

Arabidopsis SUMO E3 ligase AtMMS21 regulates root meristem development

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The small ubiquitin modifier (SUMO) conjugation/deconjugation is an important regulatory progress in plant development and responses to abiotic stresses. However, much less is known about the roles of sumoylation in plant root development. Cytokinin and auxin play crucial roles in determining the balance between cell proliferation and cell differentiation in Arabidopsis roots. The SUMO E3 ligase AtMMS21 is a homologue of human NSE2/MMS21, which modulates DNA damage and DNA repair in human cells. This addendum summarizes our recent paper on the AtMMS21 mediating cytokinin signaling to regulate the root meristem cell proliferation. The *mms21-1* roots had reduced responses to exogenous cytokinins and decreased expression of the cytokinin-induced genes *ARR3*, *ARR4*, *ARR5* and *ARR7*, compared with the wild type. Furthermore, the expression of *CRE1* and *ARR1*, which are both the receptor and positive regulator of cytokinin signaling, was also reduced in the *mms21-1* mutant plants.

All organisms use a variety of chemical modifiers for post-translational control of proteins that affect development, growth and homeostasis. Ubiquitin is one such polypeptide that was first described to attach covalently to other proteins upon completion of their synthesis. SUMOs (small ubiquitin-like modifiers) are ubiquitin-like proteins with three-dimensional structures that are similar to ubiquitin.¹ Similar to ubiquitination, sumoylation of substrates is catalyzed by a cascade of enzymes: the E1 SUMO-activating

enzyme, the E2 conjugating enzyme and the E3 SUMO ligases.² Sumoylation of target proteins in yeast and metazoans has been implicated in the regulation of innate immunity, cell cycle progression and mitosis, DNA repair, chromatin stability, cell division, nucleo-cytoplasmic trafficking, subnuclear targeting, ubiquitination antagonism and transcriptional regulation.^{3,4} PIAS/Siz proteins are SUMO E3 ligases that mediate the final step of SUMO conjugation.² Transcription factors are direct targets of SUMO conjugation mediated by PIAS/Siz proteins.⁴ The yeast SUMO E3 ligases Siz1 and Siz2 facilitate cell division at low temperatures.⁵ The yeast MMS21 and its human homologue of MMS21 contain an SP-RING domain that is related to the PIAS family of SUMO E3 ligases that autSUMOylate, and are required for the prevention of DNA damage-induced apoptosis by facilitating DNA repair.^{6,7} SUMO conjugation/deconjugation in plants has been implicated in responses to heat shock, oxidative stress, hypoxia, phosphate limitation, ABA, flowering and pathogen defense.⁸⁻¹³

Recently, the Arabidopsis SUMO E3 ligase SIZ1 has been shown to participate in responses to phosphate starvation, salicylic acid-mediated signaling in plant pathogen defense, ABA signaling and basal thermotolerance, and in the development progress of flowering.¹¹⁻¹⁶ Until now, the function of sumoylation in regulating the plant root development has not reported.

Here we demonstrated the role of Arabidopsis SUMO E3 ligase AtMMS21 in apical root meristem development. *AtMMS21* null mutant *mms21-1* exhibited severe short primary root phenotype

Key words: *Arabidopsis thaliana*, AtMMS21, cytokinin, root meristem, SUMO E3 ligase

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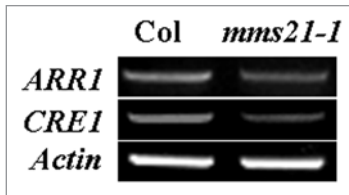


Figure 1. Expression of *CRE1* and *ARR1* genes were reduced in *mms21-1* loss-of-function mutant plants.

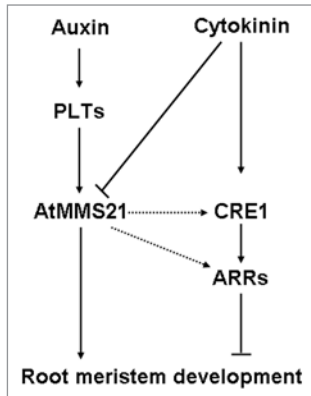


Figure 2. Proposed model of AtMMS21 function in root meristem development. AtMMS21 acts as a node element of cytokinin and auxin interaction. It plays role positively in the PLTs-mediated auxin signaling pathway to promote the root meristem cell division on one hand.¹⁸ AtMMS21 is inhibited by cytokinin to modulate the root meristem cell differentiation on the other hand.¹⁷ AtMMS21 also may stimulate cytokinin signaling by inducing the expression of *CRE1* and *ARRs* genes to sustaining the homeostasis between cytokinin and auxin signaling in the progress of root meristem development.

caused by apical root meristem cell proliferation defect that linked to impaired expression of the cell division marker *CYCB1:GUS*. The *mms21-1* roots had reduced sensitivity to exogenous cytokinins, and decreased expression of the cytokinins-induced genes *ARR3*, *ARR4*, *ARR5* and *ARR7*. Interesting, the expression of *AtMMS21* was downregulated obviously by exogenous cytokinin.¹⁷ We also detected that the expressions of the cytokinin receptor gene *CRE1* and B-type ARR genes *ARR1* were decreased obviously in the *mms21-1* loss-of-function mutant allele plants (Fig. 1). These results establish that AtMMS21 is involved in cytokinin signaling in regulating root

cell division and meristem development. Meanwhile, AtMMS21 (also named as HIGH PLOID2, HPY2) was considered to function as a repressor of endocycle onset in Arabidopsis meristems by regulating the cell cycle progression through the PLT-dependent auxin signaling pathway.¹⁸

Cytokinin and auxin play crucial roles in determining the balance between cell proliferation and cell differentiation in Arabidopsis roots. For meristem maintenance and to enable continuous root growth, the rate of cell differentiation must equal to the rate of cell division in the root meristem. It has shown that this balance is the result of crosstalk between cytokinin and auxin through *SHY2* gene, a member of the auxin repressor Aux/IAA gene family.¹⁹ Auxin promotes cell division in the root meristem and expansion/differentiation after cells have left the meristem.²⁰ Cytokinin, on the other hand, counteracts auxin to promote cell expansion/differentiation at the root meristem.^{21,22} It has demonstrated that cytokinin does not interfere with specification of the QC and the stem cell function, nor with the overall division rate in the proximal meristem. Cytokinin affects primarily the rate of meristematic cell differentiation, resulting in shortening of the meristematic zone.²¹ The negative role of cytokinin on root growth has been proven by both exogenous cytokinin application and overexpression of the *ISOPENTENYLTRANSFERASE (IPT)* gene.^{23,24} Interestingly, the root meristem was reduced in the triple cytokinin receptor mutant *abh2, abh3, abh4* and multiple mutant in *ahp* members of the signal transduction cascade.²⁵⁻²⁸ Based on these phenotypes, it has supposed that the optimal cytokinin concentration is needed for the root meristem development.²⁹ However, the specific function pathways of auxin and cytokinin and their interaction mechanisms in the root meristem are still unclear.

In wild-type roots, the transcription of *AtMMS21* is decreased by cytokinin application. Furthermore, the *mms21-1* loss-of-function mutant allele displays smaller root meristems, mimicking the effects of cytokinin application and auxin lack. Hence, application of exogenous cytokinin to the *mms21-1* loss-of-function

mutant allele did not trigger an obvious decrease in the root meristem size. Meanwhile, AtMMS21 can regulate positively the cytokinin receptor gene expression such as *CRE1*, and the ARR genes expression such as *ARR1*, *ARR3*, *ARR4*, *ARR5* and *ARR7*. These results indicate that the AtMMS21 protein is both necessary and sufficient for the cytokinin-mediated control of meristem size. Conversely, AtMMS21-mediated auxin signaling positively regulates the root meristem size by promoting the root meristem cell division. These regulation progresses sustaining the homeostasis between cytokinin and auxin signaling, and keeping the balance of root meristem cell division and differentiation. Based on these results, it can be speculated that AtMMS21 is a node component between cytokinin and auxin signaling to regulate the Arabidopsis root meristem development (Fig. 2).

Therefore, the SUMO E3 ligase AtMMS21 plays important roles in the root meristem maintenance and growth. But the mechanisms of AtMMS21-mediated cytokinin signaling to regulate root meristem growth are yet to be established. Further researches on the identification of AtMMS21 target proteins, and their functions in cytokinin signaling and root meristem growth, are necessary to elucidate the mechanisms of AtMMS21-mediated sumoylation modulate the progress of root meristem growth and development.

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