MORPHEUS' MOLECULE1 Is Required to Prevent Aberrant RNA Transcriptional Read-Through in Arabidopsis[1][C][W][OA]

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Several pathways function to remove aberrant mRNA in eukaryotic cells; however, the exact mechanisms underlying the restriction of aberrant mRNA transcription are poorly understood. In this study, we found that MORPHEUS' MOLECULE1 (MOM1) is a key component of this regulatory machinery. The Arabidopsis (*Arabidopsis thaliana*) *mom1-44* mutation was identified by luciferase imaging in transgenic plants harboring a cauliflower mosaic virus *35S* promoter-*LUCIFERASE* transgene lacking the 3′-untranslated region. In the *mom1-44* mutant, transcriptional read-though occurred in genes with an aberrant RNA structure. Analysis of an *RNA-dependent RNA polymerase2 mom1* double mutant revealed that the RNA-directed DNA methylation pathway is not involved in this regulatory process. Moreover, the prevention of aberrant mRNA transcriptional read-through by MOM1 is gene locus and transgene copy number independent.

Transcription is tightly controlled in eukaryotic cells. Most mRNA regulatory elements are located in the 5′- and 3′-untranslated regions (UTRs) of genes rather than in the coding region. The 5′-UTR functions mainly in the regulation of translation, whereas the 3′-UTR plays important roles in nuclear export, cytoplasmic localization, translation efficiency, and mRNA stability (Proudfoot et al., 2002). The highly conserved sequences in 3′-UTR are recognized by a poly(A) complex that promotes transcript cleavage at the poly(A) site and polyadenylation (Proudfoot et al., 2002; Moore and Proudfoot, 2009), which in turn directs RNA polymerase II-mediated termination (Whitelaw and Proudfoot, 1986). Recent studies have shown that transcriptional termination enhances pre-mRNA processing and protein expression (West and Proudfoot, 2009), whereas transcripts bearing an aberrant or missing 3′-UTR are often improperly terminated and unpolyadenylated, leading to degradation via RNA-dependent RNA polymerase6 (RDR6)-mediated posttranscriptional gene silencing (Herr et al., 2006; Luo and Chen, 2007). Several RNA surveillance pathways, including nonsense-mediated mRNA decay, exist for the removal of aberrant RNAs (Behm-Ansmant et al., 2007; Chang et al., 2007; Isken and Maquat, 2007; Kim et al., 2009); however, it is not fully understood how aberrant transcription and/or transcriptional read-through is controlled during the transcription initiation/elongation stage.

MORPHEUS' MOLECULE1 (MOM1) mediates transcriptional gene silencing (TGS) in a methylation-independent manner (Amedeo et al., 2000). The reactivation of targets with an aberrant RNA structure, including transcriptionally silent information (TSI) and 5S rRNA genes, has been shown in plants lacking MOM1 (Steimer et al., 2000; Habu et al., 2006). Recent studies suggest that MOM1 and RNA polymerase V mediate TGS together. MOM1 functions in small interfering RNA (siRNA) accumulation and is genetically linked to RNA-directed DNA methylation (RdDM; Yokthongwattana et al., 2010); MOM1 may help transduce RdDM signals to repress histone modification in the core region (Numa et al., 2010). In this study, we found that MOM1 plays a role in preventing aberrant RNA transcriptional read-through in Arabidopsis (*Arabidopsis thaliana*) plants.

RESULTS

Identification of a Mutant That Expresses a Cauliflower Mosaic Virus *35S* Promoter-*LUCIFERASE* Transgene Lacking the 3′-UTR

To study the machinery responsible for preventing aberrant RNA transcriptional read-through, we con-

structed two chimeric genes: the coding sequence (CDS) of firefly *LUCIFERASE* (*LUC*) under the control of the cauliflower mosaic virus (CaMV) *35S* promoter (*35SP*) in the presence (*35SP-LUC-3'UTR*) or absence (*35SP-LUC*) of a CaMV *35S* 3'-UTR fragment. The resulting chimeras were cloned into pCAMBIA1301 containing *GUS* as a reporter gene driven by the CaMV *35SP* (Fig. 1A). Thus, the only difference between the resulting plasmids was the presence or absence of the 3'-UTR fragment (Fig. 1A). The plasmids were transformed into the Columbia (Col-0) background, and positive plants were selected based on hygromycin resistance. Two types of stable transgenic lines, LUC (35SP-LUC-3'UTR) and the wild type (35SP-LUC), were thus produced. In contrast to the LUC plants, of which 90% were LUC luminance positive, no LUC was detected in more than 80% of the wild-type plants. However, GUS activity was detected in the wild-type seedlings by histologic analysis (Fig. 1B).

One LUC-negative wild-type line carrying a single copy of the *35SP-LUC* transgene was mutagenized with ethyl methanesulfonate and screened for LUC activity using a high-throughput luminescence imaging system. A mutant designated M-44 was obtained (Fig. 1, C and D). Backcrossing of the M-44 mutant with wild-type plants produced F1 plants, all of which exhibited a wild-type phenotype. The ratio of luminescent to non-luminescent plants in the F2 generation was 1:3, indicating that the M-44 phenotype was controlled by a recessive mutation in a single nuclear gene.

Map-Based Cloning of *MOM1*

To identify the gene affected in M-44, we crossed the M-44 mutant (Col-0) with wild-type Landsberg *erecta*

(L*er*) plants and allowed the F1 progeny to self. F2 seedlings with LUC activity were then selected. Using simple sequence-length polymorphism markers, we mapped the M-44 locus to the upper arm of chromosome I between F24B9 and T23G18. We then sequenced all of the CDSs in the region. A single nucleotide substitution (C to T) located 400 bp downstream of the *MOM1* (At1g08060) translation initiation codon was identified that introduced a premature stop codon in place of Arg-134, leading to early truncation of the MOM1 protein (Fig. 2, A and B). Thus, we renamed our mutant *mom1-44*.

To determine whether the LUC phenotype of *mom1-44* was due to the absence of MOM1, two *mom1* mutant lines (SAIL_610_G01/*mom1-2* and SALK_141293/*mom1-3*) with a T-DNA insertion in *MOM1* were obtained from the Arabidopsis Biological Resource Center (ABRC; Alonso et al., 2003). The T-DNA insertions in exon 2 and intron 5 of *MOM1* were confirmed by PCR using a *MOM1*-specific primer and T-DNA left border primers. The absence of a full-length *MOM1* transcript in the *mom1-2* and *mom1-3* mutants was confirmed by reverse transcription (RT)-PCR analysis (Fig. 2C). These mutants were then crossed to wild-type or *mom1-44* plants, and the F1 seedlings were subjected to luminescence imaging. Introduction of the *mom1* mutation (*mom1-2* or *mom1-3*) into wild-type or *mom1-44* plants had no significant effect on the level of LUC activity (Fig. 2D), suggesting that M-44/*mom1-44* is allelic to *mom1-2* and *mom1-3*. The *MOM1* CDS under the control of the native *MOM1* promoter (2.09 kb) in the presence of a *MOM1* 3'-UTR fragment (1.5 kb) was generated, and the resulting construct rescued the LUC activity of the *mom1-44* mutant to the level seen in wild-type plants (Fig. 2E).
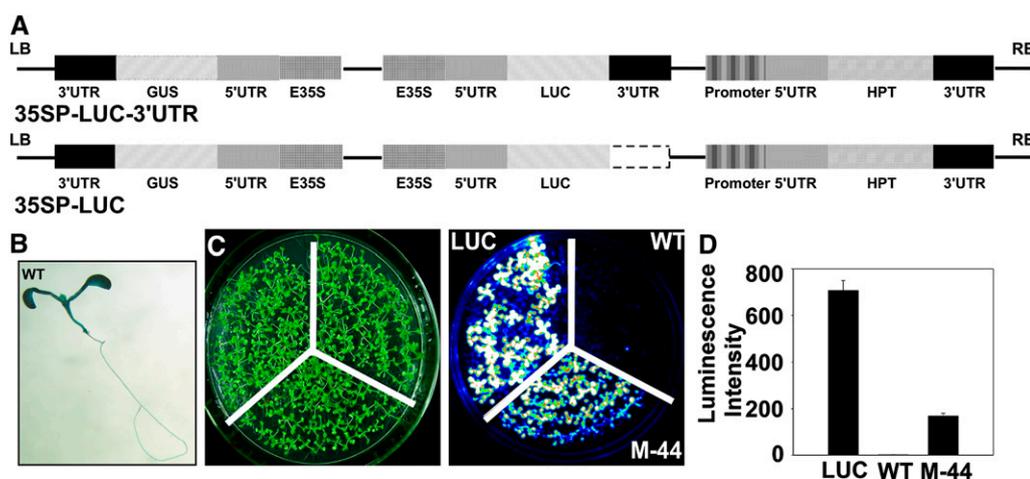

Figure 1. Identification of the M-44 mutant. A, Schematic diagram of the *LUC* constructs in the binary vector pCAMBIA1301. Both T-DNA regions contain the complete *GUS* and *HPT* genes driven by the CaMV *35SP* and the *35SP*-controlled *LUC* CDS with (*35SP-LUC-3'UTR*) or without (*35SP-LUC*) the 3'-UTR. E35S, CaMV *35SP*; 3'-UTR, *35S* terminator; LB, left border; RB, right border. B, GUS staining of a wild-type (WT) seedling. C, Luminescence images showing *LUC* expression in M-44 mutant, wild-type (*35SP-LUC*), and LUC (*35SP-LUC-3'UTR*) transgenic plants. D, Luminescence intensity indicating *LUC* expression in C. One representative experiment of three replicates is shown. Error bars represent SD (*n* = 20).

Figure 2. Positional cloning and confirmation of *MOM1*. A, Genetic mapping delimited M-44 to bacterial artificial chromosome clone (BAC) T6D22. B, Structure of *MOM1* and position of the M-44/*mom1-44* mutation. Boxes, exons; lines, introns. The *mom1-44* mutation and T-DNA insertions in *mom1-2* and *mom1-3* are indicated by arrows. The M-44 mutation is located 400 bp downstream of the *MOM1* transcription start site. C, Expression of *MOM1* in Col-0, *mom1-2*, and *mom1-3* as shown by RT-PCR. *ACTIN* was used as a control. D, M-44/*mom1-44* is allelic to *mom1*. M-44/*mom1-44* was crossed to wild-type (WT), *mom1-2*, or *mom1-3* plants. LUC imaging was done using 7-d-old F1 wild-type or *mom1* mutant seedlings. E, Complementation of *mom1-44* as shown by LUC imaging. [See online article for color version of this figure.]
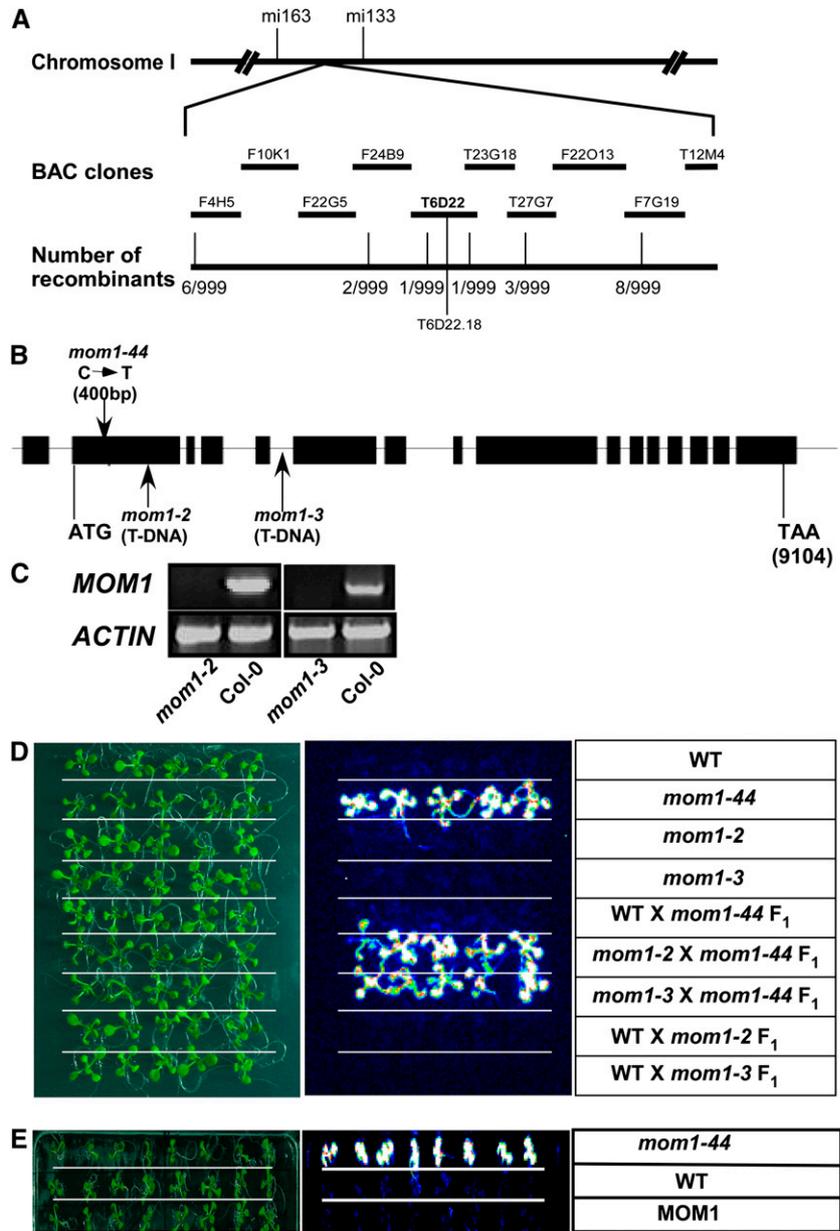
Reduced *LUC* Transcriptional Activity in Wild-Type Plants Lacking the 3′-UTR and Phenotypic Rescue by the *mom1* Mutation

To determine whether the transcriptional activity of *LUC* in the wild-type plants was altered, we performed nuclear run-on assays. *LUC* pre-mRNA was detected at a level similar to that of *GUS* in the wild-type plants; in comparison, the *mom1-44* mutation increased the transcriptional activity of both *GUS* and *LUC* (Fig. 3A).

We next examined the accumulation of *LUC* and *GUS* mRNA and protein by northern and western blotting. *LUC* mRNA and LUC protein were not detected in the wild-type plants, whereas *GUS* transcripts and protein were identified in the same assays, although the levels were significantly reduced compared with those seen in *mom1-44* (Fig. 3, B and C). These results are consistent with the observation of a GUS but not a LUC signal in the wild-type seedlings, and they suggest that without the 3′-UTR, although *LUC* can be transcribed at a low level, it is quickly degraded in wild-type plants. Surprisingly, a 4-kb *LUC* transcript was produced in the *mom1-44* plants, which is longer than its regular size (2 kb) in the LUC plants (Fig. 3B). The 4-kb *LUC* transcript was translated into a normal-sized and functional LUC protein (Fig. 3C), resulting in the recovery of LUC activity in the *mom1-44* seedlings. These results suggest that MOM1 prevents the formation of aberrant mRNAs rather than functioning in their degradation. MOM1 mediates TGS through a dynamic process (Tariq et al., 2002)
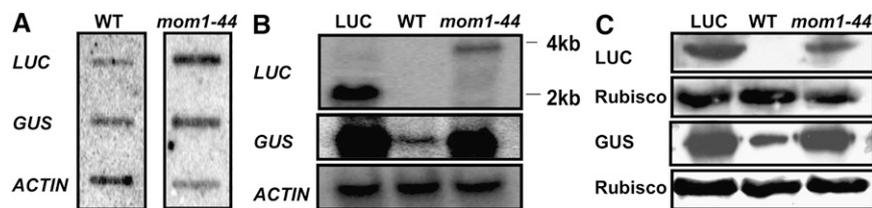
Figure 3. *LUC* and *GUS* expression in wild-type (WT) and *mom1-44* plants. A, Nuclear run-on assays of *LUC* and *GUS. ACTIN* was used as a control. B, Transcript levels and sizes of *LUC* and *GUS. ACTIN* was used as a control. C, Protein expression of LUC and GUS in wild-type and *mom1-44* seedlings. Rubisco was used as a control.

that may cause the suppression of *LUC* and *GUS* transcription in wild-type plants.

The *LUC* Transcript Was Unpolyadenylated in Wild-Type Plants But Polyadenylated in *mom1-44* Due to the 3′-UTR of *HPT*

In the *mom1* mutant, a number of endogenous targets have been found to produce aberrantly long transcripts (Steimer et al., 2000; Habu et al., 2006; Yokthongwattana et al., 2010), but how these long transcripts are generated is unknown. To determine how the extended *LUC* transcript was formed in *mom1-44* plants, we first examined the polyadenylation status of *LUC* in wild-type and *mom1-44* plants by

RNA gel blotting using total and poly(A)$^+$ RNA extracted from LUC, wild-type, and *mom1-44* plants. In both types of RNA, an extended *LUC* transcript was detected in *mom1-44*, whereas the regular *LUC* transcript was detected in LUC; neither transcript was detected in the wild-type samples (Fig. 4A). Consistent with our northern-blotting results, cDNA reverse transcribed from the wild-type and *mom1-44* RNA samples using oligo(dT) or random primers revealed the presence of *LUC* mRNA in *mom1-44* from both cDNA pools. *LUC* was weakly detected in cDNA produced from the total RNA of the wild-type plants using random primers, but only if the number of PCR cycles was increased (Fig. 4B). The relative amount of *LUC* in the cDNA pool generated by RT using random primers
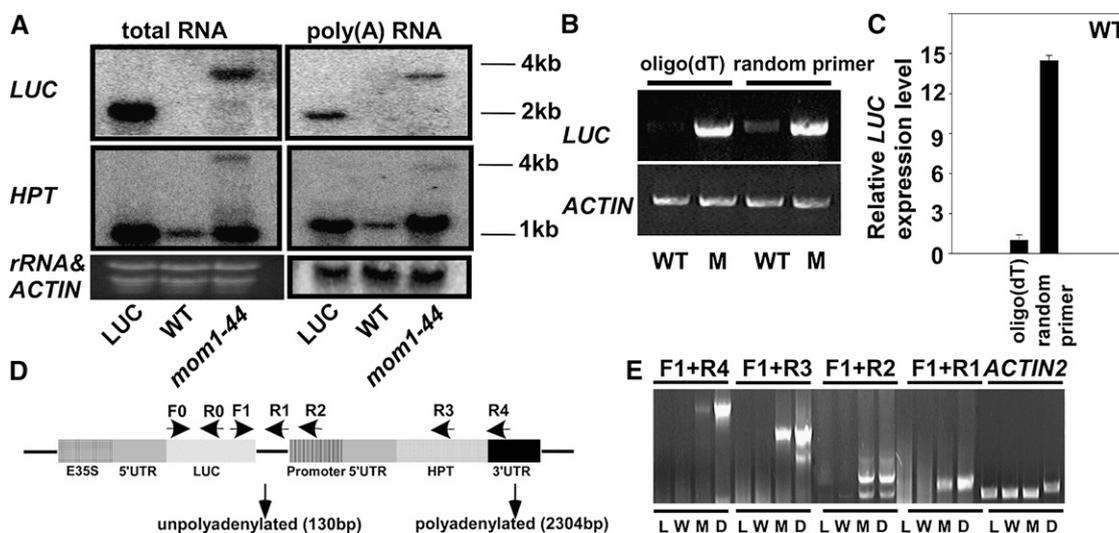

Figure 4. *LUC* transcript status in wild-type (WT) and *mom1-44* plants. A, Expression analysis of *LUC* and *HPT* in LUC, wild-type, and *mom1-44* plants by northern blotting using poly(A)$^+$ (right) or total (left) RNA. B, Expression analysis of *LUC* in wild-type and *mom1-44* plants by RT-PCR using total RNA reverse transcribed using oligo(dT) (left) or random (right) primers. Primers used were F0 and R0. *ACTIN* was used as a loading control. C, Relative *LUC* expression in wild-type plants as shown by real-time PCR using total RNA reverse transcribed using oligo(dT) (left) or random (right) primers. Primers used were F0 and R0. *ACTIN* was used as an internal control. D, Structure of the *35SP-LUC* and *35SP-HPT-3′ UTR* clusters. The positions of the primers used are indicated by arrows. Two forms of *LUC* mRNA were observed in the *mom1-44* mutant (the positions are indicated by arrows): an unpolyadenylated transcript located 130 bp downstream of the *LUC* translation stop codon and a polyadenylated transcript produced using the *HPT* 3′-UTR located 2,304 bp downstream of the *LUC* translation stop codon. E, *LUC* transcriptional read-through was detected in *mom1-44*. The 4-kb *LUC* transcript was polyadenylated and terminated using the *HPT* 3′-UTR. The cDNAs were reverse transcribed from LUC plants (L), wild-type plants (W), and *mom1-44* (M) plants. D indicates genomic DNA from *mom1-44*.

was about 14 times that generated from the poly(A)$^+$ RNA pool (Fig. 4C). Our results suggest that *LUC* mRNA was largely unpolyadenylated in the wild-type plants and that the unpolyadenylated mRNA was unstable and not translated. In contrast, the extended *LUC* transcript existed mainly in the polyadenylated state in the *mom1-44* mutant. Northern-blot analysis using *hygromycin phosphotransferase* (*HPT*) as a probe revealed the presence of two *HPT* transcripts in *mom1-44* (Fig. 4A). The lower, more intense band, which corresponds to *HPT*, was also observed in the LUC and wild-type plants, while the upper band, which was similar in size to the 4-kb *LUC* transcript, was detected in *mom1-44*. The *HPT* gene was located immediately behind *LUC* in the *35SP-LUC* plasmid (Fig. 4D); thus, the calculated length from the translation start codon of *LUC* to the translation stop codon of *HPT* was about 4 kb. This indicates that the extended *LUC* transcript in *mom1-44* was due to transcriptional read-through and that *LUC* was terminated by the *HPT* 3′-UTR. To confirm this hypothesis, RT-PCR analyses were performed using a forward primer specific for the *LUC* CDS (F) with reverse primers located between *LUC* and *HPT* (R1), in the *HPT* promoter (R2), in *HPT* (R3), or in the *HPT* 3′-UTR (R4) using oligo(dT) reverse-transcribed cDNA (Fig. 4D). Products were produced using all four primer combinations only from *mom1-44* cDNA, and they were similar in size to the product amplified from *mom1-44* genomic DNA (Fig. 4E). To rule out DNA contamination of the cDNAs, intron 1 of *ACTIN2* was amplified from the same samples. As shown in Figure 4E, *ACTIN2* cDNA was amplified from all of the samples, whereas a genomic *ACTIN2* fragment containing the intron was amplified only from *mom1-44* DNA. Our data indicate that the recovery of LUC activity in the wild-type plants by mutations in *mom1* was due to stable read-through of the *LUC* transcript.

To further assess the termination status of *LUC* mRNA in wild-type and *mom1-44* plants, we carried out a 3′-RACE assay. Following the addition of a 3′-RNA linker, we detected fewer unpolyadenylated *LUC* transcripts in the *mom1-44* mutant, which terminated 130 bp downstream of the *LUC* stop codon (Fig. 4D). Although we successfully amplified *LUC* (Fig. 4B), we were unable to obtain a *LUC* RACE product in the wild-type case. A possible explanation for this is that *LUC* mRNA was rare and unstable in the wild-type plants; in addition, the efficiency of addition of the 3′-RNA linker may have been low. We also found that the *LUC* transcript was terminated at the 3′-UTR of *HPT*, corresponding to the 4-kb *LUC* transcript detected in *mom1-44* (Fig. 4E). Our results demonstrate that deletion of the 3′-UTR led to the production of improperly terminated, unpolyadenylated *LUC* mRNA in wild-type plants. Mutations in *MOM1* caused transcriptional read-through resulting in extended (4-kb) *LUC* transcripts terminated by the *HPT* 3′-UTR. The *HPT* gene within this cluster served as part of the 3′-UTR and had little effect on *LUC* translation. Consistent with this observation, a 7-kb-long transcript of MULE-F19G14, a mutator-like element with an abnormal RNA structure, was only detected in *mom1-44* terminated by the 3′-UTR of downstream gene *CYCLOPHILIN40* (*CYP40*; Fig. 5). It is well known that elements in the 3′-UTR direct the addition of a poly(A) tail (Colgan and Manley, 1997; Wahle and Rüegsegger, 1999; Zhao et al., 1999), which plays an important role in the termination of transcription (Proudfoot, 1989). The polyadenylation of mRNAs is critical both for export into the cytoplasm and translation (Eckner et al., 1991; Huang and Carmichael, 1996, 2001). The 4-kb *LUC* mRNA in *mom1-44* was structurally complete and therefore was more stable and translatable.

MOM1 Regulates CaMV *35SP*-Directed Small RNA Production Independent of Its Inhibitory Function in Transcriptional Read-Through

GUS and *HPT* expression was reduced in the wild-type plants compared to that in *mom1-44* (Figs. 3B and 4A). Notably, the *GUS*, *HPT*, and *LUC* genes in *35SP-*

Figure 5. Endogens MULE-F19G14 transcript status in wild-type (WT) and *mom1-44* plants. A, Scheme of the chromosomal region containing MULE-F19G14 and *CYP40*. The gray box represents MULE-F19G14; dark and open boxes represent exons and introns of *CYP40*, respectively. B, A 7-kb-long transcript of MULE-F19G14 was only detected in *mom1-44* by northern-blotting assay. rRNA was used as a loading control. C, Relative MULE-F19G14 expression in wild-type plants as shown by real-time PCR using total RNA reverse transcribed using oligo(dT) (left) or random (right) primers. *ACTIN* was used as an internal control. D, Expression analysis of MULE-F19G14 in wild-type and *mom1-44* plants by RT-PCR using total RNA reverse transcribed using oligo(dT) (left) or random (right) primers.
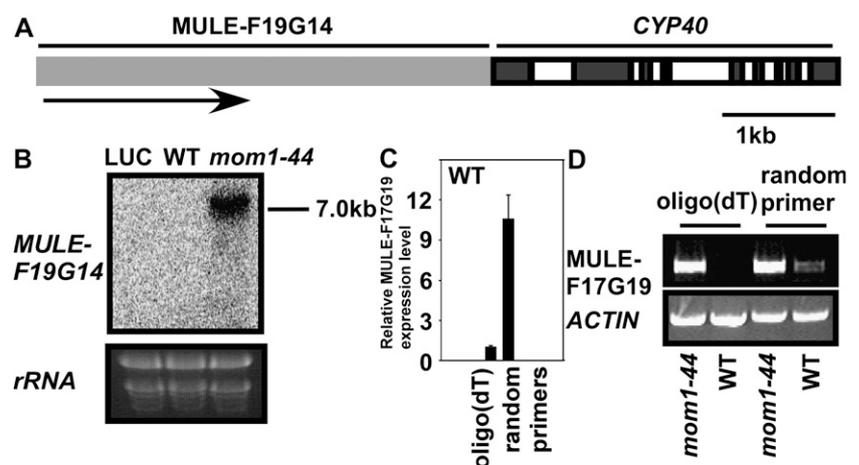
LUC were all under the control of the *35SP*. It is possible that the presence of multiple *35SPs* led to the production of siRNAs, which in turn repressed gene expression in the wild-type plants. This is in agreement with the notion that MOM1 helps mediate siRNA accumulation at the core regions of RdDM (Yokthongwattana et al., 2010). To confirm whether *35SP*-directed siRNA was generated with a role in the transcription of aberrant mRNA in wild-type plants, we tested for the accumulation of siRNA in *mom1-44* and wild-type plants. As shown in Figure 6A, a *35SP*-directed siRNA was indeed generated in the wild-type plants and at a reduced level in the *mom1-44* mutant. These data may explain why the transcription of *LUC*, *GUS*, and *HPT* was reduced in the wild-type plants compared with *mom1-44*. We also monitored the accumulation of other endogenous small RNAs: the microRNA miR166 and RDR2-dependent siRNA02 (Axtell and Bartel, 2005). The expression levels of these small RNAs were similar between the wild-type and *mom1-44* mutant plants (Fig. 6A), suggesting that MOM1 is not universally involved in small RNA generation; rather, it helps regulate the expression of specific siRNAs. This hypothesis is consistent with previous findings (Elmayan et al., 2005; Vaillant et al., 2006; Yokthongwattana et al., 2010). RDR2 is a key component of the RdDM pathway (Matzke et al., 2009). We crossed wild-type or *mom1-44* plants with *rdr2* plants to generate *35SP-LUC/rdr2* and *35SP-LUC/rdr2 mom1-44* mutant plants and tested whether RDR2 regulates *35SP*-directed siRNA generation and aberrant *LUC* transcription. In the *rdr2* and *rdr2 mom1-44* mutants, no *35SP*-directed siRNA was detected (Fig. 6A), indicating that *35SP*-directed siRNA was generated by the RDR2 pathway in wild-type and *mom1-44* plants. We next assessed the level of LUC activity in the *35SP-LUC/rdr2* and *35SP-LUC/rdr2 mom1-44* plants. As shown in Figure 6B, the loss of RDR2 function did not affect LUC activity in either the wild-type or *mom1-44*

plants. This observation was confirmed by an RNA gel-blot assay (Fig. 6C). Taken together, the siRNA generated due to the presence of multiple CaMV *35SPs* had no effect on the aberrant RNA transcriptional read-through.

The Transcriptional Read-Through of Genes with an Aberrant 3′-UTR Occurs Genome Wide in *mom1* Plants

It was previously shown that MOM1 mediates TGS mainly in intermediate heterochromatin (Habu et al., 2006). To test whether the regulation of aberrant RNA transcriptional read-through by MOM1 is a universal event that is independent of gene position and copy number, we transformed the *35SP-LUC* or *35SP-LUC-3′ UTR* plasmid into Col-0, *mom1-2*, *mom1-3*, and *rdr2* plants. More than 100 independent T1 hygromycin-resistant plants from each transformation were subjected to LUC luminescence imaging. As shown in Figure 7A, 20.9%, 81.7%, 85.4%, and 21.2% of the *35SP-LUC* transgenic plants produced in the Col-0, *mom1-2*, *mom1-3*, and *rdr2* backgrounds were LUC positive, respectively. However, in all four backgrounds, about 90% of the transgenic plants harboring *35SP-LUC-3′ UTR* were LUC positive. These results indicate that the prevention of aberrant RNA transcription by MOM1 is independent of gene position, gene copy number, and RDR2.

We randomly selected LUC-negative seedlings in the Col-0 background to examine the relationship between *LUC* and *GUS* expression by RNA gel-blot analysis. *LUC* expression was undetectable in the Col-0 background but was rescued in the *mom1-44* mutant by generating a 4-kb stable *LUC-HPT* transcript, and its expression level was not correlated with that of *GUS* in the same plant (Fig. 7B). These results suggest that the regulation of transcriptional read-through (*LUC*) and TGS (*LUC*, *GUS*, and *HPT*) by MOM1 are independent events.
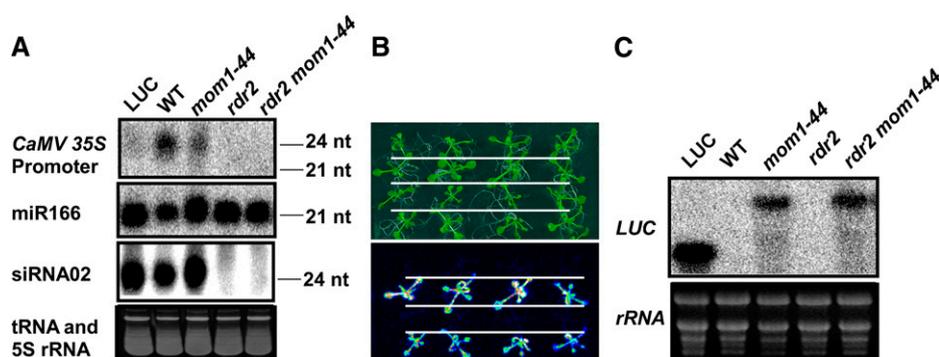

Figure 6. *35SP*-directed siRNA production in wild-type (WT) and *mom1-44* plants is not related to *LUC* transcriptional read-through. A, Detection of CaMV *35SP*-directed siRNA, miR166, and siRNA02 in the indicated genotypes. An ethidium bromide-stained gel corresponding to tRNA and 5S rRNA was used as a loading control. The positions of the size markers are indicated (24 or 21 nucleotides [nt]). B, LUC luminescence in *mom1-44*, *rdr2*, and *rdr2 mom1-44* mutant plants. The plants shown (from top to bottom) are as follows: wild type, *mom1-44*, *rdr2*, and *rdr2 mom1-44*. C, Expression of *LUC* in LUC, wild-type, *mom1-44*, *rdr2*, and *rdr2 mom1-44* plants. Ethidium bromide-stained rRNA was used as a loading control. [See online article for color version of this figure.]
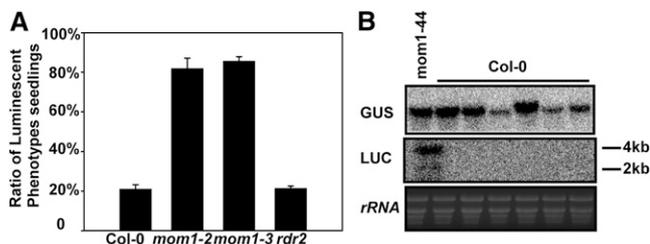
Figure 7. *LUC* transcriptional read-through in *mom1* is independent of the insertion position, copy number, and RDR2. A, Luminescence intensity (*LUC* expression) in various genetic backgrounds. Error bars represent SD (*n* = 20). B, Expression of 4-kb *LUC* and *GUS* transcripts in LUC-negative *35S-LUC* transgenic seedlings (six independent lines) produced in the Col-0 background. rRNA was used as a loading control.

DISCUSSION

In eukaryotic cells, the 3′-UTR plays important roles in transcription and translation. Polyadenylation is directed by the 3′-UTR and contributes to the termination of transcription (Proudfoot et al., 2002; Moore and Proudfoot, 2009; West and Proudfoot, 2009). Lack of the 3′-UTR results in the production of an improperly terminated transcript that is rapidly degraded by the cell's quality control system through such methods as nonsense-mediated mRNA decay (Kim et al., 2009). However, the molecular mechanisms that serve to prevent aberrant RNA transcription are not well understood. In this study, we found that MOM1 is a key player in the repression of aberrant RNA transcriptional read-through.

MOM1 Plays an Important Role in the Prevention of Transcriptional Read-Through

The regulation of transcription read-through is not well understood. Many factors, such as cis/trans-acting regulators and TGS regulators, could be involved in this process. Read-through transcription is found in at least 2% to 5% of human genes and 1% of plant genes (Münk et al., 2008; Xing et al., 2010).

Eukaryotic genomes contain large numbers of transposons, and the regulation of their transcription is important to maintain genome integrity. Most of the up-regulated targets in *mom1* mutant plants are located in regions of intermediate heterochromatin (Numa et al., 2010; Yokthongwattana et al., 2010). A number of endogenous targets of MOM1 exist in an extended form in which the transcript is longer than the expected size, including TSI (Steimer et al., 2000), MULE-F19G14 (Habu et al., 2006), and 5S genes (Vaillant et al., 2006). In the *mom1* mutant, TSI exists in two forms: as a long polyadenylated transcript and as a short unpolyadenylated transcript. The MULE-F19G14 transcript is inverted relative to its original direction, and the antisense transcript is terminated by the downstream gene *CYP40*. A long transcript (210

bp) is generated at 5S rDNA loci that is longer than its regular size of 120 bp (Vaillant et al., 2006). However, the molecular mechanism involved in generating these extended transcripts is not understood. In this study, we found that the absence of the 3′-UTR of *LUC* led to the production of a transcript that was unpolyadenylated and degraded in wild-type plants; in contrast, in *mom1*, the corresponding transcript was polyadenylated and stable and was found to be 4 kb in length due to the use of the *HPT* 3′-UTR. TSI, MULE-F19G14, and 5S genes all contain aberrant RNA structures and form extended, stable transcripts in *mom1*.

Regulation of Transcriptional Read-Through by MOM1 Is Independent of the RdDM Pathway

MOM1 regulates TGS in a DNA methylation-independent manner (Amedeo et al., 2000). In the *mom1* mutant, histone modification of the coding regions of MOM1 targets is intact (Habu et al., 2006; Vaillant et al., 2006), and nuclear organization appears to be unaltered (Probst et al., 2003). A recent study showed that RNA polymerase V interacts genetically with MOM1 in the control of gene silencing and that MOM1 contributes to the accumulation of small RNAs at promoter regions (Yokthongwattana et al., 2010). The transcriptional activation of various genes in the *mom1* mutant is associated with a reduction in dimethylated histone H3 Lys-9 in the promoter region, and MOM1 transduces RdDM signals to repress histone modification (Numa et al., 2010). We detected small RNAs generated from the CaMV *35SP*. The siRNA produced did not affect *LUC* expression or transcriptional read-through, because in the *rdr2* mutant the *LUC* gene was still silenced, whereas in the *rdr2 mom1* double mutant we were able to detect a 4-kb *LUC* transcript when the siRNA was removed. The ratio of luminescent to nonluminescent plants among the *35SP-LUC* transgenic lines produced in a *mom1-2* or *mom1-3* background was greater than that in the Col-0 and *rdr2* backgrounds (Fig. 7A). These data indicate that the function of MOM1 in the repression of transcriptional read-through differs from its function in the regulation of TGS, and this regulation is independent of the gene locus and transgene copy number and instead is related to the structural integrity of the gene. Twenty percent of the *35SP-LUC* transgenic lines in Col-0 contained the 4-kb-long transcript and were LUC positive. This may indicate that preventing transcriptional read-through is regulated by multiple genes, and *MOM1* is one of them.

It has been known that aberrant transcripts trigger RDR6-mediated RNA silencing (Luo and Chen, 2007). MOM1 maintains the transcriptionally inactive status of TGS targets; however, this activity does not correlate with releasing the aberrant transcript (Amedeo et al., 2000). Our results only imply that the effect of MOM1 on transcriptional read-through is independent of the RdDM pathway. However, we cannot rule out the possibility that, for example, MOM1 may control

the TGS of one or more genes such as RDR6, which in turn can promote transcriptional read-through.

MATERIALS AND METHODS

Plant Materials and Mutant Isolation

For LUC imaging, 1-week-old Arabidopsis (*Arabidopsis thaliana*) seedlings grown on Murashige and Skoog agar plates under constant light at 21°C ± 1°C were used. Plants were grown in soil in growth chambers at 70% relative humidity with 16 h of light at 22°C and 8 h of dark at 18°C per day.

To construct the *35SP-LUC* and *35SP-LUC-3'UTR* plasmids, we extracted the CaMV *35SP-LUC* coding region 3'-UTR with *Hin*dIII and *Eco*RI and the CaMV *35SP-LUC* coding region with *Hin*dIII and *Sac*I from pZH01 LUC+ and subcloned them into the binary vector pCAMBIA1301 at the corresponding sites. Arabidopsis (Col-0) expressing the *35SP-LUC* (3'-UTR deletion) and *35S-LUC-3'UTR* reporter genes are referred to as wild-type and LUC plants, respectively, in this study. The wild-type plants were mutagenized with ethyl methanesulfonate. Mutants were identified by luminescence imaging and GUS staining. Two T-DNA insertion lines, SAIL_610_G01 (*mom1-2*) and SALK_141293 (*mom1-3*), were obtained from the ABRC, and the insertion site in each was confirmed by PCR using *MOM1*- and left border-specific primers.

Positional Cloning

For map-based cloning, homozygous *mom1-44* mutants produced in the Col-0 background were crossed to L*er* plants and the F1 progeny were allowed to self. The level of LUC activity in the F2 plants was determined by luminescence imaging to select homozygous *mom1-44* plants. A total of 999 homozygous *mom1-44* mutant plants were selected for mapping with molecular markers that are polymorphic between L*er* and Col-0. Genetic mapping was performed as described previously (Guo et al., 2001). Using simple sequence-length polymorphism markers, *mom1-44* was first mapped to chromosome 1 between mi163 and mi133. Markers F22G5, T27G7, F24B9, and T23G18 were then used to pinpoint the *mom1-44* mutation to bacterial artificial chromosome clone T6D22. To identify the mutation in *mom1-44*, candidate genes from *mom1-44* mutant plants were sequenced.

RNA Analysis

Total RNA was isolated from 2-week-old wild-type or mutant seedlings using RNAVzol (Vigorous), and poly(A)$^+$ RNA prepared using an Oligotex mRNA Kit (Qiagen) according to the manufacturer's protocol was used for RNA gel-blot or RT-PCR analysis.

Small RNAs were enriched by precipitation with 5% polyethylene glycol (M_r =8,000) and 0.5 M NaCl, separated on a 15% polyacrylamide-7 M urea gel in 1× Tris-borate/EDTA, and transferred to a Hybond N$^+$ Nucleic Acid Transfer Membrane (Amersham). The [^{32}P]*35SP* probe was labeled using a Random Primer Kit (Takara), while the [^{32}P]miR166 and [^{32}P]siRNA02 probes were labeled using T4 Polynucleotide Kinase (New England Biolabs).

The primers used for RNA analysis are described in Supplemental Table S1.

Western Blotting

Total protein was extracted from 2-week-old wild-type and *mom1-44* mutant seedlings. Equal amounts of total protein were separated by 12% (w/v) SDS-PAGE and blotted onto a polyvinylidene difluoride membrane (Millipore). The blots were then probed with primary anti-LUC (Sigma-Aldrich; L2164) or anti-GUS (made by National Institute of Biological Sciences; http://www.nibs.ac.cn/antibody/zxjs) antibodies; chemiluminescence was detected using x-ray film.

Run-On Assay

We blotted 1 μg of *LUC*, *GUS*, or *ACTIN* cDNA on a Hybridization Nitrocellulose Filter (Millipore) using a Bio-Dot SF Blotting Apparatus (Bio-Rad). Nuclei were isolated from 2-week-old seedlings. Nuclear run-on assays were then carried out as described previously (Folta and Kaufman, 2006). The primers used for probe amplification are described in Supplemental Table S2.

3'-RACE

3'-RACE was performed using a Clontech cDNA Amplification Kit according to the manufacturer's protocol. A 3' linker was added using T4 RNA Ligase 2, Truncated (New England Biolabs). The primers used were 5'-CCC-GCTTTCCAGTCGGGAAACCTGTC-3' (LUC-GSP) and 5'-CCCACTATCC-TTCGCAAGACCTTCCTC-3' (nested primer LUC-NGSP); the sequence of the 3' linker was 5'-CGACGTAAAGCAGAAGACGGCATACGA-3'.

Complementation

A 2-kb *ACTIN2* promoter fragment spanning the transcription start site was cloned into the binary vector pCAMBIA2300 at the *Bst*XI-*Nco*I sites to drive *NPTII* expression. A 2-kb *MOM1* promoter fragment upstream of the transcription start site followed by the *MOM1* CDS and a 1.5-kb DNA fragment downstream of the *MOM1* transcription stop site was assembled in series by cloning into the *Sal*I-*Bam*HI, *Bam*HI-*Kpn*I, and *Kpn*I-*Eco*RI sites in pCAMBIA2300, respectively. The primers used for complementation are provided in Supplemental Table S3.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. Primer list for northern blot and PCR.

Supplemental Table S2. Primer list for run-on assays.

Supplemental Table S3. Primer list for the *MOM1* gene.

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