Plant Signaling & Behavior 6:3, 434-436; March 2011; © 2011 Landes Bioscience

MOS1 epigenetically regulates the expression of plant *Resistance* gene *SNC1*

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Key words: MOS1, SNC1, Resistance gene, plant immunity, BAT2, epigenetic regulation

Submitted: 12/13/10

Accepted: 12/13/10

DOI: 10.4161/psb.6.3.14495

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Addendum to: Li Y, Tessaro M, Li X, Zhang Y. Regulation of the expression of plant resistance gene SNC1 by a protein with a conserved BAT2 domain. Plant Physiol 2010; 153:1425–34. PMID: 20439546; DOI: 10.1104/pp.110.156240.

***MOS1 (MODIFIER OF snc1)* was identified through a genetic screen for suppressors of *snc1*, an autoimmune mutant caused by a gain-of-function mutation in a TIR-NB-LRR-type *Resistance* gene. Loss of MOS1 function completely suppresses *snc1*-mediated autoimmunity. The MOS1 protein contains a BAT2 domain and regulates the expression of *SNC1* in a locus-specific manner, but the mechanism on how MOS1 epigenetically regulates *SNC1* gene expression is unclear. Here, we report the gene expression pattern and subcellular localization of MOS1. In addition, we analyze and discuss the roles of DNA and histone methylation in *mos1*-mediated suppression of *SNC1* expression.**

Plants have evolved suites of *Resistance (R)* genes to defend against microbial pathogens.[1] Activation of R proteins leads to physiological changes that enhance disease resistance in the plant. Constant activation of *R* genes may lead to dwarfism as well as fitness cost.[2] For example, a gain-of-function mutation in *SUPRESSOR OF npr1-1, CONSTITUTIVE 1* (*SNC1*), a TIR-NB-LRR-type *R* gene, leads to constitutive activation of SNC1 protein.[3] Homozygous *snc1* plants are dwarf and have curly leaves because of autoimmunity.[4] Because of the detrimental effects caused by hyperactivation of R proteins, plants must have delicate mechanisms to fine-tune the expression of *R* genes, although little is known about these processes. Previously we showed that MOS1, a BAT2-domain containing protein, functions as an epigenetic regulator of *SNC1* expression.[5]

To determine the expression pattern of *MOS1*, a 1.8 kb fragment of the *MOS1* promoter upstream of the translation start site ATG was cloned into pBI101 to generate *pMOS1::GUS* transgenic plants. GUS staining of the transgenic plants showed that the reporter gene was expressed at low levels in the shoot apical meristem (**Fig. 1A**), suggesting that MOS1 is mainly expressed there. In addition, GUS staining was also observed in floral meristem and lateral shoot meristem (**Fig. 1B**), the first of which coincides with the late flowering phenotype of *mos1* mutants.[5] It remains to be determined whether MOS1 regulates the expression of genes known to be regulators of flowering time.

The protein level of MOS1 is very low in Arabidopsis. We were not able to detect MOS1 protein using polyclonal anti-MOS1 antibodies. In addition, western blot analysis failed to detect MOS1 proteins in transgenic plants expressing MOS1-3xFLAG or MOS1-GFP fusion protein under either the constitutive 35S promoter or its native promoter using commercial anti-FLAG and anti-GFP antibodies. No GFP fluorescence was observed in the transgenic plants expressing MOS1-GFP either. To determine the subcellular localization of MOS1, a construct expressing the full length MOS1 with a C-terminal GFP tag under the 35S promoter was transformed into Arabidopsis mesophyll protoplasts. We found no GFP fluorescence in the transformed protoplasts. However, we were able to express a truncated MOS1 protein (a.a. 614–1,412) containing the predicted nuclear localization signal (NLS) with a C-terminal GFP tag and it was found

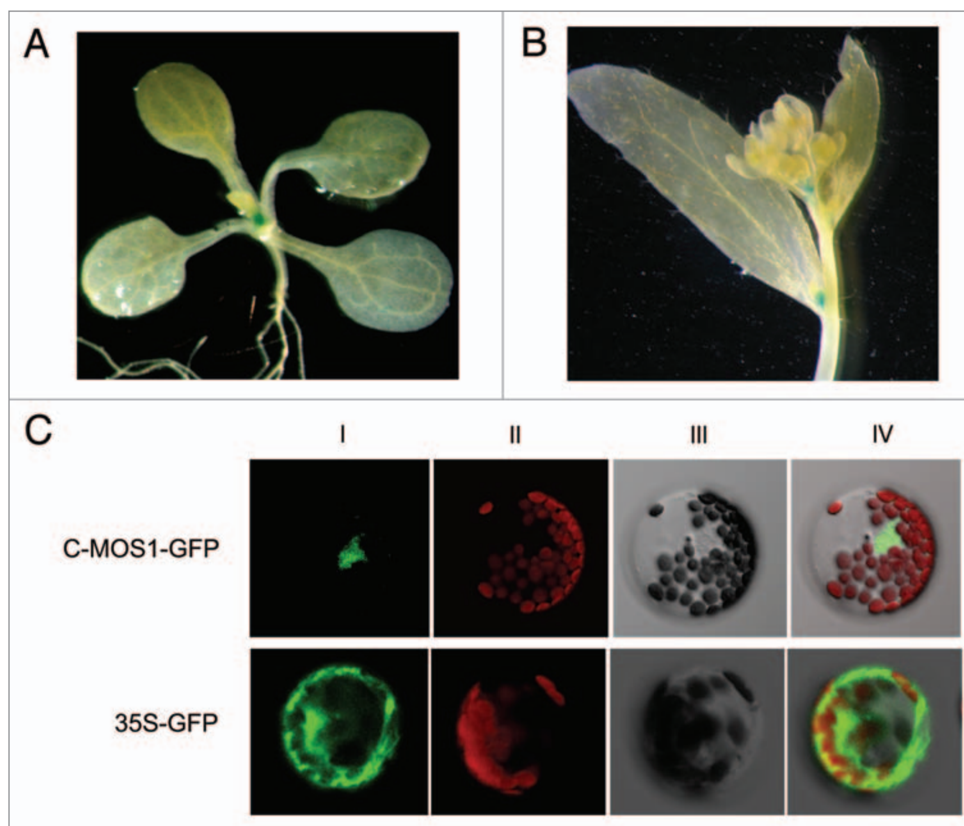

Figure 1. Expression pattern of *MOS1* and subcellular localization of truncated MOS1. (A) GUS staining of ten-day-old plate-grown seedlings expressing *pMOS1-GUS* reporter gene. (B) GUS staining of six-week-old plants expressing *pMOS1-GUS* reporter gene. (C) Subcellular localization of a C-terminal fragment of MOS1 (C-MOS1). Arabidopsis mesophyll protoplasts were transfected with a construct expressing the C-terminal part of MOS1 (amino acid 614–1,412) or the control 35S-GFP construct. Samples were examined by confocal microscopy 16 h after transfection. Epiflurescence (I), autofluescence (II), bright field (III) and merged (IV).

to localize to the nucleus (**Fig. 1C**), suggesting that MOS1 is probably a nuclear protein.

In *mos1* mutants, methylation status of DNA upstream of *SNC1* was altered.[5] To test whether DNA methylation is required for suppression of *SNC1* expression in *mos1* mutants, we performed epistasis analysis between *mos1 snc1* and mutants of genes involved in DNA methylation. In Arabidopsis, METHYLTRANSFERASE1 (MET1) is required for maintenance of DNA methylation at CpG dinucleotides.[6] Chromomethylase3 (CMT3) is required for maintenance of DNA methylation at CpNpG and CpNpN sites.[7,8] DOMAINS REARRANGED METHYLASE genes *DRM 1* and *DRM2* are involved in de novo DNA methylation as well as maintenance of asymmetric and CpNpG methylation.[9,10] We found that *snc1* mutant phenotypes were still suppressed by *mos1* in *mos1 snc1 met1*, *mos1 snc1 cmt3* or *mos1 snc1 drm1 drm2* mutant plants, suggesting that DNA methylation is probably not a major contributor to the *mos1*-mediated suppression of *snc1* expression.

Histone modifications play critical roles in the regulation of gene transcription. To determine whether histone methylation is involved in the regulation of *SNC1* expression by MOS1, we analyzed levels of histone methylation in the 3 kb promoter region of *SNC1*. Chromatin immunoprecipitation (ChIP) analysis using anti-H3K9-me2, anti-H3K27-me3 and anti-H3K4-me3 showed that H3K9-me2, H3K27-me3 and H3K4-me3 levels in the *SNC1* promoter region are similar in *snc1* and *snc1 mos1* plants, suggesting that these histone methylations are not required for *mos1*-mediated repression of *snc1* expression.

Analysis of the promoter region of *SNC1* showed that DNA methylation was affected by mutations in *MOS1*. It was unclear whether the altered DNA methylation pattern contributes to the reduced expression of *snc1* in *mos1 snc1*. Our data from epistasis analysis between *mos1 snc1* and mutants affecting DNA methylations indicate that DNA methylation is not probably required for *mos1*-mediated suppression of *snc1* expression. Interestingly, the repression of *snc1* expression in *mos1 snc1* plants can be released by knocking out *DDM1*, another epigenetic regulator which functions in chromatin remodeling and DNA methylation.[11] It is thus likely that the repression of *snc1* expression in *mos1 snc1* is caused by altered chromatin structure rather than changes in DNA methylation.

MOS1 contains an evolutionarily conserved BAT2 domain that is present in proteins from a variety of eukaryotes, including *Caenorhabditis elegans*, whose genome has no DNA methylation.[12] This also suggests that MOS1 most likely does not directly control DNA methylation. However, it is still possible that MOS1

may affect DNA methylation indirectly through regulation of chromatin structure. One common mechanism of chromatin structure regulation is histone modifications. Although we did not observe alteration of H3K9-me2, H3K27-me3 and H3K4-me3 levels in the *SNC1* promoter region in *mos1 snc1*, it remains to be determined whether *mos1* alters other types of histone modification. Moreover, the tissue we used for the CHiP experiments are not meristemic, which could mask the tissue-specific regulation of transcription by MOS1. Further in-depth analysis of histone modifications in *mos1* will help us better understand the mechanism on how MOS1 epigenetically regulates *SNC1* gene expression.

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