ISSN 0026-8933, Molecular Biology, 2017, Vol. 51, No. 4, pp. 579–585. © Pleiades Publishing, Inc., 2017.
Published in Russian in Molekulyarnaya Biologiya, 2017, Vol. 51, No. 4, pp. 656–663.

MOLECULAR CELL BIOLOGY

UDC 577.218

A Betasatellite-Encoded Protein Regulates Key Components of Gene Silencing System in Plants[1]

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Received April 27, 2016; in final form, October 10, 2016

Abstract—Small circular single-stranded DNA satellites, called betasatellites, have been found in association with some monopartite begomovirus infections. The *Cotton leaf curl Multan betasatellite* (CLCuMuB) is known to influence symptom induction in cotton leaf curl disease. CLCuMuB contains a single gene, *βC1*, whose product is a pathogenicity determinant and a suppressor of RNA silencing. Although induction of RNA silencing by RNA and DNA viruses has been well documented in plants, the interactions between betasatellites and the host's silencing machinery remain poorly understood. In this study, the transgenic expression of *βC1* from CLCuMuB in *Arabidopsis thaliana* plants produced severe developmental abnormalities, which resembled those produced by mutations in the key genes of the gene silencing pathway. Analysis of transgenic plants expressing CLCuMuB βC1 using real-time PCR showed that the expression levels of both *AGO1* and *DCL1* genes were significantly increased. In contrast, the expression of *HEN1* gene in the βC1-expressing leaf tissues was similar to that of wild-type plants. The CLCuMuB βC1 protein was found to physically interact with the AGO1 protein in a yeast two-hybrid system. It is possible that specific targeting of the gene silencing key components by the CLCuMuB βC1 inhibits the RNA silencing-based host defence.

Keywords: *Arabidopsis*, *Geminivirus*, gene silencing, plant–virus interaction, satellite DNA
DOI: 10.1134/S0026893317030037

INTRODUCTION

RNA silencing is a conserved regulatory pathway that controls endogenous gene expression and also acts as a host defence system to protect plants from invading viruses [1]. This pathway results in sequence-specific inhibition of gene expression at various levels including transcription, mRNA stability, or translation. Although several mechanisms can generate double-stranded RNA (dsRNA), dsRNA processing has common biochemical steps. RNA silencing is triggered by dsRNA, a molecule that can arise from various sources including the replication forms of plant viruses [1]. The dsRNA is then diced by ribonuclease III-type Dicer-like (DCL) enzyme into 21 to 24 nucleotides (nt) called small interfering RNA (siRNA) [2, 3]. Small RNA duplexes are protected from degradation after 2'-*O*-methylation mediated by a methyltransferase, HUA ENHANCER 1 (HEN1) [4, 5] which deposits a methyl group onto the 2' OH of the 3' terminal nucleotide with a preference for 21–24 nt RNA duplexes with 2 nt overhangs [6]. The stabilized siRNAs are then incorporated into one of the ten Argonaute (AGO) proteins to form a RNA-induced silencing complex (RISC) [7]. RISC can target siRNA complementary mRNAs to induce post-transcriptional gene silencing (PTGS) by endonucleolytic cleavage or transcriptional gene silencing (TGS) by DNA methylation and chromatin modifications of the target loci [8, 9]. As a counter-defence against host RNA silencing, most plant viruses encode proteins that suppress this pathway to protect the virus infection in plants [10–12]. Plant viral suppressors use various strategies to tackle this host defense system [13–15].

Betasatellites are circular single-stranded molecules associated with some monopartite begomoviruses in the family *Geminiviridae* [16, 17]. These satellites co-infect with their helper viruses to induce severe disease in economically important crops [16, 18, 19]. The *βC1* gene encoded by the betasatellite is a pathogenicity determinant [19, 20] and also suppresses RNA silencing [21–23]. The *βC1* gene from cotton leaf curl Multan betasatellite (CLCuMuB) is a suppressor of both local [22] and systemic silencing [24]. In addition, this protein was shown to bind large single stranded and double stranded DNA as well as double-stranded RNA [21]. However, the interaction between the betasatellite and the host's silencing machinery and the step(s) of the RNA silencing pathway

Abbreviations: siRNA, small interfering RNA; VSR, viral suppressor of RNA silencing; RISC, RNA-induced silencing complex; PTGS, post-transcriptional gene silencing; TGS, transcriptional gene silencing; CLCuMuB, cotton leaf curl Multan betasatellite; DCL, Dicer-like (proteins); dsRNA, double-stranded RNA.

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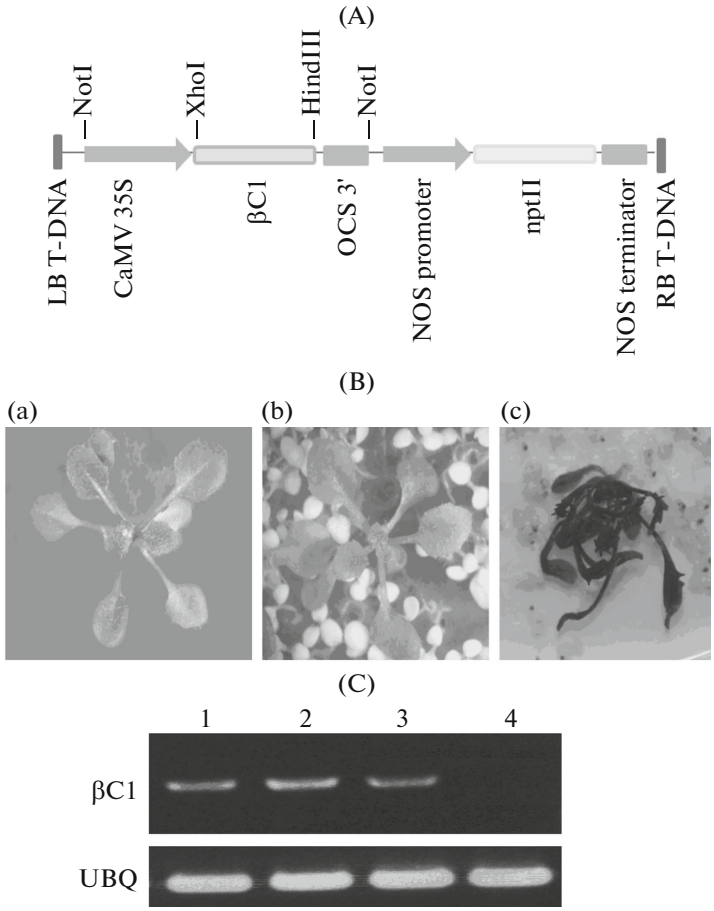

Fig. 1. Abnormal phenotypes in *Arabidopsis thaliana* plants expressing *βC1* from CLCuMuB and expression analysis for the *βC1* gene. (A) A scheme of the T-DNA construct used for expression of the *βC1* gene in transgenic plants. LB: Left border of T-DNA, CaMV 35S: 35S promoter from Cauliflower mosaic virus, OCS 3': Terminator, nptII: Neomycin phosphotransferase II gene, RB: right border of T-DNA. Restriction sites were used for cloning as shown on the top of the scheme (B) Wild-type *Arabidopsis* plant (a), plant transformed with the pART27 vector (b) and plant expressing the CLCuMuB βC1 gene (c) photographed after five weeks of growth on MS medium. (C) Agarose gel electrophoresis showing the RT-PCR products. Amplification of the *βC1* gene in three individual lines (lanes 1, 2 and 3) of *Arabidopsis* plants transformed with 35S-βC1 is shown. The negative control (lane 4) is the RT-PCR product from a control plant, transformed with the vector.

that is (are) targeted by the βC1 protein remain poorly understood. In this study, the effects of CLCuMuB βC1 on the regulation of key components of gene silencing machinery and also its physical interaction with AGO1 protein were investigated. Interaction and regulation of key components in the gene silencing pathway by CLCuMuB βC1 may explain at least partially the mechanism of suppression of gene silencing and the abnormal leaf development in plants expressing the *βC1* gene.

EXPERIMENTAL

DNA constructs and plant transformation. The *βC1* gene from CLCuMuB (Accession No. AJ298903) was amplified and cloned into a binary vector pART27 [25] as described in [19]. Figure 1A shows a scheme of the T-DNA construct for expression of *βC1* which has been made in the pART27 vector. The pART27-βC1 and pART27 were introduced into *Agrobacterium tumefaciens* GV3101 by electroporation.

Arabidopsis (Col-0 ecotype) plants were grown and transformed with each construct using the floral-dip method [26]. Seeds of primary transformants were grown on a selective Murashige and Skoog (MS) medium at 22°C with 16 h light.

RNA extraction and cDNA synthesis. Total RNA was extracted from leaf and flower tissues of transgenic *Arabidopsis thaliana* plants expressing *βC1* gene and control plants using the Plant RNA Reagent (RNAx-Plus, Sinaclon). Two micrograms of the extracted RNA were treated with DNase I and then used for preparing cDNA using the SuperScript III reverse transcriptase (Invitrogen, Carlsbad CA). The quality of the produced cDNA was tested by amplifying a fragment of a highly stable expressing polyubiquitin (*UBQ10*) gene by PCR using UBQ F/R primers (Table 1). This cDNA was used in RT-PCR and real-time PCR assays.

Table 1. Name, sequence and GenBank accession number for the oligonucleotides used in this study

Name	Sequence 5'-3'	Accession number
DCL1 F	5'-GACTTGGGAAGCTGATCATGAAAGAGATG	AF187317
DCL1 R	5'-CAACAGAATTTGTGCTGTCATAACTAGAACC	
AGO1 F	5'-AAGGAGGTCGAGGAGGGTATGG	NM_179453
AGO1 R	5'-CAAATTGCTGAGCCAGAACAGTAGG	
AGO F	5'-AGAGATCTATGGTGAGAAAGAGAAGAACG	NM_179453
AGO R	5'-CTCGAG TCAGCAGTAGAACATGAC	
HEN1 F	5'-TGGAGAGCAATCATGTGATACAGATGAC	AF411383
HEN1 R	5'-AGGATCCACTGCCAAAGACAAAGAATAG	
Beta F	5'-ACTACGCTACGCAGCAGCC	AJ298903
Beta R	5'-TACCCTCCCAGGGGTACAC	
UBQ F	5'-ATG CAG ATT TTC GTT AAG ACG TT	AT4G05320
UBQ R	5'-GTA GTC AGC CAA AGT TCT TCC AT	

Analysis of gene expression. The expression of the *βC1* gene in transgenic *A. thaliana* was tested by RT-PCR using the prepared cDNA as a template and Beta F/R primers. For internal control, the expression of ubiquitin was tested by RT-PCR using UBQ F/R primers.

The expression of *AGO1*, *DCL1* and *HEN1* genes was tested by real-time PCR (qPCR). Two microlitres of cDNA were used in each reaction containing 26.6 pmol of the forward and reverse primers (table) and Absolute QPCR SYBR Green buffer (ABgene). QPCR reactions were carried out in a RotorGene 2000 real-time PCR device (Corbett Research). The relative gene expression was calculated against a reference gene, ubiquitin, as described by Takos et al. [27]. Three biological repeats were tested for each target gene and an analysis of variance (ANOVA) for the calculated Ct values was used to statistically differentiate (Duncan's Multiple Range Test, $P \leq 0.05$) the expression of target genes using SAS (9.1) software. The identity of the PCR products was confirmed by sequencing.

Yeast two-hybrid assay. The full-length *AGO1* gene was amplified using cDNA from *A. thaliana* (Col-0 ecotype) as a template and AGO F/R primers (Table 1). The PCR product was cloned into the pGEM-T easy vector and sequenced to confirm its identity before sub-cloning into the BglII/XhoI site of the pB42 vector to produce pB42-AGO1. Construction of pLexA-βC1 was described previously [18].

Yeast (*Saccharomyces cerevisiae*) strain display YEAST-L (MATα, trp1, his3, ura3, leu2::2 LexAop-LEU2; Display Systems Biotech, Vista, CA, USA), containing a leucine biosynthesis gene downstream of the DNA recognition sequence for the LexA DNA-BD, was used in two-hybrid screening. Yeast cells were sequentially transformed with pLexA-βC1 and pB42-AGO1 constructs according to the Yeast Protocols Handbook 2001 (Clontech, Palo Alto, CA, USA). Yeast cells were selected on agar plates lacking uracil, tryptophan, histidine and leucine but containing raffinose and galactose to induce the GAL1 promoter-driven expression of the pB42-AGO1 fusions. For positive control, yeast cells were transformed with pLexA-βC1 and pB42-SlUBC3 [18]. For negative control, yeast cells were transformed with pLexA-βC1 and pB42-5A [28].

RESULTS

Developmental Abnormalities in A. thaliana Expressing the βC1 Gene

We used *A. thaliana* as a model plant to test the effect of CLCuMuB βC1 on the regulation of the key components of gene silencing machinery and plant development. Transgenic plants expressing *βC1* under the control of the 35S promoter produced narrow and deformed leaves with a rigid appearance (Fig. 1). This may have arisen from a multifunctional feature of *βC1* or its ability to target various developmental pathways in plants. In contrast, control plants transformed with the vector without an insert showed a normal phenotype. The expression of *βC1* in the transgenic plants was confirmed by RT-PCR using *βC1* gene specific primers (Fig. 1C).

CLCuMuB βC1 Regulates Key Components of Gene Silencing Machinery

CLCuMuB βC1 is a suppressor of post-transcriptional gene silencing [22, 24]. To test whether *βC1* affects gene silencing machinery by targeting the key genes of the gene silencing pathway, we tested expression levels of *AGO1*, *DCL1* and *HEN1* in *A. thaliana*

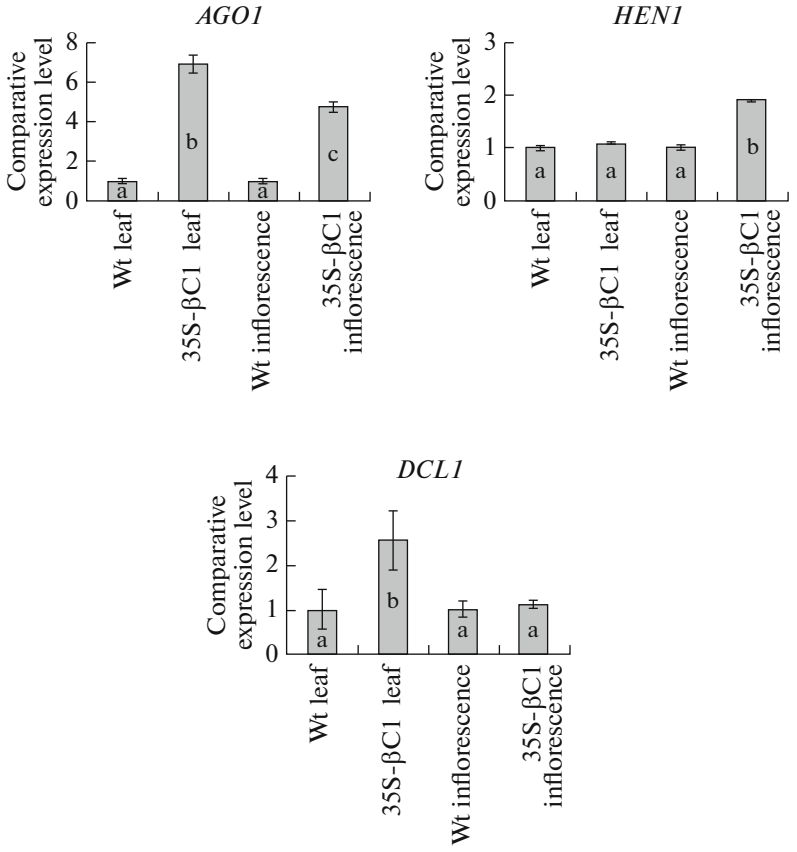

Fig. 2. Expression analysis of the key genes of the gene silencing pathway in 35S-βC1 *Arabidopsis* plants in leaves and flower tissues, using real-time PCR. The error bar shows standard deviation for three replicates for each sample. The same letter on each column indicates the absence of a statistical difference ($P < 0.05$) in expression levels.

transgenic plants expressing the *βC1* gene. QPCR analysis showed that the level of *AGO1* was significantly increased in both leaf and flower tissues of βC1 transgenic plants (Fig. 2). The *DCL1* gene was significantly ($P < 0.05$) induced only in leaf tissues (Fig. 2), whereas for *HEN1* no clear change was found in its expression levels in leaf tissues. *HEN1* gene was only induced in flower tissues in *βC1* transgenic plants as compared to the control plants.

CLCuMuB βC1 Interacts with AGO1 in Yeast

The level of *AGO1* was significantly induced in *A. thaliana* plants expressing *βC1* from CLCuMuB (Fig. 2). In addition, physical interaction with the AGO1 protein was shown for viral suppressors of RNA silencing (VSRs) including p38, P0 and 2b genes [29, 30]. To investigate the physical interaction between CLCuMuB βC1 and the AGO1 protein from *A. thaliana* in a yeast two-hybrid system, these genes were cloned into pLexA and pB42 vectors, respectively. This was to create a fusion with LexADNA-BD to generate a pLex-βC1 and a fusion with pB42 AD to generate pB42-AGO1. These constructs were co-expressed in yeast and their interactions were assayed by leucine prototrophic growth. Co-expression of pLex-βC1 and pB42-AGO1 in yeast stimulated leucine prototrophic growth. The growth level for these cells was similar to that of the positive control co-expressing pLex-βC1 and pB42-SlUBC3. This indicates a similar interaction strength between βC1 and AGO1 proteins. In the negative control, very limited growth, if any, was observed in yeast cells co-expressing a B42 fusion to 5A and LexA fusion to *βC1* gene (Fig. 3).

DISCUSSION

Most of the plant viral suppressor proteins are pathogenicity determinants [31]. They produce abnormal phenotypes, which somehow resemble those of *ago1* or *dcl1* mutants [32]. CLCuMuB βC1 is also a suppressor of RNA silencing [22–24] and produced a severe abnormal phenotype in *A. thaliana* plants (Fig. 1). This phenotype was also observed in transgenic tobacco plants expressing CLCuMuB βC1 [19]. In line with this, overexpression of other viral suppressor proteins including HCPro, p19, and p15

proteins in *A. thaliana*, has also been shown to induce developmental defects [32]. In this study, *A. thaliana* were used as model plants to investigate the effects of CLCuMuB βC1 on the regulation of key components of gene silencing machinery that may influence symptoms development, and the step(s) and element(s) of the gene silencing pathway which may be targeted by the βC1 protein.

Argonaute 1 (*AGO1*) is an essential component of the RNA silencing pathway that can also exhibit an antiviral function. [41, 33, 34]. In this study, the level of *AGO1* mRNA was significantly increased in both leaf and flower tissues of plants expressing *βC1* (Fig. 2). Consistent with this result, VSRs including p122, p19, p38, HCPro and 2b encoded by *Tobacco mosaic virus*, *Cymbidium ring spot virus*, *Turnip crinkle virus, Tobacco etch virus* and *Cucumber mosaic virus*, respectively. were shown to induce *AGO1* mRNA accumulation. However, the protein level of AGO1 was reduced in transient expression of these suppressor proteins and also in infected *A. thaliana* plants [35]. Lower levels of the AGO1 protein may support higher viral protein translation as the association of this protein with ribosomes enables translational repression [30]. In addition, low levels of the AGO1 protein in the *A. thaliana* mutant produced severe developmental abnormalities [36], what may also explain the production of abnormal phenotypes by various VSRs and possibly the CLCuMuB *βC1* gene.

Interference with the function of AGO1 through a direct interaction with VSRs has been reported. For example, p38 was shown to interfere with AGO activity and to specifically co-immunoprecipitate with AGO1 [37]. The polerovirus P0 protein was also shown to trigger AGO1 protein decay *in planta* by physically interacting with AGO1 [29]. Physical interactions with AGO1 have also been reported for the 2b protein from CMV, which specifically inhibits *AGO1* cleavage activity in RISC reconstitution assays [38]. Blocking of *AGO1* cleavage activity by the 2b inhibited miRNA pathways and attenuated RNA silencing [38]. The yeast two-hybrid system data showed that the CLCuMuB βC1 protein also interacts with AGO1 (Fig. 3). This interaction may interfere with AGO1 function in plants expressing the *βC1* gene either by reducing the AGO1 protein level as was reported for other VSRs [35] or through sequestering viral siRNA away from AGOs with antiviral functions to protect the virus. The later function was observed in plants expressing HCPro [34]. Therefore, the higher level of *AGO1* mRNA in plants expressing the *βC1* gene may not reflect a higher level or activity of the AGO1 protein. Further investigations are needed in order to confirm a direct interaction between the βC1 protein and AGO1 *in planta* and the regulation of AGO1 protein activity by βC1.

Both *AGO1* and *DCL1* were up-regulated in plants expressing the CLCuMuB *βC1*. The relative level of induction for *AGO1* was two times higher than that of *DCL1* (Fig. 2). Supporting this result, the same pattern of *AGO1* and *DCL1* up-regulation was observed in tomato plants infected with *Tomato yellow leaf curl virus* [39]. Both genes are close homologs to *A. thaliana AGO1* and *DCL1* genes. An elevated level of the *DCL1* gene was also reported in plants expressing *HCPro*, a virus-encoded suppressor of RNA silencing [30]. Induction of *AGO1* and *DCL1* by CLCuMuB βC1 and other VSRs can be explained by interference with the miRNA pathway. Both DCL1 and AGO1 mRNAs are subjected to negative regulation through the activity of mir162 and mir168, respectively [40, 41]. Therefore, βC1 may interfere with the miRNA pathway that results in the up-regulation of its key genes. This is supported by *βC1* regulation of miRNA and their target genes, including *MYB33*, in plants expressing *βC1* (Eini et al., unpublished data) [42].

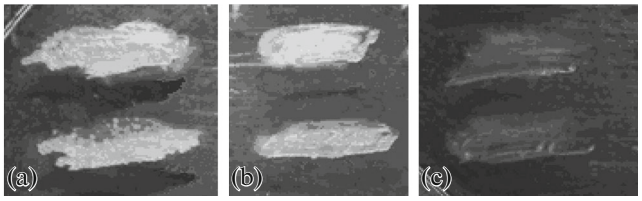

Fig. 3. Interaction between βC1 and AGO1 proteins in the yeast system. Yeast cells co-expressing AGO1 and βC1 proteins (a) were grown on SD-His-Trp-Ura medium, and the interaction was tested by leucine prototrophy on an inductive carbon source (galactose and raffinose). For a positive control, βC1 and SlUBC3 (b) were co-expressed in yeast. For the negative control, βC1 and 5A (c) were used.

The *HEN1* gene plays multiple roles in plant development and organ identity specification in flower tissues [5]. In addition, this protein participates in DCL2-mediated antiviral defense [4, 43]. For example, *A. thaliana* plants with a mutation in *HEN1* cannot survive after inoculation with *Turnip crinkle virus* [43]. Therefore, it was suggested that *HEN1* provides a genetic link between miRNA controlling development and siRNA controlling virus resistance [4]. The level of *HEN1* remained unchanged in leaf tissues expressing *βC1* (Fig. 2). This may explain why *βC1* may have no effect on the stability of small RNAs in leaf tissues. *HEN1* plays a role in flower development, and mutations in this gene result in pleiotropic phenotypes such as a reduced organ size, altered rosette leaf shape, and increased number of flowers [5]. Abnormal inflorescence was also observed in *A. thaliana* plants expressing the *βC1* gene (data not shown). Hence, *βC1* may also affect the phenotype of flower tissues by regulating the *HEN1* gene.

In conclusion, key genes in the gene silencing pathway including *DCL1* and *AGO1* were regulated in transgenic *A. thaliana* expressing the *βC1* gene from CLCuMuB. Therefore, this suppressor gene may

interfere with the function of these genes, which may explain, at least partially, the abnormal phenotypes in plants expressing the *βC1* gene. Further investigation of the effect of the CLCuMuB-derived small RNA [44] may shed some light on the interactions of the βC1 protein with the key genes of the gene silencing pathway and the effect of up-regulation of *AGO1* and *DCL1* on plant development and symptoms induction by the *βC1* gene.

ACKNOWLEDGMENTS

We would like to thank Mrs Shirzadinejad and Sarafraz (University of Zanjan) and Dr. Dry (Plant Industry, Australia) for their technical support.

This research was supported by the Research institute for Modern Biological Techniques, University of Zanjan, Zanjan.

CONFLICT OF INTEREST

Authors declare that they have no competing interest.

REFERENCES

1. Brodersen P., Voinnet O. 2006. The diversity of RNA silencing pathways in plants. *Trends Genet*. **22**, 268–280.
2. Deleris A., Gallego-Bartolome J., Bao J., Kasschau K.D., Carrington J.C., Voinnet O. 2006. Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. *Science*. **313**, 68–71.
3. Henderson I.R., Zhang X., Lu C., Johnson L., Meyers B.C., Green P.J., Jacobsen S.E. 2006. Dissecting *Arabidopsis thaliana* DICER function in small RNA processing, gene silencing and DNA methylation patterning. *Nat. Genet*. **38**, 721–725.
4. Boutet S., Vazquez F., Liu J., Beclin C., Fagard M., Gratias A., Morel J.-B., Crete P., Chen X., Vaucheret H. 2003. *Arabidopsis* HEN1: A genetic link between endogenous miRNA controlling development and siRNA controlling transgene silencing and virus resistance. *Curr. Biol*. **13**, 843–848.
5. Chen X., Liu J., Cheng Y., Jia D. 2002. HEN1 functions pleiotropically in *Arabidopsis* development and acts in C function in the flower. *Development*. **129**, 1085–1094.
6. Yang Z., Ebright Y.W., Yu B., Chen X. 2006. HEN1 recognizes 21–24 nt small RNA duplexes and deposits a methyl group onto the 2' OH of the 3' terminal nucleotide. *Nucleic Acids Res*. **34**, 667–675.
7. Hutvagner G., Simard M.J. 2008. Argonaute proteins: key players in RNA silencing. *Nat. Rev. Mol. Cell Biol*. **9**, 22–32.
8. Mette M.F., Aufsatz W., Winden J.v.d., Matzke M.A., Matzke A.J.M. 2000. Transcriptional silencing and promoter methylation triggered by double-stranded RNA. *EMBO J*. **19**, 5194–5201.
9. Sijen T., Fleenor J., Simmer F., Thijssen K.L., Parrish S., Timmons L., Plasterk R.H.A., Fire A. 2001. On the role of RNA amplification in dsRNA-triggered gene silencing. *Cell*. **107**, 465–476.
10. Kasschau K.D., Xie Z., Allen E., Llave C., Chapman E.J., Krizan K.A., Carrington J.C. 2003. P1/HC-Pro, a viral suppressor of RNA silencing, interferes with *Arabidopsis* development and miRNA function. *Dev. Cell*. **4**, 205–217.
11. Chapman E.J., Prokhnevsky A.I., Gopina*th K., Dolja V.V., Carrington J.C. 2004. Viral RNA silencing suppressors inhibit the microRNA pathway at an intermediate step. *Gene. Dev*. **18**, 1179–1186.
12. Mallory A.C., Reinhart B.J., Bartel D., Vance V.B., Bowman L.H. 2002. From the Cover: A viral suppressor of RNA silencing differentially regulates the accumulation of short interfering RNAs and micro-RNAs in tobacco. *Proc. Natl. Acad. Sci. U. S. A*. **99**, 15228–15233.
13. Ding S.-W., Voinnet O. 2007. Antiviral immunity directed by small RNAs. *Cell*. **130**, 413–426.
14. Burgyán J. 2008. Role of silencing suppressor proteins, In: *Plant Virology Protocols: From Viral Sequence to Protein Function*, vol. 451. Eds. Foster G.D., Johansen E., Hong Y., Nagy P.D. Humana Press, pp. 69–79.
15. Jiang L., Wei C., Li Y. 2012. Viral suppression of RNA silencing. *Sci. Chin. Life Sci*. **55**, 109–118.
16. Briddon R.W., Bull S.E., Amin I., Idris A.M., Mansoor S., Bedford I.D., Dhawan P., Rishi N., Siwatch S.S., Abdel-Salam A.M., Brown J.K., Zafar Y., Markham P.G. 2003. Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. *Virology*. **312**, 106–121.
17. Zhou X. 2013. Advances in understanding begomovirus satellites. *Annu. Rev. Phytopathol*. **51**, 357–381.
18. Eini O., Dogra S., Selth L.A., Dry I.B., Randles J.W., Rezaian M.A. 2009. Interaction with a host ubiquitin-conjugating enzyme is required for the pathogenicity of a geminiviral DNA β satellite. *Mol. Plant Microbe Interact*. **22**, 737–746.
19. Saeed M., Behjatnia S.A.A., Shahid M., Yusuf Z., Shahida H., Rezaian M.A. 2005. A single complementary-sense transcript of a geminiviral DNA beta satellite is determinant of pathogenicity. *Mol. Plant Microbe Interact*. **18**, 7–14.
20. Saunders K., Norman A., Gucciardo S., Stanley J. 2004. The DNA beta satellite component associated with ageratum yellow vein disease encodes an essential pathogenicity protein (ßC1). *Virology*. **324**, 37–47.
21. Saeed M., Briddon R.W., Dalakouras A., Krczal G., Wassenegger M. 2015. Functional analysis of Cotton leaf curl Kokhran virus/cotton leaf curl Multan betasatellite RNA silencing suppressors. *Biology*. **4**, 697–714.
22. Amin I., Hussain K., Akbergenov R., Yadav J.S., Qazi J., Mansoor S., Hohn T., Fauquet C.M., Briddon R.W. 2011. Suppressors of RNA silencing encoded by the components of the cotton leaf curl begomovirus-betasatellite complex. *Mol. Plant. Microbe Interact*. **24**, 973–983.
23. Cui X., Li G., Wang D., Hu D., Zhou X. 2005. A begomovirus DNA beta-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. *J. Virol*. **79**, 10764–10775.
24. Eini O., Dogra S.C., Dry I.B., Randles J.W. 2012. Silencing suppressor activity of a begomovirus DNA β

encoded protein and its effect on heterologous helper virus replication. *Virus Res.* **167**, 97–101.

25. Gleave A.P. 1992. A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. *Plant Mol. Biol.* **20**, 1203–1207.
26. Clough S.J., Bent A.F. 1998. Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735–743.
27. Takos A.M., Jaffe F.W., Jacob S.R., Bogs J., Robinson S.P., Walker A.R. 2006. Light-induced expression of a *myb* gene regulates anthocyanin biosynthesis in red apples. *Plant Physiol.* **142**, 1216–1232.
28. Dogra S., Eini O., Rezaian M., Randles J. 2009. A novel shaggy-like kinase interacts with the Tomato leaf curl virus pathogenicity determinant C4 protein. *Plant Mol. Biol.* **71**, 25–38.
29. Bortolamiol D., Pazhouhandeh M., Marrocco K., Genschik P., Ziegler-Graff V. 2007. The polerovirus F-Box protein P0 targets ARGONAUTE1 to suppress RNA silencing. *Curr. Biol.* **17**, 1615–1621.
30. Ivanov K.I., Eskelin K., Bašić M., De S., Lõhmus A., Varjosalo M., Mäkinen K. 2016. Molecular insights into the function of the viral RNA silencing suppressor HCPro. *Plant J.* **85**, 30–45.
31. Voinnet O. 2005. Induction and suppression of RNA silencing: Insights from viral infections. *Nat. Rev. Genet.* **6**, 206–220.
32. Dunoyer P., Lecellier C.H., Parizotto E.A., Himber C., Voinnet O. 2004. Probing the microRNA and small interfering RNA pathways with virus-encoded suppressors of RNA silencing. *Plant Cell.* **16**, 1235–1250.
33. Pantaleo V., Szittya G., Burgyan J. 2007. Molecular bases of viral RNA targeting by viral small interfering RNA-programmed RSC. *J. Virol.* **81**, 3797–3806.
34. Garcia-Ruiz H., Carbonell A., Hoyer J.S., Fahlgren N., Gilbert K.B., Takeda A., Giampetruzzi A., Garcia Ruiz M.T., McGinn M.G., Lowery N., Martinez Baladejo M.T., Carrington J.C. 2015. Roles and programming of *Arabidopsis* ARGONAUTE proteins during turnip mosaic virus infection. *PLoS Pathog.* **11**, e1004755.
35. Várallyay É., Havelda Z. 2013. Unrelated viral suppressors of RNA silencing mediate the control of ARGONAUTE1 level. *Mol. Plant Pathol.* **14**, 567–575.
36. Morel J.-B., Godon C., Mourrain P., Beclin C., Boutet S., Feuerbach F., Proux F., Vaucheret H. 2002. Fertile hypomorphic ARGONAUTE (AGO1) mutants impaired in post-transcriptional gene silencing and virus resistance. *Plant Cell.* **14**, 629–639.
37. Azevedo J., Garcia D., Pontier D., Ohnesorge S., Yu A., Garcia S., Braun L., Bergdoll M., Hakimi M.A., Lagrange T., Voinnet O. 2010. Argonaute quenching and global changes in Dicer homeostasis caused by a pathogen-encoded GW repeat protein. *Gene Dev.* **24**, 904–915.
38. Zhang X., Yuan Y.-R., Pei Y., Lin S.-S., Tuschl T., Patel D.J., Chua N.-H. 2006. Cucumber mosaic virus-encoded 2b suppressor inhibits *Arabidopsis* Argonaute1 cleavage activity to counter plant defense. *Gene Dev.* **20**, 3255–3268.
39. Bai M., Yang G.-S., Chen W.-T., Mao Z.-C., Kang H.-X., Chen G.-H., Yang Y.-H., Xie B.-Y. 2012. Genome-wide identification of Dicer-like, Argonaute and RNA-dependent RNA polymerase gene families and their expression analyses in response to viral infection and abiotic stresses in *Solanum lycopersicum*. *Gene.* **501**, 52–62.
40. Xie Z., Kasschau K.D., Carrington J.C. 2003. Negative feedback regulation of Dicer-Like1 in *Arabidopsis* by microRNA-guided mRNA degradation. *Curr. Biol.* **13**, 784–789.
41. Vaucheret H., Vazquez F., Crete P., Bartel D.P. 2004. The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. *Gene. Dev.* **18**, 1187–1197.
42. Amin I., Patil B.L., Briddon R.W., Mansoor S., Fauquet C.M. 2011. Comparison of phenotypes produced in response to transient expression of genes encoded by four distinct begomoviruses in *Nicotiana benthamiana* and their correlation with the levels of developmental miRNAs. *Virol. J.* **8**, 238–262.
43. Zhang X., Zhang X., Singh J., Li D., Qu F. 2012. Temperature-dependent survival of turnip crinkle virus-infected *Arabidopsis* plants relies on an RNA silencing-based defense that requires DCL2, AGO2, and HEN1. *J. Virol.* **86**, 6847–6854.
44. Wang J., Tang Y., Yang Y., Ma N., Ling X., Kan J., He Z., Zhang B. 2016. Cotton leaf curl Multan virus-derived viral small RNAs can target cotton genes to promote viral infection. *Front. Plant Sci.* **7**, 1162–1175.