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The microRNA pathway is involved in *Spodoptera frugiperda* (Sf9) cells antiviral immune defense against Autographa californica multiple nucleopolyhedrovirus infection

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ABSTRACT

The microRNA (miRNA) pathway is an epigenetic mechanism that plays important roles in various biological processes including host-virus interactions by regulating gene expression of the host and/or the virus. Previously, we showed that the cellular microRNAome in *Spodoptera frugiperda* (Sf9) cells is modulated following Autographa californica multiple nucleopolyhedrovirus (AcMNPV) infection suggesting that miRNAs may contribute in the cellular antiviral immunity. Here, we investigated the role of core components of the miRNA pathway in Sf9-AcMNPV interaction. Gene expression analyses showed that the expression levels of Dicer-1 (*Dcr1*), Argonaute-1 (*Ago1*) and Exportin-5 (*Exp5*) increased following AcMNPV infection particularly at 16 h post infection (hpi). *Ran* expression levels, however, decreased in response to virus infection. The expression levels of cellular miRNAs, miR-184 and let-7, also diminished at the post infection times further confirming differential expression of the cellular miRNAs following AcMNPV infection. To determine the role of the miRNA pathway in the interaction, we silenced key genes in the pathway using specific dsRNAs. RNAi of *Dcr1*, *Ago1* and *Ran* enhanced viral DNA replication and reduced the abundance of miR-184 and let-7 underscoring the importance of the miRNA pathway in antiviral immunity in Sf9 cells. Suppression of the miRNA pathway in mock and infected cells had no effect on *Ran* expression levels suggesting miRNA-independent downregulation of this gene after virus infection. In conclusion, our results suggest the antiviral role of the miRNA pathway in Sf9 cells against AcMNPV. To modulate this immune response, AcMNPV represses host miRNAs likely through downregulation of *Ran* to enhance its replication in the host cells.

1. Introduction

RNA interference (RNAi) is an evolutionary conserved post transcriptional gene silencing mechanism in a variety of organisms including insects. The RNAi response comprises of three pathways including small interfering RNA (siRNA), microRNA (miRNA) and Piwi-interacting RNA (piRNA). In insects, virus replication and transcription triggers antiviral responses including the RNAi machinery (Asgari, 2013). The antiviral role of siRNA has been recognized against both insect DNA (Bronkhorst et al., 2012; Jayachandran et al., 2012; Kemp et al., 2013; Karamipour et al., 2018; Mehrabadi et al., 2015) and RNA viruses (Mueller et al., 2010; van Rij et al., 2006; Zambon et al., 2006; Moon et al., 2015; Schnettler et al., 2012). However, the widespread functions of the miRNA pathway in insect-virus interaction demand more investigations.

miRNAs are small and conserved non-coding RNAs ~19–22 nucleotides (nt) in length that play important roles in the cell by regulating gene expression at the post transcriptional level (Brennecke et al., 2005; Lee et al., 2004). These endogenous non-coding RNAs are transcribed by RNA polymerase II and III (Borchert et al., 2006; Lee et al., 2002, 2004) and then processed by RNase III endonuclease Drosha into about 70 nt small hairpin RNA, named precursor miRNA (pre-miRNA), in the nucleus (Brennecke et al., 2005). They are exported into the cytoplasm in association with Ran GTP-binding nuclear protein (Ran) and Exportin-5 (Exp5). (Bohnsack et al., 2004). Thereafter, the cytoplasmic RNase III endonuclease Dicer-1 (Dcr1) generates a small double strand RNA ~22 nt in length by removing the hairpin head (Grishok et al., 2001; Lee et al., 2002). A mature miRNA is then

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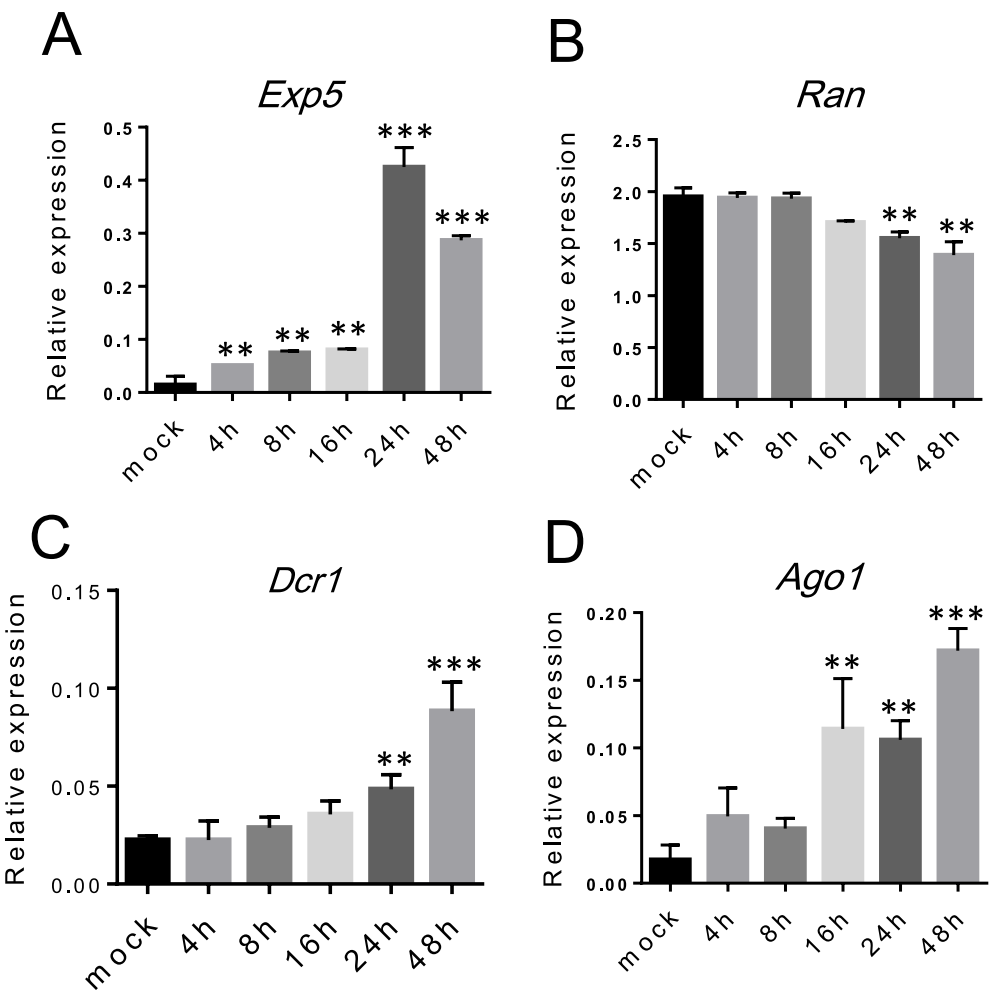

Fig. 1. Differential expressions of Sf9 miRNA pathway core genes following AcMNPV infection. A) RT-qPCR gene expression analysis showed upregulation of *Exp5* in response to AcMNPV infection. B) The transcript levels of *Ran* decreased in AcMNPV infected cells. C) The gene expression level of *Dcr1* intensified following AcMNPV infection. D) *Ago1* gene expression level was induced after AcMNPV infection in Sf9 cells. In all the experiments, RNA extractions were performed at 4, 8, 16, 24 and 48 hpi. Asterisks indicate a significant difference between the control and the infected Sf9 cells (ANOVA followed by Tukey multiple comparisons, **P < 0.01, ***P < 0.001).

produced, which is a single stranded guide RNA, selection of which is performed by the Argonate-1 (Ago1) protein as part of the miRNA Induced Silencing Complex (miRISC). The miRNA then directs the complex to target sequences by complementarity (Vaucheret et al., 2004).

The effect of miRNAs in host-virus interaction is complex. Host miRNAs may act as antiviral response for inhibition of viral replication (Bruscella et al., 2017; Hussain and Asgari, 2014; Trobaugh and Klimstra, 2017). Bantam miRNA, for example, has been shown to function as an antiviral miRNA in *Spodoptera exigua*-AcMNPV interaction (Shi et al., 2016). The presence of bee paralysis virus increases the expression of *Dcr1* and *Ago1* genes in *Bombus terrestris* (Niu et al., 2017). Moreover, the presences of viral suppressors of RNA silencing (VSRs) in some viruses that interact with the miRNA pathway component indicate the counter defense of viruses (Bruscella et al., 2017; Liu et al., 2017). On the other hand, viruses may employ host miRNAs to enhance their replication and complete their life cycle (Hussain et al., 2008; Jayachandran et al., 2013; Singh et al., 2010, 2012). These findings suggest that the miRNA pathway may either function as a host antiviral response or manipulated by viruses to favor their replication within the host highlighting their important functions in insect-virus interactions.

In our previous study, we showed overall suppression of cellular miRNAs in Sf9 cells following AcMNPV infection (Mehrabadi et al., 2013) suggesting possible role of this pathway in Sf9 cells-AcMNPV interaction. However, the importance of the miRNA pathway in this interaction remained to be functionally investigated. Therefore, in the present study we examined the expression of the key genes of the miRNA pathway including *Ran*, *Exp5*, *Dcr1* and *Ago1* in response to AcMNPV infection and determined the importance of the pathway in the interaction by knocking down the key genes. Our results suggest that the miRNA pathway contributes in the antiviral immune defense of Sf9 cells against AcMNPV infection.

2. Methods and material

2.1. Cell culture and virus infection

Spodoptera frugiperda Sf9 cells were maintained in SF900-II serum-free medium supplemented with 10% FBS. The cells were maintained as monolayer in a cell culture flask at 27 °C. To examine the expression levels of *Ran*, *Exp5*, *Ago1* and *Dcr1*genes at different times post infection, Sf9 cells were seeded in 12-well plates and infected with 500 µl of AcMNPV (MOI of 5 pfu/cell) (Mehrabadi et al., 2013). For UV inactivation of the virus, AcMNPV was exposed to a total of 12,000 mJ UV light in 4 × 3 min bursts. No infection was observed in cells inoculated with UV-inactivated viruses.

2.2. RNA extraction and RT-qPCR

To quantify the expression levels of the core components of the miRNA pathway in the mock and AcMNPV-infected Sf9 cells (at 4, 8, 16, 24 and 48 hpi), total RNA was isolated from the samples using Tri-Reagent™ according to the manufacturer's instructions (Molecular Research Center Inc.) and subsequently incubated with DNase I at 37 °C for 10 min followed by heat inactivation at 75 °C for 10 min. The RNA concentrations were measured using an Epoch microplate spectrophotometer (BioTek) and integrity was ensured through analysis of RNA on a 1% (w/v) agarose gel. First strand cDNA was generated using 2 µg of total RNA as template and an oligo dT primer. Samples were

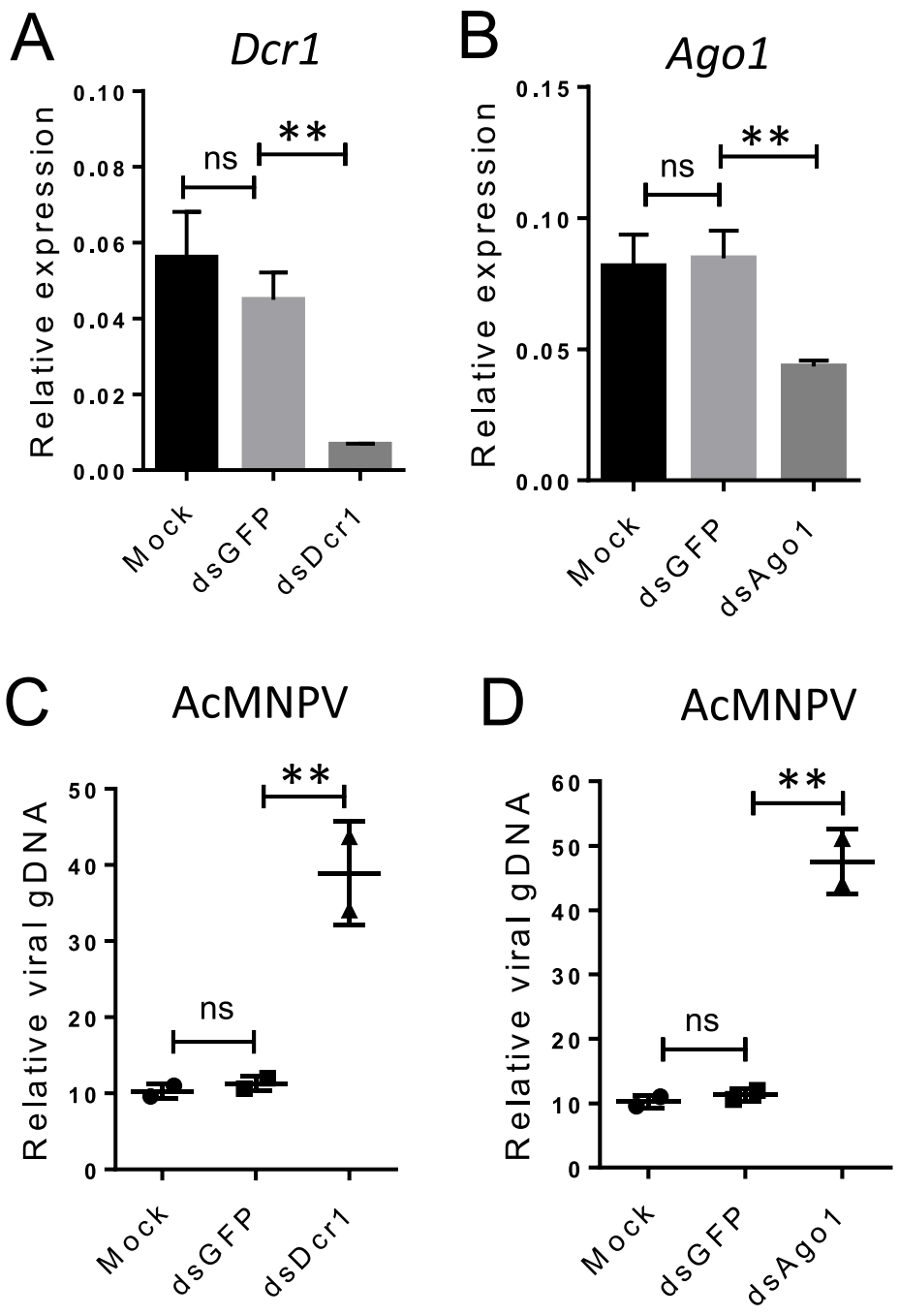

Fig. 2. RNAi of *Dcr1* and *Ago1* genes enhanced replication of AcMNPV genomic DNA. A and B) The expression of *Dcr1*and *Ago1* genes reduced in transfected cells using dsDcr1 and dsAgo1 at 24 h post transfection, respectively. C and D) QPCR analysis indicted that silencing *Dcr1* and *Ago1* genes led to improved replication levels of AcMNPV's genomic DNA (gDNA) at 24 hpi. RNAi of *Dcr1* and *Ago1* was performed using 1 μg of dsDcr1 and dsAgo1. Asterisks indicate a significant difference between the mock and dsRNA transfected Sf9 cells (ANOVA followed by Tukey multiple comparisons, **P < 0.01).

incubated at 42 °C for 60 min followed by heating at 65 °C for 5 min. The cDNA samples were used for qPCR using gene-specific primers and *Rpl27* as reference. QPCR was performed using SYBR Green Mix without ROX (Ampliqon) with a Mic real-time PCR (BMS) under following condition: 95 °C for 15min, followed by 40 cycles of 95 °C for 10s, 57 °C for 10 s, and 72 °C for 20 s, followed by the melting curve (68–95 °C). For each experiment, three biological replicates with three technical replicates were analyzed. Primers used for qPCR are shown in Table S1.

2.3. DNA extraction and qPCR

The levels of viral DNA accumulation in the mock and AcMNPV-infected cells were determined using qPCR. To do this, DNA samples were extracted from the cells as described before (Glatz et al., 2003). DNA concentrations were measured and 20 ng total genomic DNA was used for each qPCR. Then, DNA samples were subjected to qPCR using specific primer to AcMNPV *ie-1* gene and the host *Rpl27* as reference gene. QPCR conditions were as follow: 95 °C for 15 min, and 40 cycles of 95 °C for 10 s and 60 °C for 45 s, followed by the melting curve (68–95 °C). Reactions from three biological replicates were repeated three times.

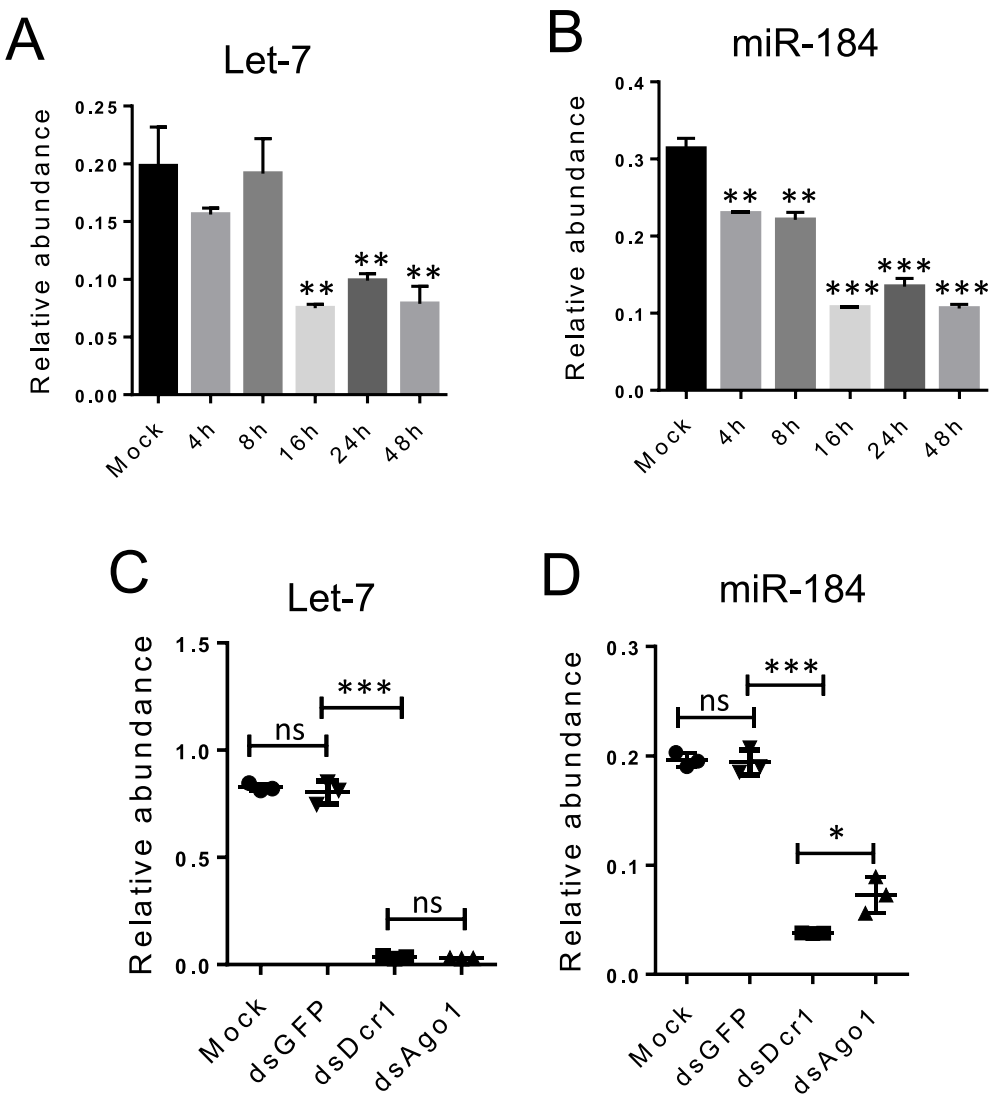

Fig. 3. AcMNPV infection suppresses expression of miR-184 and let-7. A and B) The abundance of let-7 and miR-184 reduced in response to AcMNPV infection, respectively. In all the experiments, RNA extractions were performed at 4, 8, 16, 24 and 48 hpi. C and D) The abundance of let-7 and miR-184 reduced in dsDcr1 and dsAgo1 transfected cells. Cell transfection was performed using 1 μg of dsDcr1 and dsAgo1. Small nuclear *U6* gene was used as reference gene. Asterisks indicate a significant difference between the control and treated (virus infected or dsRNA transfected) Sf9 cells (ANOVA followed by Tukey multiple comparisons, *P < 0.05, **P < 0.01, ***P < 0.001).

2.4. RNAi

To confirm the role of the miRNA pathway in AcMNPV-Sf9 interaction, RNAi was used to suppress core genes of the pathway (i.e. *Dcr1*, *Ago1* and *Ran*). To do this, the gene-specific primers contained T7 promoter sequences (Table S1) at their 5′ end were designed for *in vitro* dsRNA synthesis. dsRNAs (~500 bp) were generated using T7 MEGAScript kit according to the manufacturer's instructions (Ambion). Then, dsRNA purification was performed for each fragment. Also, dsRNA of the green fluorescent protein gene (dsGFP) was generated as a control. The concentrations of dsRNA samples were determined spectrophotometrically and their synthesis were also confirmed by running dsRNA samples on an agarose gel. For transfection of dsRNAs, Sf9 cells were seeded onto a 12-well plate and after adherence of the cells in the wells, the transfection medium consisted of 0.5 ml SF-900II, 8 μl Cellfectin (Invitrogen), and 1 μg dsRNA either for gene of interest or GFP, was added to the cells. Twenty-four (for *Dcr1* and *Ran*) or 48 h later (for *Ago1*), cells in each well were then infected with 200 μl of AcMNPV inoculum. QPCR was carried out at 24 hpi to quantify viral DNA accumulation level. RNA samples from the cells were also extracted at 24 hpi to confirm gene silencing.

2.5. MicroRNA expression analysis

To quantify the expression levels of the two of the most abundant miRNAs (let-7 and miR-184) in Sf9 cells (Mehrabadi et al., 2013), total RNA samples were isolated from the mock and AcMNPV infected cells at 4, 8, 16, 24 and 48 hpi and cDNA was synthesized with stem-loop primers as described previously (Chen et al., 2005). U6 gene was used as reference gene in the experiment. The miRNA expression was measured using SYBR Green Mix under the following condition: 95 °C for 15min, followed by 40 cycles of 95 °C for 10s, 60 °C for 10 s followed by the melting curve (68–95 °C).

2.6. In silico miRNA-target analysis

To find the homologous BmNPV miRNAs (Singh et al., 2012) in AcMNPV, we mapped the BmNPV pre-miRNAs onto the AcMNPV genome by BLAST software (Altschul et al., 1990). Secondary structures of pre-miR-1 in AcMNPV and BmNPV were determined by MFold program (Zuker, 2003). RNAHybrid software (Krüger and Rehmsmeier, 2006) was also used to evaluate potential targets of viral miRNAs in *S. frugiperda Ran* gene.

3. Results

3.1. AcMNPV infection modulates expression of the miRNA pathway core genes in Sf9 cells

Analysis of expression levels of the miRNA pathway core genes

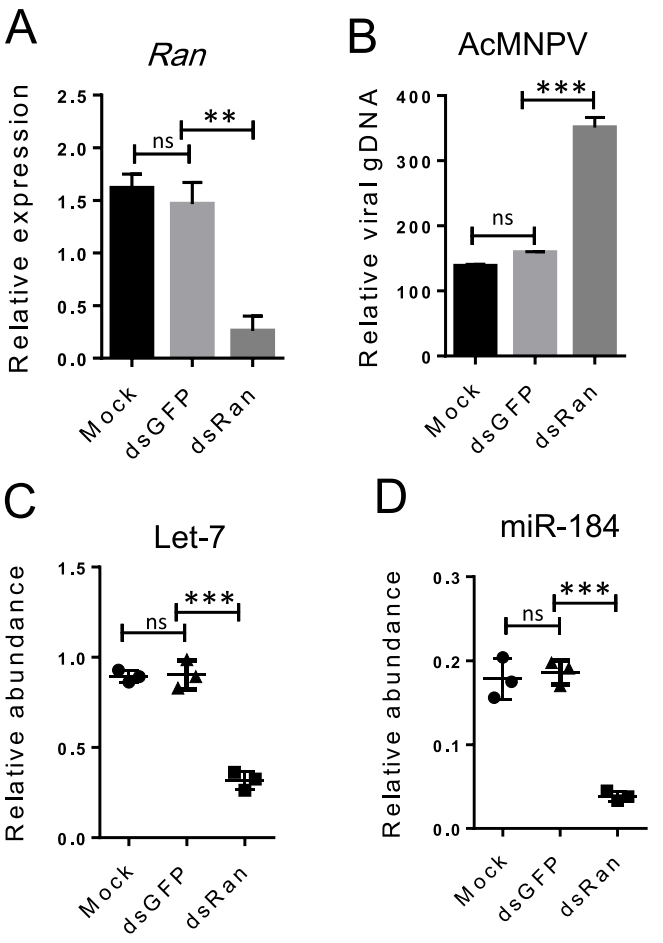

Fig. 4. *Ran* silencing enhanced replication of AcMNPV's genomic DNA and reduced cellular miRNA levels in Sf9 cells. A) RT-qPCR revealed significant reduction in the transcript levels of *Ran* using dsRan in Sf9 cell 24 h post transfection. B) AcMNPV gDNA replication increased at 24 hpi after silencing *Ran* in Sf9 cells. Cell transfection was performed using 1 μg of dsRan. Asterisks indicate a significant difference (ANOVA followed by Tukey multiple comparisons, *P < 0.05, **P < 0.01).

showed differential expression of the genes in Sf9 cells following AcMNPV infection. The mRNA levels of *Exp5* increased from 4 hpi reaching the highest level at 24 hpi (Fig. 1A). However, expression levels of the *Ran* gene decreased from 24 hpi (Fig. 1A). The transcript levels of *Dcr1* and *Ago1* genes significantly increased from 24 to 16 hpi, respectively (Fig. 1C and D).

3.2. RNAi of Dcr1 and Ago1 improved viral DNA replication

Modulation of the miRNA pathway core genes in response to AcMNPV infection implied possible role of the pathway in the AcMNPV-Sf9 interaction. To determine the role of the miRNA pathway in the interaction, we silenced *Ago1* and *Dcr1* genes and evaluated their consequences on the viral DNA accumulation in Sf9 cells. RT-qPCR showed reduction of *Dcr1* and *Ago1* mRNA levels after transfection of specific-dsRNAs into the cells (Fig. 2A and B). RNAi of *Dcr1* and *Ago1* enhanced viral genomic DNA levels compared to the mock and control (dsGFP transfected) cells (Fig. 2C and D). There was no difference in virus replication between the control and mock cells.

3.3. Abundance of let-7 and miR-184 decreased following AcMNPV infection and after suppression of the miRNA pathway

To determine the effect of AcMNPV infection on the abundance of

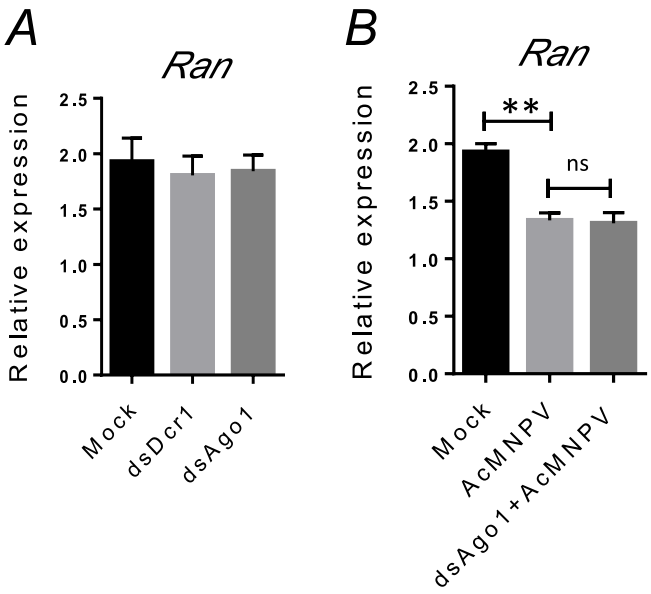

Fig. 5. RNAi of *Ago1* had no effect on the expression levels of *Ran* in mock and AcMNPV-infected cells. A) The expression levels of *Ran* remained unchanged in response to RNAi of *Dcr1* and *Ago1* in mock-infected Sf9 cells as revealed by RT-qPCR. B) RNAi of *Ago1* did not change the expression levels of *Ran* following AcMNPV infection at 24 hpi. RNAi of *Dcr1* and *Ago1* was performed using 1 of dsDcr1 and dsAgo1. The RT-qPCRs were performed on RNA samples that were extracted from transfected cells after 24 h. Asterisks indicate a significant difference (ANOVA followed by Tukey multiple comparisons, *P < 0.05, **P < 0.01).

cellular miRNAs, two miRNAs with high expression levels in Sf9 cells were selected and the expression of these miRNAs was determined in AcMNPV infected cells. The abundance of let-7 significantly decreased from 16 hpi (Fig. 3A). miR-184's abundance also diminished from 4 hpi compared to mock cells (Fig. 3B). RNAi of *Dcr1* and *Ago1* also reduced the abundance of let-7 and miR-184 indicating they are produced through the canonical miRNA biogenesis pathway (Fig. 3C and D). To find out whether the down-regulation of sfr-miR-184 following AcMNPV infection is due to virus replication or entry, AcMNPV virions were inactivated by UV radiation and used to infect Sf9 cells. The results showed no noticeable change in sfr-miR-184 concentrations when inoculated with the inactivated virus (Fig. S1), which suggests that the miRNA down-regulation is linked to virus replication.

3.4. RNAi of Ran reduced miRNA expression and enhanced AcMNPV replication

Our results showed that expression of *Ran* and miRNAs were similarly reduced after AcMNPV infection, while the other core miRNA genes were upregulated. To determine the role of *Ran* in the interaction, we silenced *Ran* and assessed the levels of let-7 and miR-184, and AcMNPV replication. The results showed that suppression of *Ran* enhanced AcMNPV replication (Fig. 4A and B), and reduced the abundance levels of let-7 and miR-184 (Fig. 4C and D).

3.5. Reduction of Ran expression following AcMNPV infection is independent of the miRNA pathway

To examine the possibility of *Ran* regulation by the miRNA pathway, we silenced *Dcr1* and *Ago1* in Sf9 cells and quantified the transcript levels of *Ran*. Our results showed no change in expression of *Ran* after suppressing the miRNA pathway (Fig. 5A). We also analyzed *Ran* expression following AcMNPV infection in the cells that the miRNA pathway was already suppressed by RNAi of *Ago1*. We found no difference between the expression of *Ran* in AcMNPV-infected cells in the

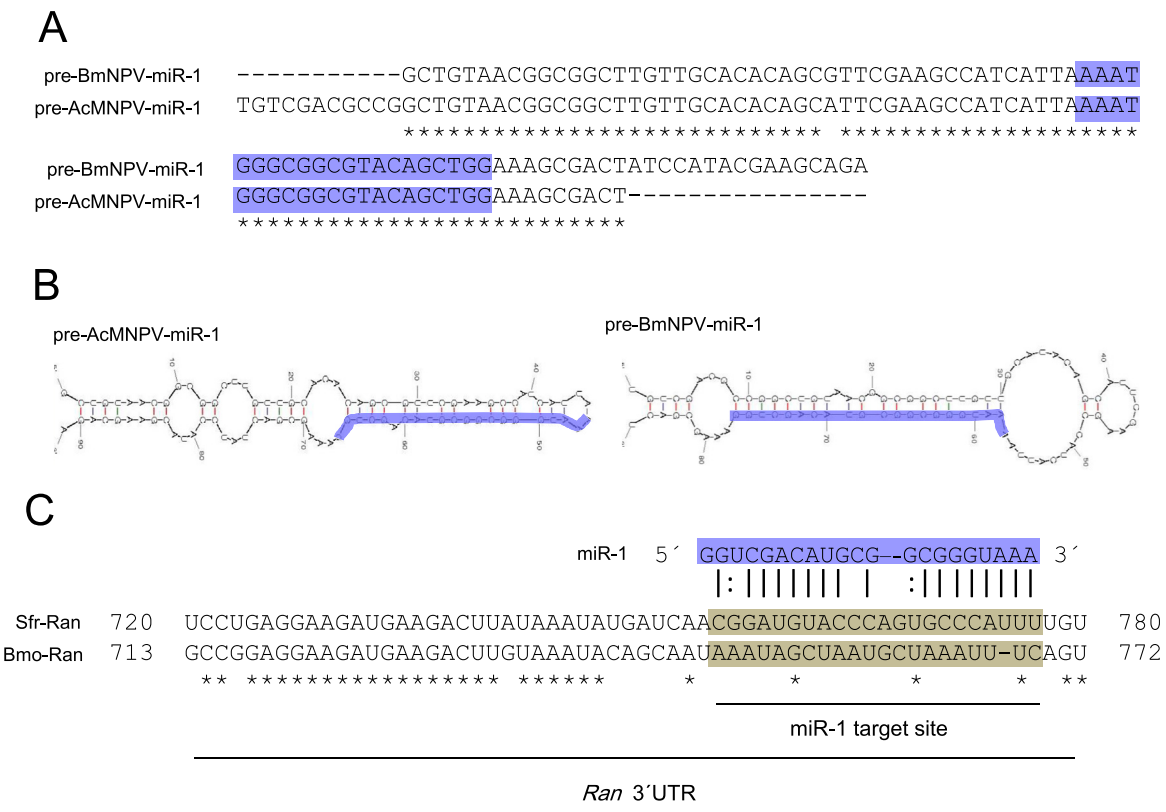

Fig. 6. Comparison of pre-miR-1 in BmNPV and AcMNPV and its target gene, *Ran,* in *B. mori* and *S. frugiperda* (Sf9). A) Pairwise alignment of pre-miR-1 sequences in AcMNPV and BmNPV showed high level of similarity. The position of mature miRNAs is shown in purple. B) Comparison of secondary structures of pre-miR-1 in AcMNPV and BmNPV. The position of mature miRNAs is shown in purple. C) Pairwise alignment of *Ran* 3′ UTR in *B. mori* and *S. frugiperda* showed low similarity, resulting in no binding site for miR-1 on the *S. frugiperda Ran* 3′ UTR. The position of miRNAs binding site is shown in tan. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

absence of the miRNA pathway with the control cells (i.e. with normal *Ago1* expression) (Fig. 5B). To determine if there is any AcMNPV-derived miRNA binding site in the *Ran* mRNA, we first identified homologues of five Bombyx mori nucleopolyhedrovirus pre-miRNAs in the genome of AcMNPV (Fig. 5, S2). Then, using RNAhybrid analysis we examined for possible binding sites on Sf9 *Ran* mRNA and found no optimal target site for the predicted miRNAs on the *Ran* gene even for *bmnpv-miR-1* (Fig. 6B), which was previously reported as BmNPV-derived miRNA suppressing *Ran* expression in *B. mori* larvae. This is due to differences in the 3′UTRs of the *Ran* genes in *B. mori* and Sf9 cells. These data also revealed miRNA-independent regulation of *Ran* expression in Sf9 cells.

4. Discussion

In insects, virus infection induces antiviral responses including the RNAi pathways that target viral genome and transcripts (Bronkhorst and van Rij, 2014). Host miRNAs, produced through a sub-pathway of RNAi, may play important roles in host insect-virus interactions through regulation of either host insect or viral gene expression at the post-transcriptional level (Hussain and Asgari, 2014). The outcome of the interaction could be either in favor of the host insect by attenuating virus infection or facilitate virus replication within the host insect. Changes in insect miRNA profile are commonly observed upon virus infection. For example, the abundance of miRNAs was altered in host insect cells following infection with insect viruses including Heliothis virescens ascovirus (HvAV3e) (Hussain and Asgari, 2010), Helicoverpa armigera single nucleopolyhedrovirus (HaSNPV) (Jayachandran et al., 2013), and Bombyx mori cytoplasmic polyhedrosis virus (BmCPV) (Wu et al., 2013).

In a previous study, we showed that AcMNPV infection alters the abundance of most Sf9 miRNAs (Mehrabadi et al., 2013) suggesting that the miRNA pathway might play a role in AcMNPV-Sf9 cells interaction. Therefore in the present study, the role of the miRNA pathway in the interaction was further investigated. Gene expression of core component of the miRNA pathway revealed upregulation of *Dcr1*, *Ago1* and *Exp5* genes in AcMNPV-infected cells, while *Ran* expression level declined. The expression levels of two highly abundant miRNAs, let-7 and miR-184, also decreased following AcMNPV infection indicating that although the majority of the miRNA pathway core genes were upregulated in response to AcMNPV infection, the abundance of cellular miRNAs can be affected by *Ran* downregulation. The expression level of *Ago1* increased prior to *Dcr1* following virus infection while *Ago1* functions downstream of *Dcr1*. Most mature miRNAs associate with Ago1 to guide translational repression on their target genes, therefore, it is likely that host cells induce the expression of *Ago1* following virus infection to accelerate targeting of virus transcripts even before biogenesis of new miRNAs. In addition, Ago1 might be involved in processes other than the miRNA pathway. RNAi silencing of *Dcr1*, *Ago1* and *Ran* independently led to lower cellular miRNA abundance and higher viral titer that revealed an antiviral role for the miRNA pathway in Sf9 cells. The antiviral role of insect miRNAs have also been reported in some studies. For instance, in *Helicoverpa zea* (HzFB) cells, miR-24 targets DNA dependent RNA polymerase II and DdRP β subunits of HvAV3e. This miRNA is suppressed following HvAV3e infection that could be considered as counteract of the virus to facilitate its replication (Hussain and Asgari, 2010). Blocking of the cellular miRNA miR-8 in *B. mori* that targets transcripts of BmNPV resulted in higher viral titer (Singh et al., 2012). The antiviral role of the miRNA pathway has also been proven in mammals. Mice with defect in *Dcr1* were more susceptible to vesicular somatic virus infection (Otsuka et al., 2007). Also, silencing of *Dcr1* or *Drosha* improved human immunodeficiency virus

replication (Triboulet et al., 2007). Furthermore, our *in silico* prediction of cellular miRNA targets in AcMNPV ORFs showed that there are many targets for cellular miRNAs on AcMNPV genome (Data not shown). Considering antiviral function of cellular miRNAs, it is likely that host cells try to increase the expression of the miRNA pathway core gene to improve antiviral immunity as we observed in the case of the majority of the miRNA pathway genes.

Ran-GTPase is a nucleocytoplasmic transport protein, which is necessary for dsRNA transport through the Exportin-5 protein (Bohnsack et al., 2004). Our results showed reduction in *Ran* gene expression in AcMNPV-infected cells. Silencing of *Ran* in Sf9 cells reduced the abundance of miRNAs and increased viral titer. The antiviral role of *Ran* has also been observed in *B. mori* infected with BmNPV (Singh et al., 2012). A BmNPV encoded miRNA (bmnpv-miR-1) has been shown to downregulate the expression of the host GTP-binding nuclear protein Ran, thereby inhibits small RNA transport from the nucleus into the cytoplasm (Singh et al., 2012). Upregulation of other core miRNA pathway genes, *Exp5* in particular, which we observed in this study, could be considered as a compensatory action of the cells to increase the abundance of antiviral miRNAs. Considering our results with AcMNPV and the previous report from BmNPV, it can be hypothesized that downregulation of *Ran* may be a common strategy used by baculoviruses (at least in NPVs) to reduce host insect's antiviral response by interrupting the miRNA pathway to promote their replications.

We also investigated the effect of the miRNA pathway on expression of Sf9 *Ran*. Our results showed that suppression of the miRNA pathway core genes had no effect on the expression levels of *Ran* compared to that in the control cells. This result suggests that cellular miRNAs do not regulate *Ran* expression in Sf9 cells. Also, the possibility of the effect of AcMNPV miRNAs on *Ran* expression was investigated by suppressing the miRNA pathway through RNAi of *Ago1* followed by AcMNPV infection. We observed no change in the expression levels of *Ran* after suppression of the miRNA pathway. This result indicates that *Ran* expression is not regulated by viral miRNAs either. In accordance with these results, *in silico* analysis also showed that none of the AcMNPV predicted miRNAs have target sites in Sf9 *Ran* gene even miR-1, which was reported as a negative regulator of *Ran* gene in *B. mori* infected with BmNPV (Singh et al., 2012). This could be due to differences in nucleotide sequence of the 3′UTR of *Ran* genes (miR-1 target site) between *B. mori* and *S. frugiperda*.

In conclusion, our results showed that AcMNPV infection induced the expression of *Dcr1*, *Ago1* and *Exp5* genes, but *Ran* expression was reduced. Downregulation of *Ran* would contribute in reduction of the abundance of cellular miRNAs. Further, RNAi of *Ago1*, *Dcr1* and *Ran* genes facilitated AcMNPV infection through suppression of biogenesis of the host miRNA. We also showed that the miRNA pathway obviously is not involved in regulation of *Ran* expression in normal and infected Sf9 cells revealing miRNA-independent downregulation of *Ran* in Sf9 cells following AcMNPV infection. While the mechanism of *Ran* downregulation remains unknown, this downregulation as a result of virus infection benefits the virus in suppressing the host's miRNA biogenesis, which is apparently part of the host antiviral response. This study provides evidence for critical role of the miRNA pathway in AcMNPV-Sf9 interactions and antiviral immunity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibmb.2019.103202.

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