MOS11: A New Component in the mRNA Export Pathway

Hugo Germain1,2,3, Na Qu1,3, Yu Ti Cheng1, EunKyong Lee4, Yan Huang1, Oliver Xiaoou Dong1, Patrick Gannon1, Shuai Huang3, Pingtao Ding3, Yingzhong Li3, Fred Sack4, Yuelin Zhang3, Xin Li4,*

1 Michael Smith Laboratories, University of British Columbia, Vancouver, Canada, 2 Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Stn. Sainte-Foy, Canada, 3 National Institute of Biological Sciences, Beijing, China, 4 Department of Botany, University of British Columbia, Vancouver, Canada

Abstract

Nucleocytoplasmic trafficking is emerging as an important aspect of plant immunity. The three related pathways affecting plant immunity include Nuclear Localization Signal (NLS)-mediated nuclear protein import, Nuclear Export Signal (NES)-dependent nuclear protein export, and mRNA export relying on MOS3, a nucleoporin belonging to the Nup107–160 complex. Here we report the characterization, identification, and detailed analysis of Arabidopsis modifier of snc1, 11 (mos11). Mutations in MOS11 can partially suppress the dwarfism and enhanced disease resistance phenotypes of snc1, which carries a gain-of-function mutation in a TIR-NB-LRR type Resistance gene. MOS11 encodes a conserved eukaryotic protein with homology to the human RNA binding protein CIP29. Further functional analysis shows that MOS11 localizes to the nucleus and that the mos11 mutants accumulate more poly(A) mRNAs in the nucleus, likely resulting from reduced mRNA export activity. Epistasis analysis between mos3-1 and mos11-1 revealed that MOS11 probably functions in the same mRNA export pathway as MOS3, in a partially overlapping fashion, before the mRNA molecules pass through the nuclear pores. Taken together, MOS11 is identified as a new protein contributing to the transfer of mature mRNA from the nucleus to the cytosol.

Introduction

Plants utilize a two-layered immune system to recognize and combat pathogens. Conserved pathogen-associated molecular patterns (PAMPs), which are detected by plasma membrane localized plant pattern recognition receptors (PRRs), trigger a low amplitude defense response termed PAMP-triggered immunity (PTI) [1]. To suppress PTI, pathogens have evolved a suite of effectors (also called Avirulence or Avr proteins) [2–5]. The second layer of the plant immune response is initiated by the recognition of effectors or their effects, by cognate Resistance (R) proteins, leading to a strong defense response termed effector-triggered immunity (ETI) that culminates with hypersensitive response (HR) [1]: a programmed cell death event believed to restrict pathogen growth. Most cloned R genes encode Nucleotide Binding Leucine Rich-Repeat (NB-LLR) proteins with either a Toll/Interleukin1 receptor (TIR) or a Coiled-Coil (CC)-domain at their N terminus [6].

Mutant snc1 (suppressor of npr1-1, constitutive 1) plants carry a gain-of-function mutation in a TIR-NB-LRR R gene. This mutation renders the snc1 protein auto-active without the presence of pathogens. As a consequence, snc1 plants constitutively express Pathogenesis Related (PR) defense marker genes, accumulate high levels of defense phytohormones salicylic acid (SA), and are more resistant to the virulent bacterial pathogen Pseudomonas syringae maculicola (P.s.m.) ES4326 and the oomycete pathogen Hyaloperonospora arabidopsidis (H.a.) Noco2. Like most TIR-type R proteins, snc1 is fully dependent on EDS1 (Enhanced Disease Susceptibility 1) and PAD4 (Phytoalexin Deficient 4) for its function [7,8].

The snc1 mutant results from a point mutation in the linker region between the NB and LRR domains. This gain-of-function mutation causes activation of the ETI pathways mediated by TIR-NB-LRR R proteins. The extreme dwarf morphology of snc1 also makes it an ideal candidate to carry out genetic screens to investigate ETI components downstream of SNC1. Utilizing the unique autoimmune phenotypes of snc1, we conducted genetic screens to search for components contributing to R protein mediated immune responses. From the MOS (modifier of snc1) genetic screens, three nucleocytoplasmic pathways have been shown to affect plant immunity: Nuclear Localization Signal (NLS)-mediated nuclear protein import, Nuclear Export Signal (NES)-dependent nuclear protein export and mRNA export. MOS6, an importin α homolog involved in NLS-dependent protein import was shown to contribute to plant immunity [9]. More recently we identified MOS7, an integral nuclear pore component homologous to Nucleoporin88 (Nup88). Partial loss-of-function mos7-1 mutant plants showed increased NES-dependent protein export and exhibited reduced nuclear accumulation of the important defense regulators EDS1, NPR1 (Non-expressor of Pathogenesis Related 1) and snc1 [10]. The contribution of mRNA export to plant immunity was demonstrated in the mos3 mutant which exhibits defects in basal and R protein mediated immunity [10,11]. MOS3/SAR3/AtNup96 is an integral nuclear pore component in the conserved Nup107–160 complex and was shown to be required for mRNA export [12].

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* E-mail: xinli@interchange.ubc.ca (XL); zhangyuelin@nibs.ac.cn (YZ)

These authors contributed equally to this work.

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In eukaryotic cells, mRNAs are synthesized in the nucleus where they are processed (capped, spliced, poly-adenylated, and carried toward the nuclear pore) prior to being exported to the cytoplasm for translation. Insights into mRNA export mechanisms have been gained mostly from studies on yeast and humans, while little is known about plant mRNA export. In a genetic screen for suppressors of the Arabidopsis autoimmune mutant snc1, we isolated and identified mos11 (modifier of snc1, 11). MOS11 encodes a protein with unknown function in the plant kingdom, and its closest relative in animals is the human RNA binding protein CIP29, whose function is unclear. We demonstrate that MOS11 is a nuclear protein and that mos11 mutants accumulate more poly(A) mRNA in the nucleus, likely resulting from reduced mRNA export. Our data suggest that MOS11 functions before nuclear pore entry in the mRNA export pathway.

The same mRNA export pathway as MOS3.

Identification of the molecular lesion in mos11-1

The T-DNA insertion causing the mos11-1 snc1 phenotype was identified in the 5' UTR of At5g02770 using inverse PCR (Figure 2A). To confirm that the mutant phenotypes in mos11-1 snc1 were caused by the T-DNA insertion, we cloned the wild type DNA of At5g02770 with 1992 base pairs (bp) upstream of the start codon and transformed it into mos11-1 snc1 plants. At5g02770 fully complemented the mos11-1 snc1 morphology (Figure 2B). When the T2 transgenic plants were challenged with Ha. Noco2, they were as resistant as snc1 plants (Figure 2C). These data confirmed that MOS11 is At5g02770.

Another T-DNA insertion allele (SAIL_266_E03) in the first exon of At5g02770 was available from the Arabidopsis Stock Center (ABRC), which we named mos11-2 (Figure 2A). The mos11-1 single mutant was obtained from a backcross between mos11-1 snc1 and the wild type Columbia ecotype (Col-0). The mos11-1 and mos11-2 single mutants had identical morphology but were slightly different than Col-0. When mos11-1 was crossed with mos11-2, the two alleles failed to complement each other in all 29 F1 plants (data not shown), confirming that mos11-2 and mos11-1 carry lesions in the same gene. When mos11-2 was crossed with snc1, the mos11-1 plus snc1 double mutant exhibited a similar level of snc1 suppression as mos11-1 (data not shown), further supporting that At5g02770 is MOS11.

Subcellular localization of MOS11

MOS11 is a single copy gene that encodes a 214 amino-acid protein with unknown function. Using BlastP, the human protein CIP29 (cytokine induced protein 29 kDa) was found to share 38% identity and 50% similarity over the entire length of the MOS11 protein. It is striking that islands of positively charged residues, arginine and lysine, are highly conserved (see alignment in Figure S1). Although MOS11 does not have predicted NLS (Nuclear Localization Signal) motifs, it is predicted by WoLF PSORT (http://wolfpsort.org/) to be a nuclear protein.

To determine the biochemical function of MOS11, we first investigated its subcellular localization. When MOS11-GFP with its native promoter was transformed into mos11-1 snc1, among the 9 T1 progeny, all showed snc1-like morphology (Figure 3A). The T2 transgenic plants also displayed snc1-like enhanced resistance to Ha. Noco2 (Figure 3B). These data indicate that MOS11-GFP largely reflects endogenous MOS11 in the transgenic line, and therefore, the subcellular localization of MOS11-GFP should be identical to MOS11. As shown in Figure 3C, in contrast to the MOS3-GFP control, which localizes to the nuclear envelope, strong MOS11-GFP signal was observed in the nuclei of leaf epidermal and root cells, however it appears to be excluded from the nucleolus. Nuclear localization of the MOS11-GFP signal was observed in guard cells as well. Thus we conclude that MOS11 is a nuclear protein.

mos11 partially suppresses the autoimmune phenotypes of snc1

The mos11-1 mutant was identified by T-DNA tagging in the snc1 background [13]. Normally snc1 plants are dwarf, have twisted dark green leaves, accumulate high levels of the defense phytohormone SA and are resistant to the virulent oomycete Ha. Noco2 and P. syringae ES4326 bacteria. mos11-1 partially suppressed the snc1 morphology (Figure 1A). The mos11-1 plus snc1 plants never grew to wild type size and their leaves remained curly (Figure 1A). In addition, semi-quantitative RT-PCR confirmed that endogenous PR1 and PR2, defense markers acting downstream of R gene activation that are constitutively expressed in snc1, are greatly reduced in mos11-1 snc1 (Figure 1B). In order to investigate how the mos11 mutation suppresses snc1 morphology and PR gene expression, we assessed whether the snc1 protein levels were affected in mos11-1 snc1 plants. We observed a slight but consistent reduction in snc1 levels in mos11-1 snc1 compared with snc1 plants (Figure 1C). Total SA levels in mos11-1 snc1 were drastically reduced compared with snc1 plants, but were still significantly higher than in wild type plants (Figure 1D). To assess whether the mos11-1 mutation affects snc1-mediated resistance to virulent pathogens, we performed infection assays with the obligate biotrophic oomycete Ha. Noco2 and P. syringae ES4326 bacteria. While snc1 plants were resistant to Ha. Noco2 and showed very low sporulation, mos11-1 snc1 displayed intermediate susceptibility to Ha. Noco2 (Figure 1E). mos11-1 snc1 supported 13-fold more sporulation than snc1, however it was still significantly more resistant than the wild type (Figure 1E). This trend was also observed when we investigated susceptibility to P. syringae ES4326 (Figure 1F). Taken together, these results show that mos11-1 partially suppresses all the phenotypes of snc1.
48-mer oligo d(T) 5’-end labeled with Alexa 488. Poly(A) signals were observed in the nucleus of Col-0 and the cytosol of all labeled cells (Figure 4C). When mos11-1 and mos11-2 were analyzed, most poly(A) signal was found in the nucleus, indicating that mRNA export is diminished in mos11 plants, resulting in mRNA accumulation in the nucleus (Figure 4C and Figure S2). Thus MOS11 is likely a contributing factor in the mRNA export pathway. Note that nucleolar oligo d(T) signal is weak in all in situ experiments (Figure 4C and Figure 5E), a region where MOS11-GFP was also absent (Figure 3C).

MOS11 functions in the same mRNA export pathway as MOS3 in a partially overlapping manner

Since mos3 was previously shown to affect mRNA export [12], we investigated the relationship between mos3-1 and mos11-1 using epistasis analysis. The morphological phenotypes associated with both single mutants are quite different; mos3-1 shows long and narrow leaves and flowers early, whereas mos11-1 has mostly wild type-like morphology. The mos3-1 mos11-1 double mutant displayed a mos3-like morphology with leaves that are slightly narrower and longer than those of the mos3 single mutant, but flowered slightly earlier than mos3-1 or mos11-1 (Figure 5A and 5B). These data suggest that in regard to flowering time regulation, MOS3 and MOS11 seem to play partially overlapping roles.

In order to assess the relationship between mos3 and mos11 in immunity, we evaluated the response of mos3-1, mos11-1 and mos11-1 mos3-1 to the virulent bacteria P.s.m. ES4326. Virulent pathogens, ones not carrying Avirulence genes recognized by the plant R proteins, were used to evaluate the contribution of basal resistance to immunity. Both mos3-1 and mos11-1 mos3-1 plants exhibited enhanced susceptibility when infiltrated with virulent P.s.m. ES4326 and supported higher bacterial growth, whereas the response of the single mos11-1 mutant was similar to that of the wild type (Figure 3C), suggesting that MOS11 is not involved in basal resistance. No further enhanced susceptibility was observed for mos11-1 mos3-3, indicating that these two genes function in the same pathway to regulate immunity. It should be noted that due to
Figure 2. Complementation of mos11-1 snc1 by At5g02770. (A) Gene structure of At5g02770. Exons are represented by black boxes and introns and UTRs are shown as solid lines. Locations of mos11-1 and mos11-2 (SAIL_266_E03) insertions are as indicated. (B) Morphology of WT, snc1, mos11-1 snc1 and a representative transgenic line with MOS11 (At5g02770) driven by its own promoter transformed into mos11-1 snc1. (C) Growth of H.a. Noco2 on WT, snc1, mos11-1 snc1 and MOS11 in mos11-1 snc1. The infection was carried out as in Figure 1E. Data were analyzed using one-way ANOVA. Different letters indicate statistically significant differences (p-value <0.001).

Discussion

To search for components in R protein mediated immunity, we performed genetic screens for suppressors of the autoimmune mutant snc1. mos11-1 was identified from a T-DNA mutagenized snc1 population. Mutation in MOS11 partially abolishes the dwarf stature, elevated SA levels, and enhanced resistance to H.a. Noco2 and P.s.m. ES4326 of snc1. MOS11 encodes a protein with unknown function in the plant kingdom, however, it shares 50% similarity with human CIP29. Similarities and identities are more striking in islands of conserved positively charged residues; namely lysine and arginine (Figure S1). These basic islands are known to be present in nucleic acid binding proteins directly involved in DNA/RNA binding [18–20].

There are a limited number of reports on human CIP29, which was initially named HCC-1 after being isolated from the hemofiltrate of patients with chronic renal failure [21] and later renamed CCL14a [22]. Overexpression of CIP29 in human embryonic kidney cell line results in slower growth. Biochemical characterization demonstrated that CIP29 could bind RNA on its own [23]. In yeast two-hybrid assays, CIP29 interacted with two DEAD-box RNA helicases, BAT1 and DDX39 [24].

Hints on the potential biological functions of CIP29 came from the study by Sugiiura et al. (2007). They showed that the ATP-dependent DEAD-box RNA helicases DDX39 can co-immunoprecipitate CIP29, confirming the earlier yeast two-hybrid interaction reported by Leaw et al. (2004). Additionally they demonstrated that CIP29 can bind RNA on its own [23], and more importantly, it enhances the RNA unwinding activity of DDX39. The same group also identified FUS/TL, a nucleic acid binding protein participating in both transcription and splicing as a physical interactor of CIP29 [23]. In addition, they detected ALY, a well-characterized RNA export factor, in the DDX39 immunoprecipitate. CIP29 localized mainly to the nucleus although small amounts were also detected in the cytosol [25]. Very recently, Dufu et al. (2010) reported that CIP29 physically associates with ALY via UAP56 in an ATP-dependent manner to form a trimeric complex. In addition they demonstrated that efficient recruitment of CIP29 to the mRNA is splicing- and cap-dependent [26]. The functions of the CIP29 interactors, its affinity for RNA and the conserved island of positively charged residues suggest a potential role of CIP29 in RNA processing and/or RNA export, although mRNA export defects have not been shown for a cip29 mutant. Through knockout analysis with mos11 mutants, we demonstrate that MOS11 is indeed required for mRNA export. We speculate that its human counterpart CIP29 probably has a similar function.

In eukaryotic cells, transcription and translation take place in two separate compartments, which allows fine-tuned regulation of biological processes. For proteins to be translated in the cytosol, the mRNA molecules must first be properly processed, assembled...
into an export competent mRNA ribonucleoprotein (mRNP) particle and transferred through the nuclear pore. This assembly starts at the onset of transcription. Several proteins bind to the nascent pre-mRNA. These proteins can be involved in transcription [27], capping, splicing [28], polyadenylation [29] or binding to nuclear pore proteins [30]. Most of the knowledge on mRNA export has been gained from studies of human and yeast cells. Little is known about the mechanisms of plant mRNA export (for reviews see [31,32]), although it is believed to share high similarity with yeast and human pathways.

In plants, several nuclear pore complex mutants have been shown to exhibit defects in mRNA export [12,31–37]. In Arabidopsis, previously reported components shown to participate in mRNA export all localize to the nuclear envelope; these include MOS3/AtNup96, AtNup160, LOS4, AtNup1 and AtTHP1. LOS4 encodes a DEAD-box RNA helicase enriched at the nuclear rim and it is also present in the cytosol. Two point mutation alleles of los4/cryophyte were shown to affect mRNA export [38]. More recently orthologs of the TREX-2 complex, which is necessary for mRNA export in yeast and humans, have been identified in Arabidopsis. The TREX-2 complex, which is tethered to the nuclear pore complex via Nup1 in yeast, is composed of Thp1, Sac3, Cdc31 and Sus1. AtNUP1 and AtTHP1 were shown to be necessary for mRNA export, but the other three proteins do not appear to be involved in mRNA export despite evidence of a physical interaction with other TREX-2 subunits [36]. Another protein, TEX1, which is not part of the THO/TREX core complex but part of a complex associated with the TREX complex is also found in Arabidopsis [37]. In Drosophila and human, TEX associates with the helicase UAP56 and the mRNA export factor ALY [39,40]. However, co-IP experiments using HA-tagged AtTEX did not pull-down ALY or UAP56 in Arabidopsis, but they did retrieve THO1, THO2, THO5, THO6 and THO7 [37].

Subcellular localization of MOS11-GFP revealed that MOS11 is a nuclear protein. In mos11-1, mos11-2 and mos3-1, mRNA export was similarly affected. Epistasis analysis between mos11-1 and mos3-1 suggested that these two genes function in the same mRNA export pathway. Thus we drew a simplified model of the mRNA export pathway in plants (Figure 6) where MOS11 likely functions in the early steps of the mRNA export before mRNA exits the nucleus through the nuclear pore. This is consistent with their respective subcellular localization where MOS11 is nuclear and MOS3 is limited to the nuclear rim. This indicates that MOS11 has access to the mRNA prior to MOS3. Since MOS11 is conserved in eukaryotes, its human homolog CIP29 probably also functions in a similar step in mRNA export as suggested by Dufu et al. [26].

The reason why mos11 suppresses sncl phenotypes is not clear. mos11 single mutant plants, although slightly different from the wild type, grow to full stature, produce seeds and complete their life cycle successfully. This shows that the observed impairments in mRNA export in mos11 are not sufficient to significantly affect ontogeny. The sncl mutation involves an extensive transcriptional reprogramming that results in the dramatic phenotypes displayed...
mos11-1 membrane and hybridized by 32P-ATP labeled 18-mer oligo dT. The
mos11-1 (B) Dot blot of WT, snc1

mRNA export leading to less mRNA for translation of the protein (Figure 1C).

The detailed mechanism of MOS11 in mRNA export is not clear. Since CIP29 was recently shown to interact with ALY and UAP56 in an ATP-dependent manner [26], and it was also shown to enhance the RNA unwinding activity of DDX39 [23], we speculate that the function of MOS11 is likely to act as a co-chaperone to plant homologs of UAP56 and ALY to co-catalyze mRNA unwinding prior to its nuclear export. The specific targets of MOS11 await further investigation.

Materials and Methods

Plant growth, SA measurement, RT-PCR analysis, pathogen infections, and T-DNA screens

All plants were grown under a 16 h light/8 h dark regime. SA extractions and measurements were carried out as previously described [41]. RNA was extracted from 2-week-old plate-grown seedlings on half Murashige and Skoog (½ MS) media using the Totally RNA kit (Ambion). 200 ng of RNA was used for reverse transcription using Superscript II (Invitrogen). RT-PCR analysis of PR1 and PR2 expression was carried out as previously described [8]. Tubulin was used as an uninduced loading control with primers 5’ACGTATCGATGCTATTTCCAGG3’ and 5’ATATCGTAGAGCCCTATTGCTC3’. Pathogen assays with H.a. Noco2 and P.s.m. ES4326 were carried out as previously described [7]. T-DNA screen in snc1 was described previously [13].

Total protein extraction and Western blot

Total protein was extracted from 4-week-old soil-grown plants using the extraction buffer containing 100 mM Tris-HCl (pH 8), 0.2% SDS and 2% β-mercaptoethanol. 30 µl of total protein was separated on 8% SDS-PAGE and transferred onto nitrocellulose membrane. For SNC1 total protein level, the membrane was probed with purified anti-SNC1 antibody, which was generated against a SNC1-specific peptide in rabbit [42].

MOS11-GFP subcellular localization

The full-length genomic MOS11 gene plus 1992 bp upstream of its start codon was cloned in frame to the N-terminus of GFP in a modified pCambia1305 vector in which the GUS gene had been replaced by GFP. Plant transformation was carried out by Agrobacterium in planta dipping [43] and transformants were selected on ½ MS supplemented with 30 µg/ml hygromycin. T1 and T2 transgenic plants were then observed with a confocal microscope with propidium iodine as a cell wall/dying nuclei marker.

Dot blot hybridization

The total RNA was extracted by RNAiso Plus (Takara, Cat No. D9108A). The RNA samples were quantified by spectrophotometry and confirmed by agarose gel electrophoresis. The RNA samples were prepared and applied to the Hybond-N membrane (Amersham Cat. No. RPN303B). The membrane was cross-linked for 2 minutes at 70000 micro-joules/cm2 and baked for 2 hours at 80°C. The membrane was pre-hybridized for 1 hour at 37°C in Hyb-50 (Mylab Corporation, Beijing, P. R. C.). The 18-mer oligo-dT was labeled with 32P by the T4 poly-nucleotide kinase (NEB, Cat. No. M0201) and added to the pre-hybridization buffer. After 16 hours of hybridization, the membrane was washed following Amersham Hybond-N+ manual. The signals were detected by phosho-imager using a Typhoon scanner.
We adopted a protocol similar to that described in Parry et al. (2006). Briefly, 4–7 day-old plate-grown seedlings were immersed in 1 ml fixation cocktail (50% fixation buffer consisting of 120 mM NaCl, 7 mM Na$_2$HPO$_4$, 3 mM NaH$_2$PO$_4$, 2.7 mM KCl, 0.1% Tween 20, 80 mM EGTA, 5% formaldehyde, 10% DMSO and 50% heptane) in a glass container and gently agitated for 30 minutes at room temperature. The sample was dehydrated twice for 5 minutes each in 100% methanol, three times for 5 minutes each in 100% ethanol and incubated for 30 minutes in ethanol:xylene (50:50) with gentle agitation. The samples were washed twice in ethanol for 5 minutes each and twice in methanol for 5 minutes each before the samples were incubated 5 minutes in methanol: fixation buffer without formaldehyde (50:50) and fixed in fixation buffer with 5% formaldehyde for 30 minutes. Samples

**Figure 6.** A proposed plant mRNA export pathway. DNA helicase unwinds DNA and RNA polymerase II and co-activators proceed with transcription and mRNA maturation. Pre-mRNA is capped, polyadenylated, and spliced. Mature mRNA is then carried by MOS11 and its partners to TREX2, which associates with NUP1. LOS4 then binds the mRNA and passes the mature mRNA through the nuclear pores with the help of the NUP107–160 complex to the cytosol where it can be translated.

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**Whole mount in situ total mRNA localization**

We adopted a protocol similar to that described in Parry et al. (2006). Briefly, 4–7 day-old plate-grown seedlings were immersed in 1 ml fixation cocktail (50% fixation buffer consisting of 120 mM NaCl, 7 mM Na$_2$HPO$_4$, 3 mM NaH$_2$PO$_4$, 2.7 mM KCl, 0.1% Tween 20, 80 mM EGTA, 5% formaldehyde, 10% DMSO and 50% heptane) in a glass container and gently agitated for 30 minutes at room temperature. The sample was dehydrated twice for 5 minutes each in 100% methanol, three times for 5 minutes each in 100% ethanol and incubated for 30 minutes in ethanol:xylene (50:50) with gentle agitation. The samples were washed twice in ethanol for 5 minutes each and twice in methanol for 5 minutes each before the samples were incubated 5 minutes in methanol: fixation buffer without formaldehyde (50:50) and fixed in fixation buffer with 5% formaldehyde for 30 minutes. Samples
were rinsed twice for 5 minutes each with fixation buffer without formaldehyde and incubated for 5 minutes with PerfectHyb Plus (Sigma). 1 ml of new hybridization buffer was added and pre-hybridized at 50 °C for 1 hour. Finally, 5 pmol of 5’ end-labeled Alexa-488 40-mer oligo-d(T) (Invitrogen) was added and incubated overnight in the dark. The final samples were mounted in water and fluorescence was observed with a spinning disc microscope at a magnification of 63X using the following settings: Velocity 5.1 software, Hamamatsu C9100-50 camera, the exposure time was set to 100 ms and the sensitivity was adjusted to 176. Settings were the same for all samples.

Supporting Information
Figure S1 Amino acid sequence alignment of MOS11 and its homologs in human, mice, rice, corn, poplar, and grape vine. Identical amino acids are shaded dark and similar amino acids are shaded light. Asterisks indicate highly conserved positively charged residues. Found at: doi:10.1371/journal.pgen.1001250.s001 (1.38 MB TIF)

Figure S2 Quantification of the amount of fluorescent signal in the nucleus of the genotypes described in Figure 4C and Figure 5E, reflecting the relative amount of mRNAs.

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Author Contributions
Conceived and designed the experiments: HG NQ YZ XL. Performed the experiments: HG NQ YTC EKL YH OXD PG SH PD YL. Analyzed the data: HG NQ YTC EKL YH OXD PG SH PD YL FS YZ XL. Contributed reagents/materials/analysis tools: FS YZ XL. Wrote the paper: HG YZ XL.

MO51/CIP29 in mRNA Export

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Figure S3 Growth of P. syr. DC3000 carrying respective Avr effectors in 5-week-old plants. The infection was carried out as in Figure 1F. Data were analyzed using one-way ANOVA. Different letters indicate statistically significant differences (p-value <0.00001). Found at: doi:10.1371/journal.pgen.1001250.s003 (0.26 MB TIF)
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