significantly shorter (Fig. 6). The *rps6b* mutant presented the same phenotype (Fig. S11). These observations clearly show that QC function is impaired in the *rps6a* mutant compared with the WT and that the slow root growth of *rps6a* is due to reduced meristem activity.

4 Discussion

The ribosome plays a central role in protein translation[14]. In this study, we performed phenotypic analysis, cell biological experiments and transcriptome profiling to investigate the role of *RPS6A* in plant growth and development. The *rps6a* mutant presented shortened primary roots, fewer lateral roots, defective cotyledon veins and fewer root meristem cells, which are all auxin-deficient phenotypes. Notably, *rps6b* and *rps6a* presented similar phenotypes in terms of primary root length, lateral root number, and root meristem cell number. Thus, on the basis of the above results and previous studies, we believe that *RPS6A* and *RPS6B* are functionally redundant and interchangeable[5].

The DR5 signal revealed that the auxin concentration was reduced at the tip of the *rps6a* mutant. Notably, the *rps6a* mutant was relatively insensitive to auxin treatment,

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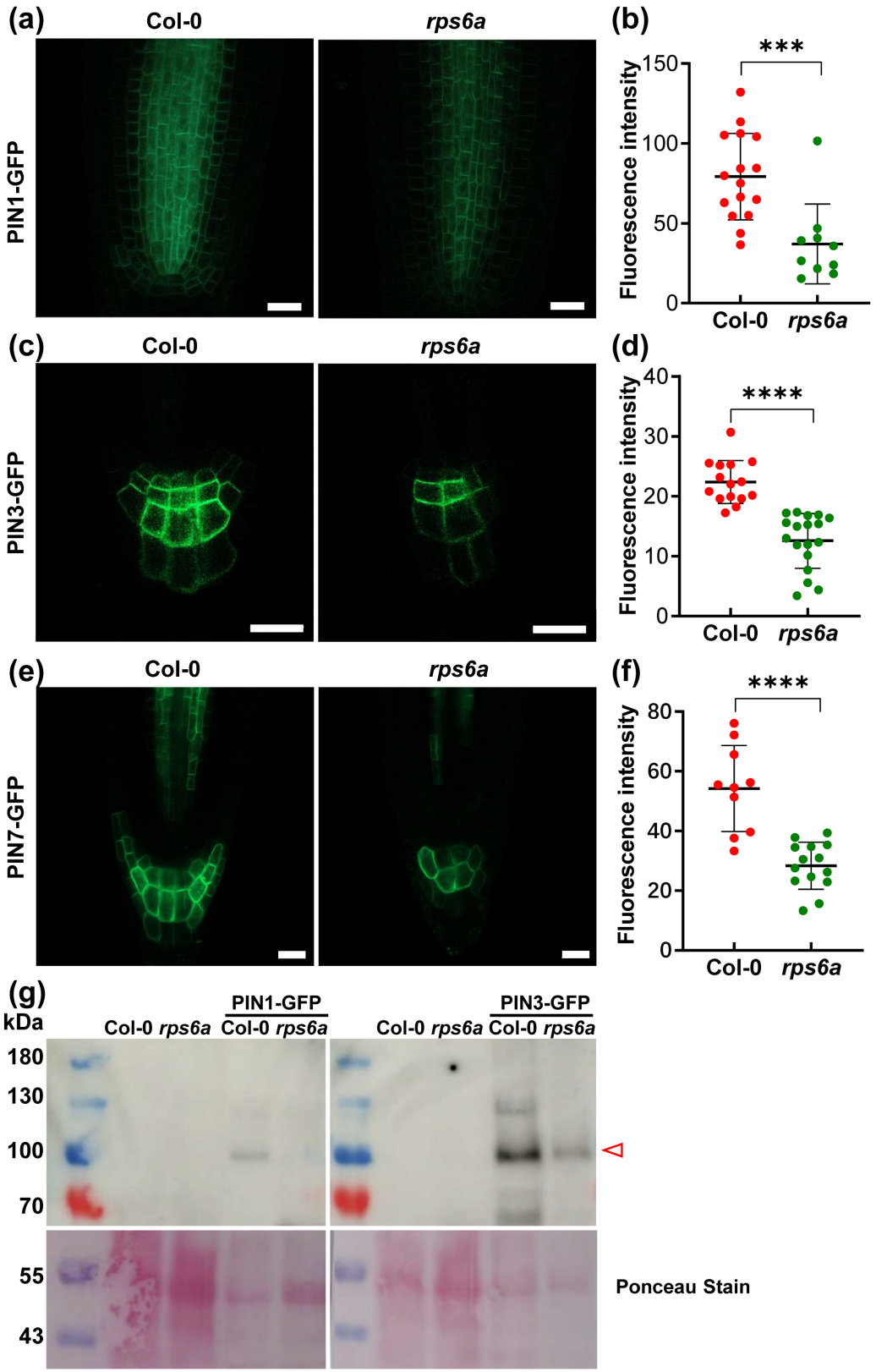

Fig. 4. The PIN1-GFP, PIN3-GFP, and PIN7-GFP fluorescence signals of *rps6a* decreased. (a, c, e) The PIN1-GFP, PIN3-GFP, and PIN7-GFP signals decreased in the *rps6a* mutant. Four-day-old *PIN1-GFP*, *PIN3-GFP*, and *PIN7-GFP* Col-0 and *rps6a* seedlings were grown on MS media. Scale bar, 20 μm. (b, d, f) The GFP signal in (a, c, e) was measured to indicate the fluorescence intensity. n = 16, 10, 15, 18, 10, and 14. The dots represent individual values, and the lines indicate the means ± SDs. ***p<0.001, and ****p<0.0001 according to Welch's t test. (g) Western blot analysis revealed that the abundance of PIN1-GFP and PIN3-GFP decreased in the *rps6a* mutant. The sample was detected by an anti-GFP antibody.

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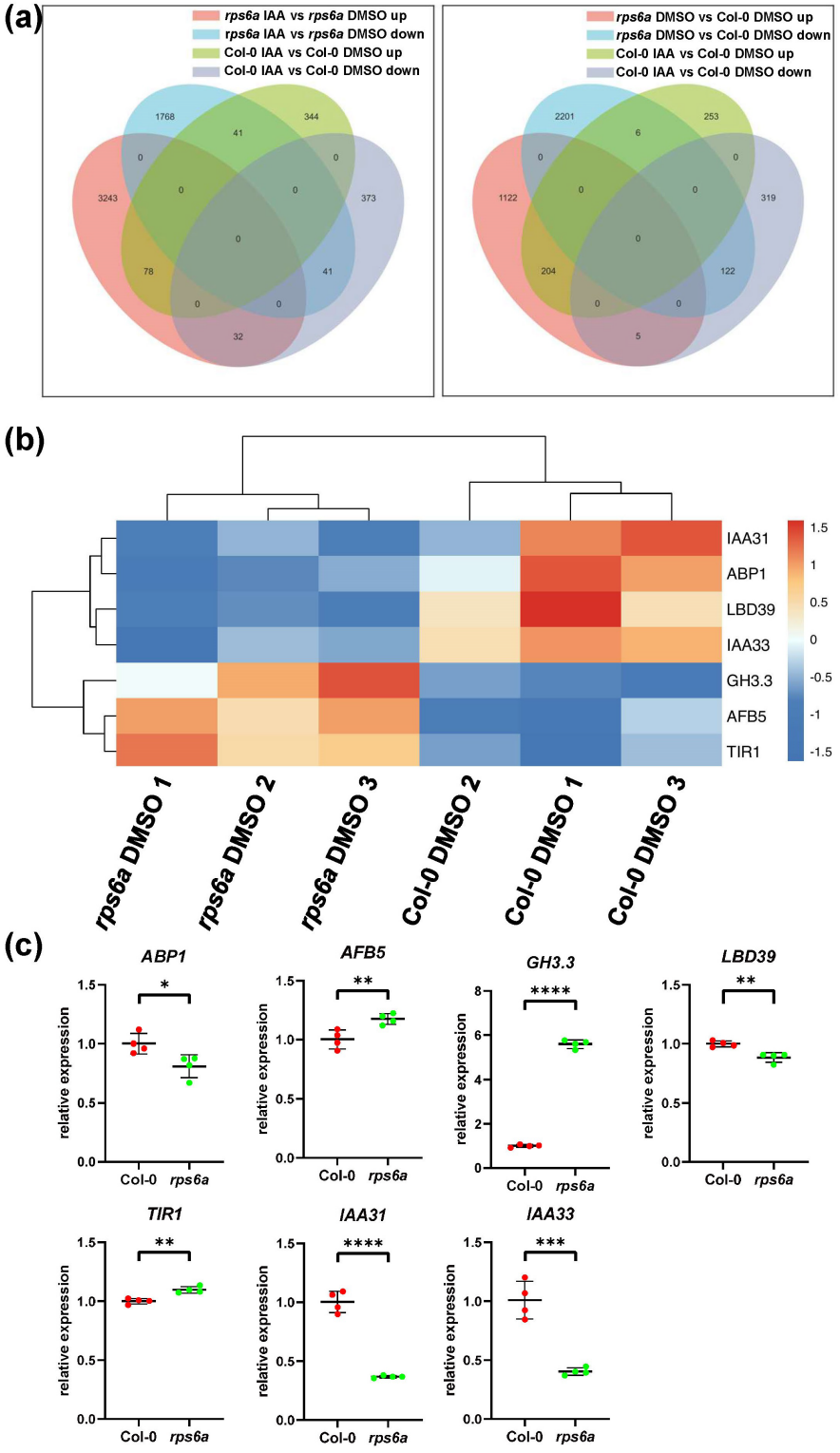

Fig. 5. The RNA-seq results revealed that gene expression changed in the mutant after auxin treatment. (a) Venn diagram showing the number of DEGs (FDR< 0.05, versus Col-0) in various pairwise comparisons. (b) Heatmap showing the expression patterns of the auxin-related genes *ABP1*, *AFB5*, *GH3.3*, *TIR1*, *LBD39*, *IAA5*, *IAA19*, *IAA31*, and *IAA33* in the *rps6a* mutant and Col-0. Seven-day-old Col-0 and *rps6a* seedlings were grown vertically on MS media and treated with 1 μM IAA or DMSO (as the mock control) for 3 h. Hierarchical clustering of $\log_2$FC values (Col-0 vs. *rps6a*) was performed via DESeq2 analysis. (c) RT–qPCR analysis showing the relative expression levels of the auxin-related genes *ABP1*, *AFB5*, *GH3.3*, *TIR1*, *LBD39*, *IAA5*, *IAA19*, *IAA31*, and *IAA33*. The dots represent individual values (Col-0 represents red dots, and *rps6a* represents green dots), and the lines indicate the means ± SDs. **p*<0.05, ***p*< 0.01, ****p*<0.001, and *****p*<0.0001 according to Welch's *t* test.

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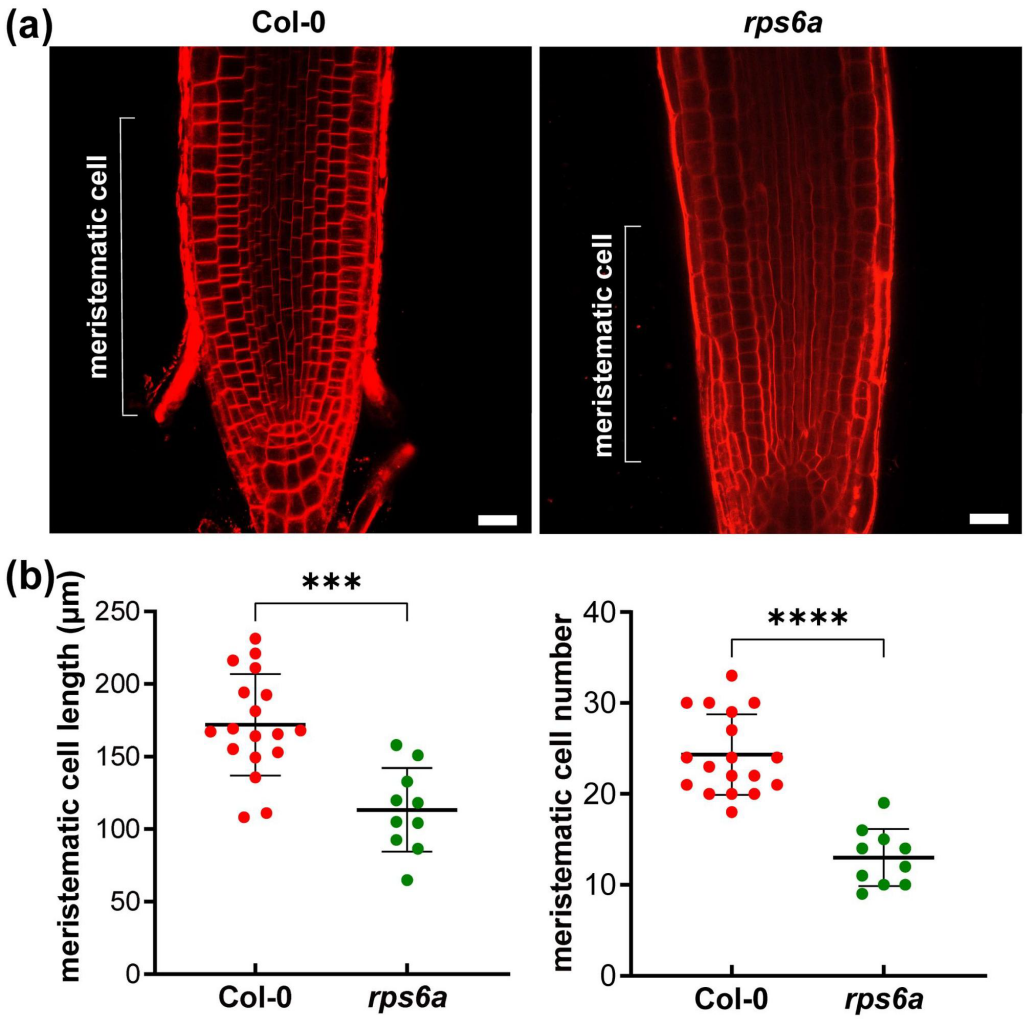

Fig. 6. Meristematic cells in the roots of the *rps6a* mutant were reduced. (a) Four-day-old Col-0 and *rps6a* seedlings were stained with FM4-64 for 15 min. Scale bar, 20 μm. (b) Quantification of meristematic cell length and cell number in (a), n=18 and 10. The dots represent individual values, and the lines indicate the means ± SDs. ****p<0.0001 according to Welch's t test.

suggesting a defect in auxin signaling. Further subcellular localization observations revealed that the abundance of the PIN1, PIN3 and PIN7 proteins was lower than that of Col-0. When we observed the DR5rev::GFP signal, the morphology of the QC region was abnormal in the *rps6a* mutant, and a double layer of QC cells appeared. This may be due to the lower auxin concentration in the root tip.

The process of protein expression involves many regulatory processes, such as transcriptional regulation, posttranscriptional regulation, and translational regulation. In most cases, scientists tend to focus on RNA-seq results to infer gene expression levels. However, only approximately 40% of mRNA abundances are clearly correlated with their translated protein abundances[48]. Different protein or rRNA compositions of the ribosome can affect the translation of specific mRNAs[49]. Here, we found that RPS6A deficiency resulted in a decrease in the abundance of the auxin transporters PIN1, PIN3, and PIN7, which led to a decreased auxin concentration and an auxin-deficient phenotype. These results suggested that RPS6A might regulate plant growth and development by modulating the protein translation efficiency of auxin-containing components.

5 Conclusions

In conclusion, this study revealed that the ribosomal protein RPS6A in *Arabidopsis* regulates auxin signaling and root development by modulating the abundance of PIN auxin transporters (PIN1, PIN3, and PIN7). These results reveal the important role of RPS6A in ribosomal function and auxin-mediated development, linking protein synthesis to auxin signaling in plants.

Further investigations are needed to explore the molecular mechanisms by which RPS6A affects global protein translation and plant developmental processes. The role of RPS6A phosphorylation and its interaction with signaling pathways, such as the TOR-S6K cascade, should be examined to understand how RPS6A integrates environmental and developmental cues. These studies help bridge the connection between ribosomal proteins and auxin signaling, revealing how the protein synthesis machinery directly impacts hormone-regulated growth and development in plants.

Supporting information

The supporting information for this article can be found

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online at https:// doi.org/10.52396/JUSTC-2024-0148. The supporting information includes 11 supplemental figures, 4 supplemental tables, and 6 supplemental datasets.

Disclosures

The chemicals used in this study, including TCA, acetone, acrylamide, methanol, and TEMED, are corrosive, toxic, and/or flammable. All procedures involving these chemicals were performed under ventilated conditions with appropriate personal protective equipment, including gloves, masks, and lab coats.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] Ben-Shem A, Garreau de Loubresse N, Melnikov S, et al. The structure of the eukaryotic ribosome at 3.0 Å resolution. *Science*, **2011**, *334*: 1524–1529.
[2] Rabl J, Leibundgut M, Ataide S F, et al. Crystal structure of the eukaryotic 40*S* ribosomal subunit in complex with initiation factor 1. *Science*, **2011**, *331*: 730–736.
[3] Warner J R, McIntosh K B. How common are extraribosomal functions of ribosomal proteins. *Molecular Cell*, **2009**, *34*: 3–11.
[4] Lindström M S. Emerging functions of ribosomal proteins in gene-specific transcription and translation. *Biochemical and Biophysical Research Communications*, **2009**, *379*: 167–170.
[5] Creff A, Sormani R, Desnos T. The two *Arabidopsis* RPS6 genes, encoding for cytoplasmic ribosomal proteins S6, are functionally equivalent. *Plant Molecular Biology*, **2010**, *73*: 533–546.
[6] Watson K L, Chou M M, Blenis J, et al. A *Drosophila* gene structurally and functionally homologous to the mammalian 70-kDa S6 kinase gene. *Proceedings of the National Academy of Sciences of the United States of America*, **1996**, *93*: 13694–13698.
[7] Mancera-Martínez E, Dong Y, Makarian J, et al. Phosphorylation of a reinitiation supporting protein, RISP, determines its function in translation reinitiation. *Nucleic Acids Research*, **2021**, *49*: 6908–6924.
[8] Williams A J, Werner-Fraczek J, Chang I F, et al. Regulated phosphorylation of 40S ribosomal protein S6 in root tips of maize. *Plant Physiology*, **2003**, *132*: 2086–2097.
[9] Obomighie I, Lapenas K, Murphy B E, et al. The role of ribosomal protein S6 kinases in plant homeostasis. *Frontiers in Molecular Biosciences*, **2021**, *8*: 636560.
[10] McCready K, Spencer V, Kim M. The importance of TOR kinase in plant development. *Frontiers in Plant Science*, **2020**, *11*: 16.
[11] Dobrenel T, Mancera-Martínez E, Forzani C, et al. The Arabidopsis TOR kinase specifically regulates the expression of nuclear genes coding for plastidic ribosomal proteins and the phosphorylation of the cytosolic ribosomal protein S6. *Frontiers in Plant Science*, **2016**, *7*: 1611.
[12] Hirashita T, Hirashita Y, Iwashita Y, et al. S6 ribosomal protein phosphorylation is associated with malignancy of intraductal papillary mucinous neoplasm of the pancreas. *Annals of Gastroenterological Surgery*, **2020**, *4*: 571–579.
[13] Ruvinsky I, Meyuhas O. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. *Trends in Biochemical Sciences*, **2006**, *31*: 342–348.
[14] Chen G H, Liu M J, Xiong Y, et al. TOR and RPS6 transmit light signals to enhance protein translation in deetiolating *Arabidopsis* seedlings. *Proceedings of the National Academy of Sciences of the United States of America*, **2018**, *115*: 12823–12828.
[15] Turkina M V, Klang Årstrand H, Vener A V. Differential phosphorylation of ribosomal proteins in *Arabidopsis thaliana* plants during day and night. *PLoS ONE*, **2011**, *6*: e29307.
[16] Enganti R, Cho S K, Toperzer J D, et al. Phosphorylation of ribosomal protein RPS6 integrates light signals and circadian clock signals. *Frontiers in Plant Science*, **2018**, *8*: 2210.
[17] Dasgupta A, Urquidi Camacho R A, Enganti R, et al. A phosphorylation-deficient ribosomal protein eS6 is largely functional in *Arabidopsis thaliana*, rescuing mutant defects from global translation and gene expression to photosynthesis and growth. *Plant Direct*, **2024**, *8*: e566.
[18] Rosado A, Li R, van de Ven W, et al. *Arabidopsis* ribosomal proteins control developmental programs through translational regulation of auxin response factors. *Proceedings of the National Academy of Sciences of the United States of America*, **2012**, *109*: 19537–19544.
[19] Wang R J, Zhao J, Jia M, et al. Balance between cytosolic and chloroplast translation affects leaf variegation. *Plant Physiology*,

CSTR: 32290.14.JUSTC-2024-0148
DOI: 10.52396/JUSTC-2024-0148
JUSTC, **2025**, 55(3): 0305

2018, *176*: 804–818.
[20] Friml J, Vieten A, Sauer M, et al. Efflux-dependent auxin gradients establish the apical–basal axis of *Arabidopsis*. *Nature*, **2003**, *426*: 147–153.
[21] Xu J, Hofhuis H, Heidstra R, et al. A molecular framework for plant regeneration. *Science*, **2006**, *311*: 385–388.
[22] Zádníková P, Petrásek J, Marhavy P, et al. Role of PIN-mediated auxin efflux in apical hook development of *Arabidopsis thaliana*. *Development*, **2010**, *137*: 607–617.
[23] Kleine-Vehn J, Ding Z, Jones A R, et al. Gravity-induced PIN transcytosis for polarization of auxin fluxes in gravity-sensing root cells. *Proceedings of the National Academy of Sciences of the United States of America*, **2010**, *107*: 22344–22349.
[24] Geng H, Wang Y, Xu Y, et al. Data-driven optimization yielded a highly-efficient CRISPR/Cas9 system for gene editing in Arabidopsis. *bioRxiv*, **2023**. https://doi.org/10.1101/2023.10.09.561629.
[25] Clough S J, Bent A F. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal*, **1998**, *16*: 735–743.
[26] Koontz L. Chapter one - TCA precipitation. In: Lorsch J, editor. Methods in Enzymology: Vol. 541. New York: Academic Press, **2014**: 3−10.
[27] Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nature Methods*, **2012**, *9*: 676–682.
[28] Tan S T, Luschnig C, Friml J. Pho-view of auxin: reversible protein phosphorylation in auxin biosynthesis, transport and signaling. *Molecular Plant*, **2021**, *14*: 151–165.
[29] Rahman A, Nakasone A, Chhun T, et al. A small acidic protein 1 (SMAP1) mediates responses of the *Arabidopsis* root to the synthetic auxin 2, 4-dichlorophenoxyacetic acid. *The Plant Journal*, **2006**, *47*: 788–801.
[30] Dindas J, Becker D, Roelfsema M R G, et al. Pitfalls in auxin pharmacology. *New Phytologist*, **2020**, *227*: 286–292.
[31] Adamowski M, Friml J. PIN-dependent auxin transport: action, regulation, and evolution. *The Plant Cell*, **2015**, *27*: 20–32.
[32] Yang Z S, Xia J, Hong J J, et al. Structural insights into auxin recognition and efflux by *Arabidopsis* PIN1. *Nature*, **2022**, *609*: 611–615.
[33] Křeček P, Skůpa P, Libus J, et al. The PIN-FORMED (PIN) protein family of auxin transporters. *Genome Biology*, **2009**, *10*: 249.
[34] Petrášek J, Friml J. Auxin transport routes in plant development. *Development*, **2009**, *136*: 2675–2688.
[35] Marhava P, Bassukas A E L, Zourelidou M, et al. A molecular rheostat adjusts auxin flux to promote root protophloem differentiation. *Nature*, **2018**, *558*: 297–300.
[36] Xia J, Kong M J, Yang Z S, et al. Chemical inhibition of *Arabidopsis* PIN-FORMED auxin transporters by the anti-inflammatory drug naproxen. *Plant Communications*, **2023**, *4*: 100632.
[37] Tan S T, Zhang X X, Kong W, et al. The lipid code-dependent phosphoswitch PDK1-D6PK activates PIN-mediated auxin efflux in *Arabidopsis*. *Nature Plants*, **2020**, *6*: 556–569.
[38] Zhang J, Nodzynski T, Pencík A, et al. PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport. *Proceedings of the National Academy of Sciences of the United States of America*, **2010**, *107*: 918–922.
[39] Zegzouti H, Anthony R G, Jahchan N, et al. Phosphorylation and activation of PINOID by the phospholipid signaling kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, **2006**, *103*: 6404–6409.
[40] Friml J, Gallei M, Gelová Z, et al. ABP1–TMK auxin perception for global phosphorylation and auxin canalization. *Nature*, **2022**, *609*: 575–581.
[41] Dharmasiri N, Dharmasiri S, Estelle M. The F-box protein TIR1 is an auxin receptor. *Nature*, **2005**, *435*: 441–445.
[42] Wojtaczka P, Ciarkowska A, Starzynska E, et al. The GH3 amidosynthetases family and their role in metabolic crosstalk modulation of plant signaling compounds. *Phytochemistry*, **2022**, *194*: 113039.
[43] Lv B S, Yu Q Q, Liu J J, et al. Non-canonical AUX/IAA protein IAA33 competes with canonical AUX/IAA repressor IAA5 to negatively regulate auxin signaling. *The EMBO Journal*, **2020**, *39*: e101515.
[44] Roychoudhry S, Kepinski S. Auxin in root development. *Cold Spring Harbor Perspectives in Biology*, **2022**, *14*: a039933.
[45] Saini S, Sharma I, Kaur N, et al. Auxin: a master regulator in plant root development. *Plant Cell Reports*, **2013**, *32*: 741–757.
[46] Yamada M, Han X, Benfey P N. RGF1 controls root meristem size through ROS signalling. *Nature*, **2020**, *577*: 85–88.
[47] Dello Ioio R, Linhares F S, Scacchi E, et al. Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. *Current Biology*, **2007**, *17*: 678–682.
[48] Vogel C, Marcotte E M. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nature Reviews Genetics*, **2012**, *13*: 227–232.
[49] Dalla Venezia N, Vincent A, Marcel V, et al. Emerging role of eukaryote ribosomes in translational control. *International Journal of Molecular Sciences*, **2019**, *20*: 1226.

CSTR: 32290.14.JUSTC-2024-0148
DOI: 10.52396/JUSTC-2024-0148
JUSTC, **2025**, 55(3): 0305