Fish and Shellfish Immunology 92 (2019) 21–30

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Role of DCP1-DCP2 complex regulated by viral and host microRNAs in DNA virus infection

Yuechao Sun, Xiaobo Zhang*

College of Life Sciences and Laboratory for Marine Biology and Biotechnology of Qingdao National Laboratory for Marine Science and Technology, Zhejiang University, Hangzhou, 310058, People's Republic of China

ARTICLE INFO

Keywords:
DCP1-DCP2 complex
miRNA
DNA virus infection

ABSTRACT

The DCP1-DCP2 complex can regulate the antiviral immunity of animals by the decapping of retrovirus RNAs and the suppression of RNAi during RNA virus infection. However, the influence of DCP1-DCP2 complex on DNA virus infection and the regulation of DCP1-DCP2 complex by microRNAs (miRNAs) remain unclear. In this study, the role of miRNA-regulated DCP1-DCP2 complex in DNA virus infection was characterized. Our results showed that the DCP1-DCP2 complex played a positive role in the infection of white spot syndrome virus (WSSV), a DNA virus of shrimp. In the DCP1-DCP2 complex, the N-terminal regulatory domain of DCP2 was interacted with the EVH1 domain of DCP1. Furthermore, shrimp miRNA miR-87 inhibited WSSV infection by targeting the host DCP2 gene and viral miRNA WSSV-miR-N46 took a negative effect on WSSV replication by targeting the host DCP1 gene. Therefore, our study provided novel insights into the underlying mechanism of DCP1-DCP2 complex and its regulation by miRNAs in virus-host interactions.

Importance: During RNA virus infection, the DCP1-DCP2 complex can play important roles in the animal antiviral immunity by decapping retrovirus RNAs and suppressing RNAi. In the present study, the findings indicated that the silencing of DCP1 and DCP2 inhibited the infection of WSSV, a DNA virus of shrimp, suggesting that the DCP1-DCP2 complex facilitated DNA virus infection. Due to the suppressive role of the DCP1-DCP2 complex in shrimp RNAi against WSSV infection, the DCP1-DCP2 complex could promote WSSV infection in shrimp. The results showed that WSSV-miR-N46 and shrimp miR-87 could respectively suppress the expressions of DCP1 and DCP2 to affect virus infection. Therefore, our study contributed novel aspects of the DCP1-DCP2 complex and its regulation by miRNAs in virus-host interactions.

1. Introduction

Classical virus infection in the host cells is initiated by the interactions between viral capsid or envelope proteins and host cell surface receptors. The internalization of virions is either through the fusion of the viral capsid or envelope with the host plasma membrane, or through the endocytosis pathway, causing the virions to escape from the endosomes or other small vesicles and enter the cytoplasm of host cell [1]. The receptors on the surface of host cells can directly trigger the conformational changes of viral surface structure or activate some specific signaling pathways that facilitate the entry of viruses [1]. After entering the host cells, the replication of viral genomes, the synthesis of viral proteins, the assembly of viral particles, and the release of viruses from host cells depend largely on host mechanisms [1,2]. It is reported that the stability of viral mRNA is regulated by the DCP1-DCP2 complex located in the P-body (the processing bodies) [3,4]. The DCP1-DCP2 complex can trigger mRNA decapping. The DCP2-catalyzed dissection releases m7G and a single 5′ phosphorylated mRNA, which is considered to be an irreversible process. The decapped mRNAs are degraded by 5′ to 3' exonuclease Xrn1 [5]. The DCP2 protein contains N-terminal Nudix/Mut T motifs, which are usually present in pyrophosphatases and are essential for the decapping of mRNAs [4,6]. Except for the DCP2-DCP1 complex, Pat1 (decapping activator and translation repressor) [7–10], Dhh1 (decapping activator and translation repressor) [10–13] and the Lsm1-7 complex (decapping activator) [10,12,14] are involved in the decapping of mRNAs. At present, the decapping of retrovirus RNAs by the DCP2-DCP1 complex has been well characterized [3,4]. However, the role of DCP1-DCP2 complex in DNA virus infection remains unclear.

Although the DCP1-DCP2 complex affects the mRNA stability, the regulation of DCP1-DCP2 complex mediated by microRNAs (miRNAs) has not been extensively explored. In many eukaryotic cellular

* Corresponding author.
E-mail address: zxb0812@zju.edu.cn (X. Zhang).

https://doi.org/10.1016/j.fsi.2019.05.058
Received 22 February 2019; Received in revised form 23 May 2019; Accepted 25 May 2019
Available online 27 May 2019

processes, miRNAs play essential roles, such as virus-host interaction, development, apoptosis, immune response, tumorigenesis and homeostasis [15–17]. The RNA-induced silencing complex (RISC) is formed after the loading of the guiding strand of miRNA onto Argonaute (Ago) protein [18–20]. The target mRNA is bound to the miRNA and then it is cleaved by the Ago protein in the RISC [18]. Recently, it is reported that the phosphorylation and dephosphorylation of Ago2 protein in human being have a significant impact on the role of miRNA in RISC [15]. As reported, the gene expressions can be regulated by host and/or virus miRNAs in the virus-host interactions [20–31]. In shrimp, the host miRNA expression profiles are altered by the infection of white spot syndrome virus (WSSV), a virus with a double-stranded DNA genome [25,27,30,31]. Shrimp miR-7 can target the WSSV early gene wsv477, leading to the inhibition of virus infection [17], while a viral miRNA can target the shrimp caspase 8 gene to suppress the host antiviral apoptosis [25]. It has been reported that virus-originated miRNAs promote viral latency during virus infection through the miRNA editing [32]. At present, however, the impact of miRNA-mediated regulation of the DCP1-DCP2 complex on virus infection remains to be investigated.

To address the influence of DCP1-DCP2 complex on DNA virus infection and the role of the miRNA-regulated DCP1-DCP2 complex in virus infection, the shrimp DCP1-DCP2 complex and the shrimp and WSSV miRNAs targeting the DCP1-DCP2 complex were characterized in this study. The results indicated that the viral WSSV-miR-N46 (a viral miRNA) and shrimp miR-87 could suppress virus infection by targeting *DCP1* and *DCP2* genes, respectively.

2. Materials and methods

2.1. Shrimp culture and WSSV challenge

Shrimp (*Marsupenaeus japonicus*), 10–12 cm in length, were cultured in groups of 20 individuals in the tank filled with seawater at 25 °C as described before [23]. To ensure that shrimp were virus-free before experiments, PCR was performed using WSSV-specific primers (5′-TATTGTCTCTCCTGACGTAC-3′ and 5′-CACATTCT TCACGAGTC TAC-3′) to detect WSSV in shrimp [23]. The virus-free shrimp were infected with WSSV (10^5 copies/ml) by injection at 100 μl/shrimp into the lateral area of the fourth abdominal segment of shrimp [23]. At different time postinfection, three shrimp were randomly collected for each treatment. The shrimp hemocytes were collected for later use.

2.2. Analysis of WSSV copies with quantitative real-time PCR

The genomic DNA of WSSV was extracted with a SQ tissue DNA kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instruction. The extracted DNA was analyzed by quantitative real-time PCR with WSSV-specific primers and WSSV-specific TaqMan probe (5′-FAM-TGCTGCCGTCTCCAA- TAMRA-3′) as described previously [23]. The PCR procedure was 95 °C for 1 min, followed by 40 cycles of 95 °C for 30 s, 52 °C of 30 s, and 72 °C for 30 s [23].

2.3. Detection of mRNA or miRNA by Northern blotting

Total RNAs were extracted from shrimp hemocytes with mirVana miRNA isolation kit (Ambion, USA). After separation on a denaturing 15% polyacrylamide gel containing 7 M urea, the RNAs were transferred to a Hybond-N + nylon membrane, followed by ultraviolet cross-linking [23]. The membrane was prehybridized in DIG (digoxigenin) Easy Hyb granule buffer (Roche, Basel, Switzerland) for 0.5 h at 42 °C and then hybridized with DIG-labeled miR-87 (5′-GA GGGGAAAAGCC ATACGCTTA-3′), WSSV-miR-N46 (5′-AGUGCCAAGAUAAC GGUUG AAG-3′), U6 (5′-GGGCCATGCTAATCTTCTCTGTATCGTT-3′), wsv477 (5′-CGATTTCGGCAGGCCAGTTGTCAGA-3′), DCP2 (5′-CCAGAAACCCT GAA CTAAGAGAA-3′) or β-actin (5′-CTCGCTCGGCGGTGGTCGTGA AGG-3′) probe at 42 °C overnight [23]. Subsequently the detection was performed with the DIG HighPrime DNA labeling and detection starter kit II (Roche).

2.4. Silencing or overexpression of miR-87 or WSSV-miR-N46 in shrimp

To knock down miR-87 or WSSV-miR-N46, an anti-miRNA oligonucleotide (AMO) was injected into WSSV-infected shrimp. AMO-miR-87 (5′-TGTACGTTTC TGGAGC-3′) and AMO-WSSV-miR-N46 (5′-CTTC AACCGTTATCTTGGCACT -3′) were synthesized (Sangon Biotech, Shanghai, China) with a phosphorothioate backbone and a 2′-O-methyl modification at the 12th nucleotide. AMO (10 nM) and WSSV (10^5 copies/ml) were co-injected into virus-free shrimp at a 100 μl/shrimp. At 16 h after the co-injection, AMO (10 nM) was injected into the same shrimp. As controls, AMO-miR-87-scrambled (5′-TTGCATGTCTGTC GAG-3′), AMO-WSSV- miR-N46-scrambled (5′-TTGCATGTCTGTC GAG-3′), WSSV alone (10^5 copies/ml) and phosphate buffered saline (PBS) were included in the injections. To overexpress miR-87 or WSSV-miR-N46, the synthesized miR-87 (5′-TAAGCGTATGGCTTTT CCCCTC-3′) (10 nM) or WSSV-miR-N46 (5′-AGTGCCAAGATAACGGTTGAAG -3′) and WSSV (10^5 copies/ml) were co-injected into shrimp. As controls, miR-87-scrambled (5′-TATCGCATAGGCTTTTCCCCTC-3′), WSSV-miR-N46- scrambled (5′-ATTTGACAGATGCCTAGTACCAG-3′), WSSV alone (10^5 copies/ml) and PBS were used. The miRNAs were synthesized by Sangon Biotech (Shanghai, China).

At different time after treatment with AMO or miRNA, three shrimp were collected at random for each treatment. The shrimp hemocytes were collected for later use. At the same time, the cumulative mortality of shrimp was examined daily. All the experiments were biologically repeated three times.

2.5. Prediction of miRNA target genes

To predict the target genes of a miRNA, four independent computational algorithms including TargetScan 5.1 (http://www.targetscan.org), miRanda (www.microrna.org/), Pictar (http://www.pictar.mdc-berlin.de/) and miRlnspector (http://www.Imbb.Forth.gr/microinspector) were used [23]. The overlapped genes predicted by the four algorithms were the potential targets of the miRNA.

2.6. Cell culture, transfection and fluorescence assays

Insect High Five cells (Invitrogen, USA) were cultured with Express Five serum-free medium (Invitrogen) containing L-glutamine (Invitrogen) at 27 °C as previously described [23]. To determine the dosage of a synthesized miRNA, 10, 50, 100, 200, 500 or 1000 pM of miRNA was transfected into cells [23]. Then the miRNA expression in the cells was detected with quantitative real-time PCR. It was indicated that the transfection of miRNA at 100 pM or more could overexpress miRNA in cells. The insect cells were co-transfected with EGFP, EGFP-DCP2- 3′UTR, EGFP-ΔDCP2-3′UTR, EGFP-DCP1-3′UTR or EGFP-ΔDCP1-3′UTR and miRNA (miR-87 or WSSV-miR-N46). At 48 h after co-transfection, the fluorescence intensity of cells was evaluated with a Flex Station II microplate reader (Molecular Devices, USA) at 490/510 nm excitation/emission (Ex/Em) [23]. The experiments were biologically repeated three times.

2.7. Western blot analysis

Shrimp tissues were homogenized with a lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, pH7.8) and then centrifuged at 10,000 × *g* for 10 min at 4 °C. The proteins were separated by 12.5% SDS-polyacrylamide gel electrophoresis and then transferred onto a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk in TBST (10 mM Tris-HCl, 150 mM NaCl, 20% Tween 20, pH7.5) for 2 h at room temperature, followed by incubation overnight with a

primary antibody. The antibodies were prepared in our laboratory. After washes with TBST, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody (Bio-Rad, USA) for 2 h at room temperature. Subsequently the membrane was detected using a Western Lightning Plus-ECL kit (PerkinElmer, USA).

2.8. RNAi (RNA interference) assay in shrimp

To silence gene expression in shrimp, RNAi assay was conducted. The small interfering RNA (siRNA) specifically targeting the 3′ UTR of *DCP1* or *DCP2* gene was designed, generating DCP1-siRNA (5′-AAUC GCAGUUGCUAUGCGUUGGA CG-3′) or DCP2-siRNA (5′-GCGGAAGAC CGUGCCCGUAAUAUAA-3′). As a control, the sequence of DCP1-siRNA or DCP2-siRNA was randomly scrambled (DCP1-siRNA-scrambled, 5′-GACAUUAAGAUAUAUAUGG-3’; DCP2-siRNA-scrambled, 5′-CGCCU UCUGGCACGGGCAUUAUAUU-3′). All the siRNAs were synthesized with the in vitro transcription T7 kit (TaKaRa, Japan) according to the manufacturer's instructions. The synthesized siRNAs were quantified by spectrophotometry. The shrimp were co-injected with WSSV (10^4 copies/shrimp) and siRNA (4 nM). PBS and WSSV alone (10^4copies/shrimp) were included in the injections as controls [23]. At 0, 24, 36 and 48 h after infection, the hemocytes of three shrimp, randomly selected from each treatment, were collected for later use. At the same time, the cumulative mortality of shrimp was examined daily. All the experiments were biologically repeated three times.

2.9. Co-immunoprecipitation

Shrimp hemocytes were lysed with ice-cold cell lysis buffer (Beyotime). Then the lysate was incubated with Protein G-agarose beads (Invitrogen, Carlsbad, CA, USA) for 2 h at room temperature, followed by incubation with the DCP2-specific antibody overnight at 4 °C. After washes three times with ice-cold lysis buffer, the immunocomplex was subjected to SDS-PAGE with Coomassie blue staining. The proteins were identified with mass spectrometry using a Reflex IV MALDI-TOF mass spectrometer (Bruker Daltonik, Bremen, USA). The spectra were processed by the Xmass software (Bruker Daltonik, Bremen) and the peak lists of the mass spectra were used for peptide mass fingerprint analyses with the Mascot software (Matrix Science).

2.10. Cloning of full-length cDNAs of shrimp DCP1 and DCP2 genes

The full-length *DCP1* and *DCP2* cDNAs were obtained by rapid amplification of cDNA ends (RACE) using a 5’/3′ RACE kit (Roche, Indianapolis, IN, USA). RACEs were conducted according to the manufacturer's instructions using DCP1-specific primers (5′RACE, 5′-CCTG GGACACTTGAAG-3′ and 5′-GGGTAAACCAGTGCC -3’; 3′ RACE, 5′-GCCCCACAGTCCCACCCACCT-3′ and 5′-CCCAGGAGGAGC ACCAAT CTCA-3′) or DCP2-specific primers (5′ RACE, 5′-GGGAACCATTTCA GTTGCT-3′ and 5′-GCCAGAAACCCTGAACTAAG-3’; 3′ RACE, 5′-ATTG GAG AGCAGTTTGTGAGAC-3′ and 5′-TTTACATCATCCCAGGCG-3′). PCR products were cloned into pMD-19 vector (Takara, Japan) and sequenced.

2.11. Interactions between DCP1 and DCP2 domains

To explore the interaction between DCP1 and DCP2 proteins, the full-length and domain deletion mutants of DCP1 and DCP2 were cloned into pIZ/EGFP V5-FLAG and pIZ/EGFP V5-His (Invitrogen, USA), respectively. The full-length and deletion mutants of DCP1 and DCP2 were amplified by PCR with sequence-specific primers (full-length DCP1, 5′-GGAAGATCTATGCGCTAAGGTTTTATTTGGAAAAA -3′ and 5′-CCGCTCGAGTGACTTATCGTCGTCATCCTTGTAATCCAAACAAC CTTTGATAGAGAGAT-3’; DCP1 EVH1 domain, 5′-GGAAGATCTATGC GCTA AGGTTTTATTTGGAAAAA-3′ and 5′-CCGCTCGAGTGACTTATCG TCGTCAT CCTTGTAATCTATGTCCCCTCCAGGTGCCCCA-3’; DCP1 C-terminal extension region, 5′-GGAAGATCTGAATGACAAATCAAG TGA-3′ and 5′-CCGCTCGAGT GACTTAT CGTCGTCATCCTTGTAATCC AAACAACCTTTGATAGAGAGAT-3’; full-length DCP2, 5′-GGAAGATCT ATGGCCCCACCAACAGGTGGAAAA-3′ and 5′-TCCCCGCGGTTAATGGT GATGGTGATGATGCCAAGACAGCATCACATCGGCCC-3’; DCP2 N-regulatory domain, 5′-CGCGGATCCGATGAAGAACCACA TTGTTGTGCC-3′ and 5′-TCCCCGCGGTTAATGGTGATGGTGATGATGCCAA GACAGCATC ACATCGGCCC-3’; DCP2 C-terminal divergent region, 5′-CGCGG ATCC GATGAAGAACCACATTGTTGTGCC-3′ and 5′-TCCCCGCGGTTAATGG TGATGGTGATGATGCTGGCGGTCAGAGGTACTGGTG-3’; DCP2 Nudix domain, 5′-CGCGGATCCATGGCCCCACCAACAGGTGGAAAA-3′ and 5′-TCCC CGCGGCACATTAATTTTCCCTTTTGG-3′, and 5′-TCCCCGCGGAT GGCCCCA CCAACAGGTGGAAAA-3′ and 5′-TCCCCGCGGTTAATGGTG ATGGTGATGA TGCCAAGACAGCATCACATCGGCCC-3′).

The constructs were co-transfected into insect High Five cells at 70% confluence using Cellfectin transfection reagent (Invitrogen, USA) according to the manufacturer's protocol. The cells were cultured at 27 °C in Express Five serum-free medium (Invitrogen) supplemented with L-glutamine (Invitrogen). At 48 h after co-transfection, the cells were subjected to immunoprecipitation assays with anti-His or anti-FLAG antibody, followed by Western blot analysis.

2.12. Statistical analysis

All the numerical data presented were analyzed by one-way analysis of variance (ANOVA) to calculate the means and standard deviations of triplicate assays.

3. Results

3.1. Role of shrimp DCP2 in virus infection

To evaluate the role of shrimp DCP2 in DNA virus infection, the expression level of DCP2 was examined in shrimp in response to WSSV challenge. The results indicated that DCP2 was significantly upregulated in virus-challenged shrimp (Fig. 1A), suggesting that DCP2 was involved in virus infection.

To explore the influence of DCP2 on virus infection, the DCP2 expression was knocked down by sequence-specific DCP2-siRNA in shrimp (Fig. 1B). The results revealed that the DCP2 silencing resulted in significant decreases of WSSV copies compared with the controls (Fig. 1C), showing that DCP2 played a positive role in WSSV infection.

3.2. Proteins interacted with DCP2

To elucidate the mechanism of DCP2 involving in virus infection in shrimp, the proteins interacted with DCP2 were characterized. The results of co-immunoprecipitation (Co-IP) assays using shrimp DCP2-specific antibody indicated that two proteins were obtained compared with the control (Fig. 2A). Mass spectrometry identification revealed that the two proteins were DCP1 and DCP2 (Fig. 2A). These data showed that DCP2 was interacted with DCP1 in shrimp. To confirm the interaction between DCP1 and DCP2 proteins, the plasmids respectively expressing DCP1 and DCP2 were co-transfected into insect cells, followed by Co-IP using DCP2-specific antibody. Western blots revealed that the DCP1 protein was directly interacted with the DCP2 protein (Fig. 2B).

To identify which domains of DCP1 and DCP2 were interacted, the deletion mutants of DCP1 EVH1 domain (ΔEVH1, FLAG-tagged), DCP1 C-terminal region (ΔCR, FLAG-tagged), DCP2 N-terminal regulatory domain (ΔNRD, His-tagged), DCP2 Nudix domain (ΔND, His-tagged) and DCP2 C-terminal divergent region (ΔCDR, His-tagged) were constructed, respectively (Fig. 2C). The deletion constructs and the full-length DCP1 (FLAG-tagged) or DCP2 (His-tagged) were co-transfected into insect cells. The results showed that when insect cells were co-transfected with DCP2 ΔNRD and full-length DCP1, the DCP1 protein

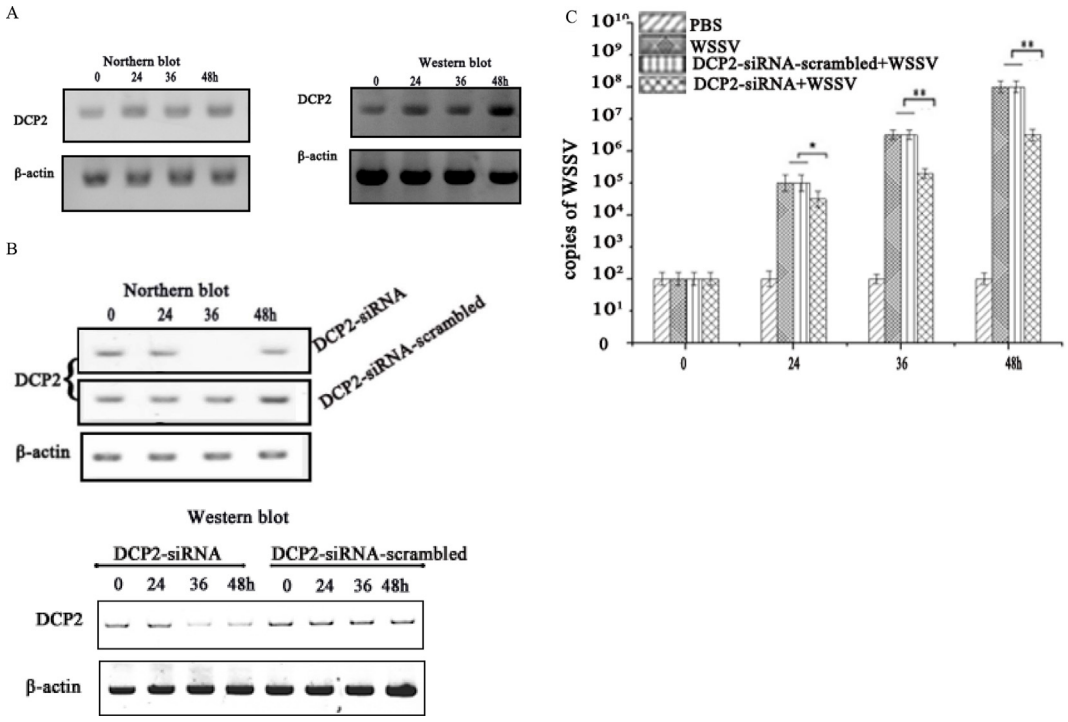

Fig. 1. Role of shrimp DCP2 in virus infection. (A) Expression level of DCP2 in shrimp in response to virus infection. Shrimp were challenged with WSSV. At different time post-infection, the expression level of DCP2 in shrimp hemocytes was examined by Northern blotting or Western blotting. Shrimp β-actin was used as a control. Numbers indicated the time post-infection. Probes or antibodies used were shown on the left. (B) Knockdown of DCP2 by siRNA in shrimp. Shrimp were injected with DCP2-siRNA to silence DCP2 expression. As a control, DCP2-siRNA-scrambled was included in the injection. At different time after injection, the *DCP2* mRNA and protein levels were examined by Northern blot and Western blot, respectively. Shrimp β-actin was used as a control. The probes or antibodies were indicated on the left. (C) Influence of DCP2 silencing on virus infection in shrimp. Shrimp were co-injected with DCP2-siRNA and WSSV. At different time post-infection, the WSSV copies were examined with quantitative real-time PCR (*, $p < 0.05$; **, $p < 0.01$).

was not detected in the immunoprecipitated product using His antibody (Fig. 2D), showing that DCP1 was interacted with DCP2 N-terminal regulatory domain. When DCP2 and DCP1 ΔEVH1 were co-transfected into cells, the DCP2 protein did not exist in the immunoprecipitated complex (Fig. 2E), indicating that DCP2 was interacted with DCP1 by binding to its EVH1 domain.

The above findings indicated that the EVH1 domain of DCP1 was interacted with the N-terminal regulatory domain of DCP2.

3.3. Impact of shrimp DCP1 on virus infection

To explore the influence of DCP1 on virus infection of shrimp, the expression profile of DCP1 was examined in hemocytes of WSSV-infected shrimp. The data of Northern blots and Western blots indicated that the DCP1 expression was significantly upregulated in shrimp in response to WSSV infection, suggesting the involvement of DCP1 in virus infection (Fig. 3A).

In an attempt to assess the role of DCP1 in virus infection, the DCP1 expression was knocked down by sequence-specific siRNA (DCP1-siRNA) in WSSV-infected shrimp, followed by the evaluation of virus infection. The results revealed that the expression of DCP1 was silenced by DCP1-siRNA (Fig. 3B). The DCP1 silencing led to significant decreases of WSSV copies compared with the controls (WSSV and WSSV + DCP1-siRNA-scrambled) (Fig. 3C). These findings indicated that DCP1 played a positive role in virus infection.

3.4. Effects of the interaction between shrimp miR-87 and DCP2 on virus infection

To reveal the miRNAs targeting shrimp *DCP2* gene, the miRNAs targeting *DCP2* were predicted. The prediction data showed that the shrimp *DCP2* gene was a potential target of shrimp miR-87 (Fig. 4A). To evaluate the interaction between miR-87 and *DCP2* gene, the plasmid pIZ/EGFP-DCP2-3′UTR containing the EGFP and the DCP2 3′UTR was co-transfected with miR-87 into insect cells. The results indicated that the fluorescence intensity of the cells co-transfected with miR-87 and pIZ/EGFP-DCP2-3′UTR was significantly decreased compared with the controls (Fig. 4B). However, the fluorescence intensity of the cells co-transfected with miR-87 and EGFP-ΔDCP2-3′UTR was similar to those of the controls (Fig. 4B). These findings revealed that miR-87 was directly interacted with *DCP2* gene. In order to examine the interaction between miR-87 and *DCP2* gene *in vivo*, miR-87 was overexpressed in shrimp, followed by the analysis of *DCP2* gene expression. It was revealed that the miR-87 overexpression led to a significant decrease of DCP2 expression at transcript and protein levels compared with the controls (Fig. 4C). However, the miR-87 overexpression had no effect on the DCP1 expression (Fig. 4D). These data indicated that miR-87 was interacted with *DCP2* gene *in vivo*.

To explore the role of shrimp miR-87 in virus infection of shrimp, the expression level of miR-87 was examined in hemocytes of WSSV-infected shrimp. Northern blots indicated that miR-87 was significantly downregulated in shrimp in response to WSSV infection, suggesting that miR-87 was involved in virus infection of shrimp (Fig. 4E).

To assess the influence of miR-87 on virus infection, the miR-87 expression was silenced or overexpressed in the WSSV-infected shrimp, followed by the evaluation of virus infection. The results showed that the expression of miR-87 was knocked down by AMO-miR-87 compared with the controls (Fig. 4F). The miR-87 silencing led to significant increases of WSSV copies and the virus-infected shrimp mortality compared with the controls (Fig. 4G and H). On the other hand, when miR-87 was overexpressed (Fig. 4I), the WSSV copies and the virus-infected shrimp mortality were significantly decreased compared with the controls (Fig. 4H and J).

Taken the above data together, these findings presented that miR-87

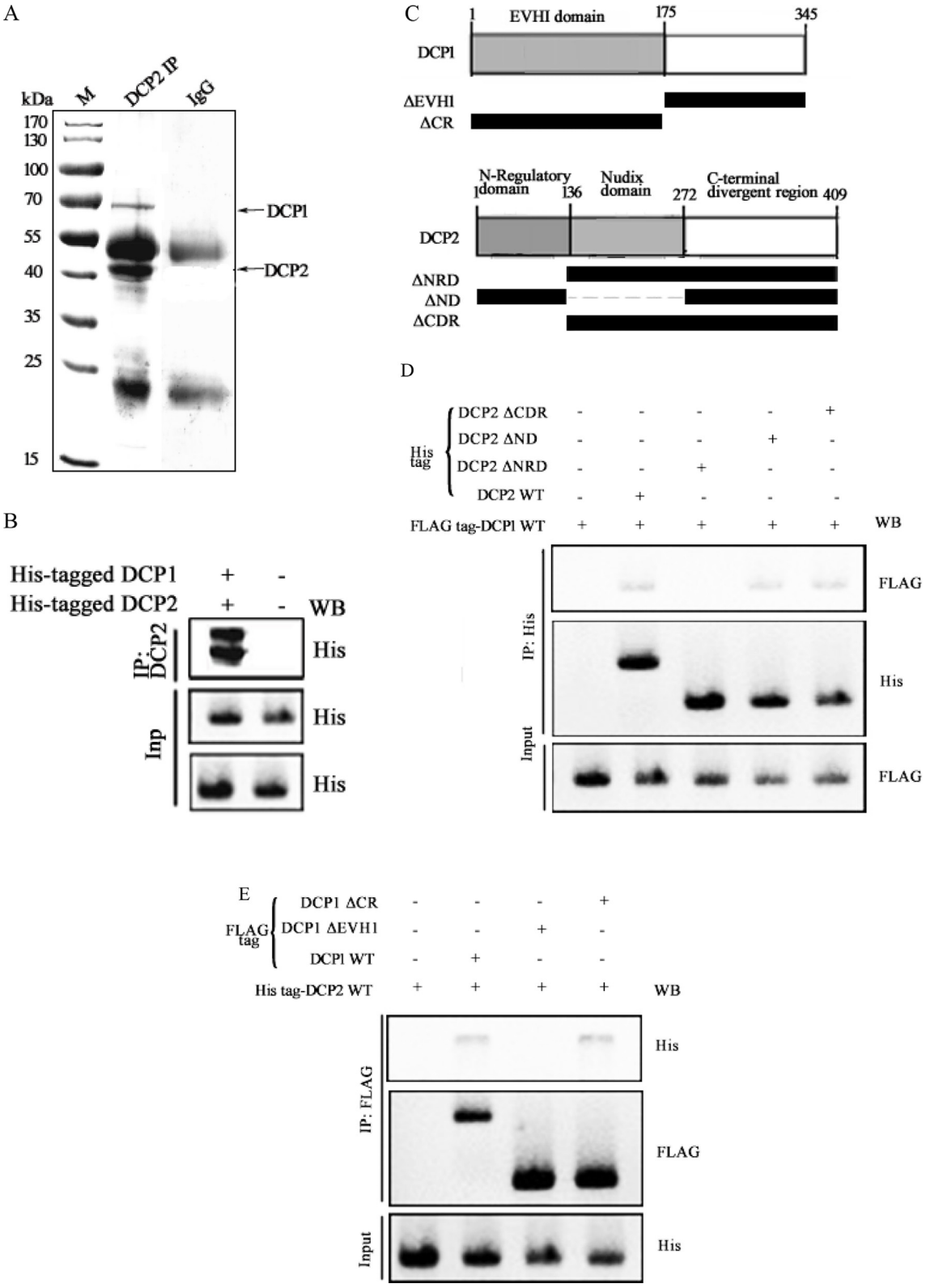

Fig. 2. Proteins interacted with DCP2. (A) The proteins bound to DCP2. Co-IP using the DCP2-specific antibody was conducted. The eluted proteins were subjected to SDS-PAGE, followed by protein identification using mass spectrometry. (B) The interaction between DCP1 and DCP2 proteins. The His-tagged DCP1 and DCP2 were co-transfected into insect cells. At 48 h after co-transfection, Co-IP was conducted using DCP2-specific antibody, followed by Western blot analysis with anti-His IgG. (C) The constructs of deletion mutants of DCP1 and DCP2 domains. (D) and (E) The interactions between DCP1 and DCP2 domains. The full-length and/or deletion mutants of DCP1 and DCP2 were co-transfected into insect cells. At 48 h after transfection, the target proteins were immunoprecipitated with anti-His (D) or anti-FLAG IgG (E), followed by Western blot analysis.

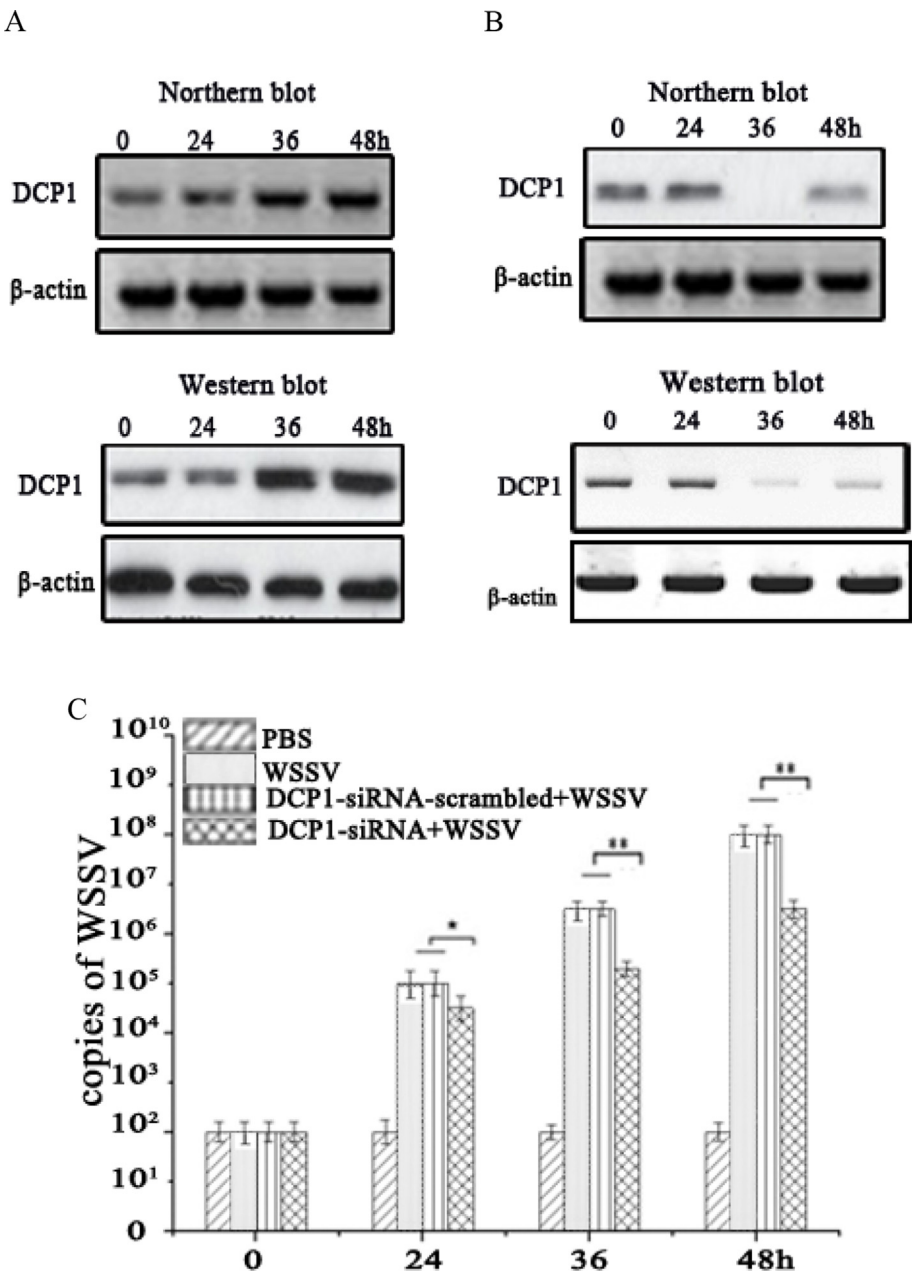

Fig. 3. Impact of shrimp DCP1 on virus infection. (A) DCP1 expression profile in shrimp in response to virus infection. Shrimp were challenged with WSSV. At different time post-infection, the expression of DCP1 was examined in shrimp hemocytes by Northern blotting or Western blotting. Shrimp β-actin was used as a control. The numbers indicated the time post-infection. Probes or antibodies used were shown on the left. (B) Silencing of DCP1 in shrimp. Shrimp were injected with DCP1-siRNA, followed by the detection of DCP1 with Northern blot or Western blot. The probes or antibodies were indicated on the left. (C) Influence of DCP1 silencing on virus infection. WSSV and DCP1-siRNA or DCP1-siRNA-scrambled were co-injected into shrimp. WSSV alone and PBS were used as controls. At different time after injection, the WSSV copies in shrimp were examined with quantitative real-time PCR (*, $p < 0.05$; **, $p < 0.01$).

could inhibit virus infection in shrimp by targeting shrimp *DCP2* gene.

3.5. Influence of viral WSSV-miR-N46 targeting DCP1 on virus infection

To characterize the miRNAs targeting *DCP1*, the miRNAs targeting *DCP1* gene were predicted. The miRNA target prediction showed that the *DCP1* gene might be the target of WSSV-miR-N46, a viral miRNA encoded by WSSV (Fig. 5A). To validate the target prediction, the synthesized viral WSSV-miR-N46 and the plasmid EGFP-DCP1-3′ UTR were co-transfected into insect cells. The results indicated that the fluorescence intensity of the cells co-transfected with WSSV-miR-N46 and EGFP-DCP1-3' UTR was significantly decreased compared with those of the controls (Fig. 5B), showing that WSSV-miR-N46 was directly interacted with *DCP1* gene. However, the WSSV-miR-N46 overexpression in WSSV-infected shrimp had no effect on the DCP2 expression (Fig. 5C).

In an attempt to reveal the role of WSSV-miR-N46 in virus infection, the expression of WSSV-miR-N46 in WSSV-challenged shrimp was examined. Northern blotting results indicated that WSSV-miR-N46 was detected at 48 h after virus infection in shrimp (Fig. 5D). Therefore, WSSV-miR-N46 was overexpressed in shrimp (Fig. 5E), followed by the evaluation of virus copy. The results revealed that the WSSV-miR-N46 overexpression significantly decreased the number of WSSV copies in shrimp (Fig. 5F), indicating that WSSV-miR-N46 played a negative role in WSSV replication.

Taken together, the findings revealed that the viral miRNA (WSSV-miR-N46) and host miRNA (miR-87) suppressed virus infection by targeting *DCP1* and *DCP2* of the DCP1-DCP2 complex, respectively (Fig. 5G).

4. Discussion

As reported, the DCP1-DCP2 complex, localized in processing bodies (P bodies), can regulate the animal immune defense against RNA virus invasion by two strategies, that is the decapping of retrovirus RNAs and the suppression of RNAi pathway [32–36]. During the process of

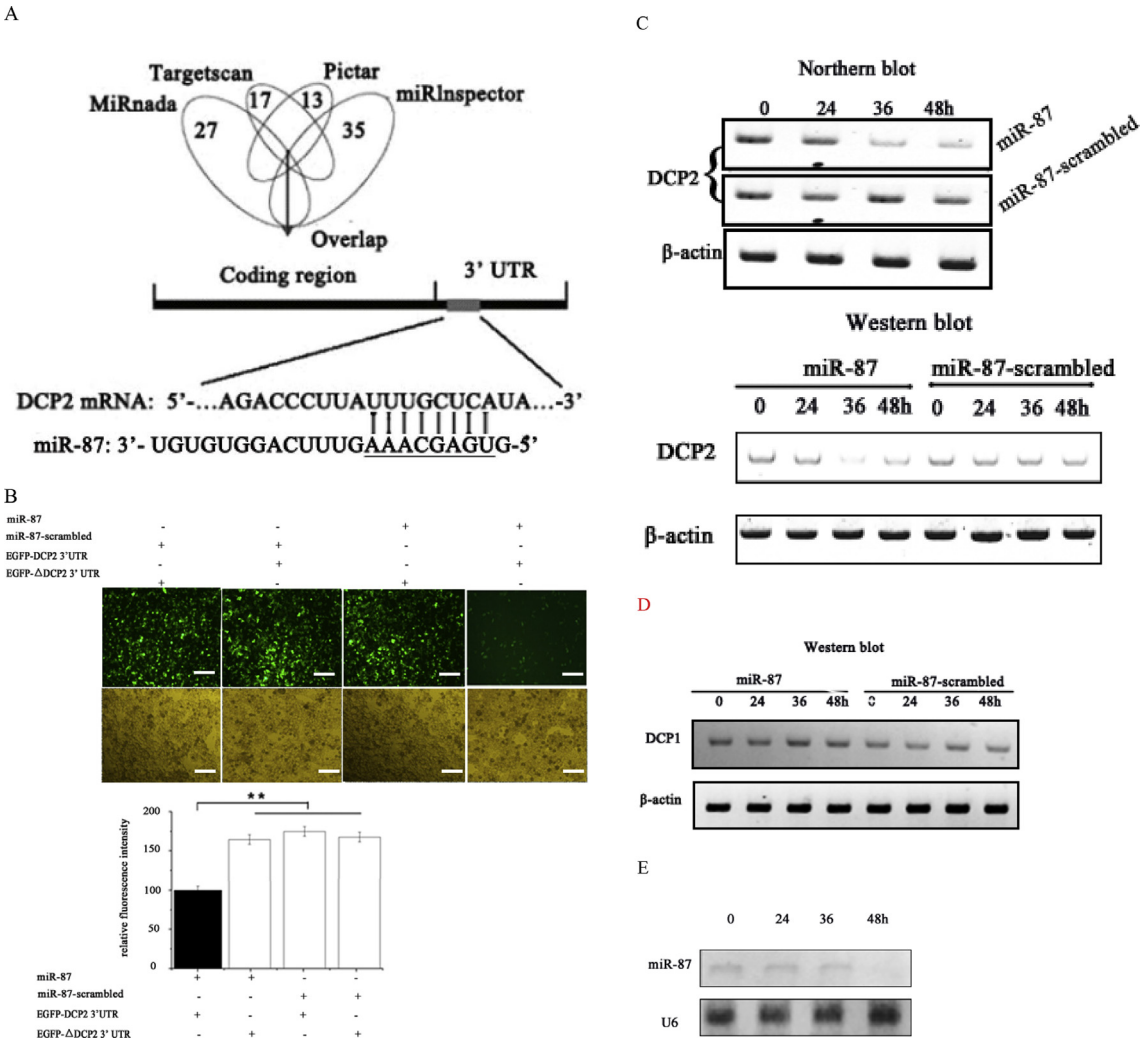

Fig. 4. Effects of the interaction between shrimp miR-87 and *DCP2* on virus infection. (A) Prediction of miRNAs targeting *DCP2*. According to the prediction, the 3′ UTR of *DCP2* gene could be targeted by miR-87. The seed sequence of miR-87 was underlined. (B) Direct interaction between miR-87 and DCP2 3′ UTR. The insect High Five cells were co-transfected with miR-87 and the plasmid EGFP-DCP2-3′UTR or EGFP-ΔDCP2-3′UTR. At 36 h after co-transfection, the fluorescence intensity of insect cells was evaluated. Scale bar, 50 μm. (C) Interaction between miR-87 and DCP2 *in vivo*. MiR-87 was overexpressed in shrimp. At different time after miR-87 overexpression, the *DCP2* mRNA and protein levels were examined by Northern blot and Western blot, respectively. As a control, miR-87-scrambled was included in the assays. Data were representatives of three independent experiments. The probes or antibodies were indicated on the left. (D) Impact of miR-87 overexpression on the DCP1 expression in shrimp. The DCP1 protein level in miR-87-overexpressed WSSV-infected shrimp was examined by Western blot. The antibodies were indicated on the left. The numbers showed the time after WSSV infection. (E) Expression level of miR-87 in virus-infected shrimp. Shrimp were challenged with WSSV. At different time post-infection, miR-87 was detected in hemocytes of virus-infected shrimp by Northern blotting. U6 was used as a control. Probes were indicated on the left. (F) Silencing of miR-87 expression in shrimp. Shrimp were co-injected with AMO-miR-87 and WSSV. As a control, AMO-miR-87-scrambled was included in the injection. At different time post-infection, miR-87 was detected by Northern blot. The probes used were indicated on the left. The numbers showed the time points post-infection. U6 was used as a control. (G) Influence of miR-87 silencing on virus copies. WSSV and AMO-miR-87 or AMO-miR-87-scrambled were co-injected into shrimp. WSSV and PBS were used as controls. At different time after injection, the WSSV copies in shrimp were examined with quantitative real-time PCR. (H) Effects of miR-87 silencing or overexpression on WSSV-infected shrimp mortality. (I) Overexpression of miR-87 in shrimp. Shrimp were co-injected with miR-87 or miR-87-scrambled and WSSV. At different time after injection, the shrimp were subjected to Northern blot with probes indicated on the left. PBS and WSSV were used as controls. (J) Impact of miR-87 overexpression on WSSV copies. Shrimp were simultaneously injected with miR-87 and WSSV. As a control, miR-87-scrambled was included in the injection. At different time post-infection, the virus copies were examined with quantitative real-time PCR. In all panels, the significant differences between treatments were indicated (*, $p < 0.05$; **, $p < 0.01$).

retrovirus infection, the canonical mRNA decapping enzyme DCP2, along with its activator DCP1, can restrict the infection of retrovirus at the level of mRNA transcription [34,35]. The host DCP1-DCP2 complex directly decapps the mRNAs of retroviruses or cellular mRNAs targeted by bunyaviruses for cap-snatching, thus creating a bottleneck for retrovirus replication [33,35]. In the infection process of Sindbis virus or Venezuelan equine encephalitis virus, the host can inhibit the infection of retrovirus through the DCP1-DCP2-mediated 5′-3′ decay pathway. During the bunyavirus infection in insects and mammals, the bunyaviruses cap their mRNAs at the 5′ ends by the "cap-snatching" machinery in the P bodies [35]. The virus-encoded nucleocapsid N protein recognize 5′ caps and 10–18 nucleotides (nt) downstream 5′ caps of cellular mRNAs and then the viral RNA-dependent RNA polymerase cleaves the mRNAs at the same position. Subsequently the cleaved 5′ caps are used for the synthesis of viral mRNAs [35]. It is reported that the silencing of DCP2 and/or DCP1 promotes RNAi in animals during RNA virus infection, showing that the DCP2-DCP1 complex takes a negative effect on the animal RNAi against RNA virus invasion [35,36]. RNAi, an important component of innate immune responses, mediated by siRNAs or miRNAs, plays crucial roles in the

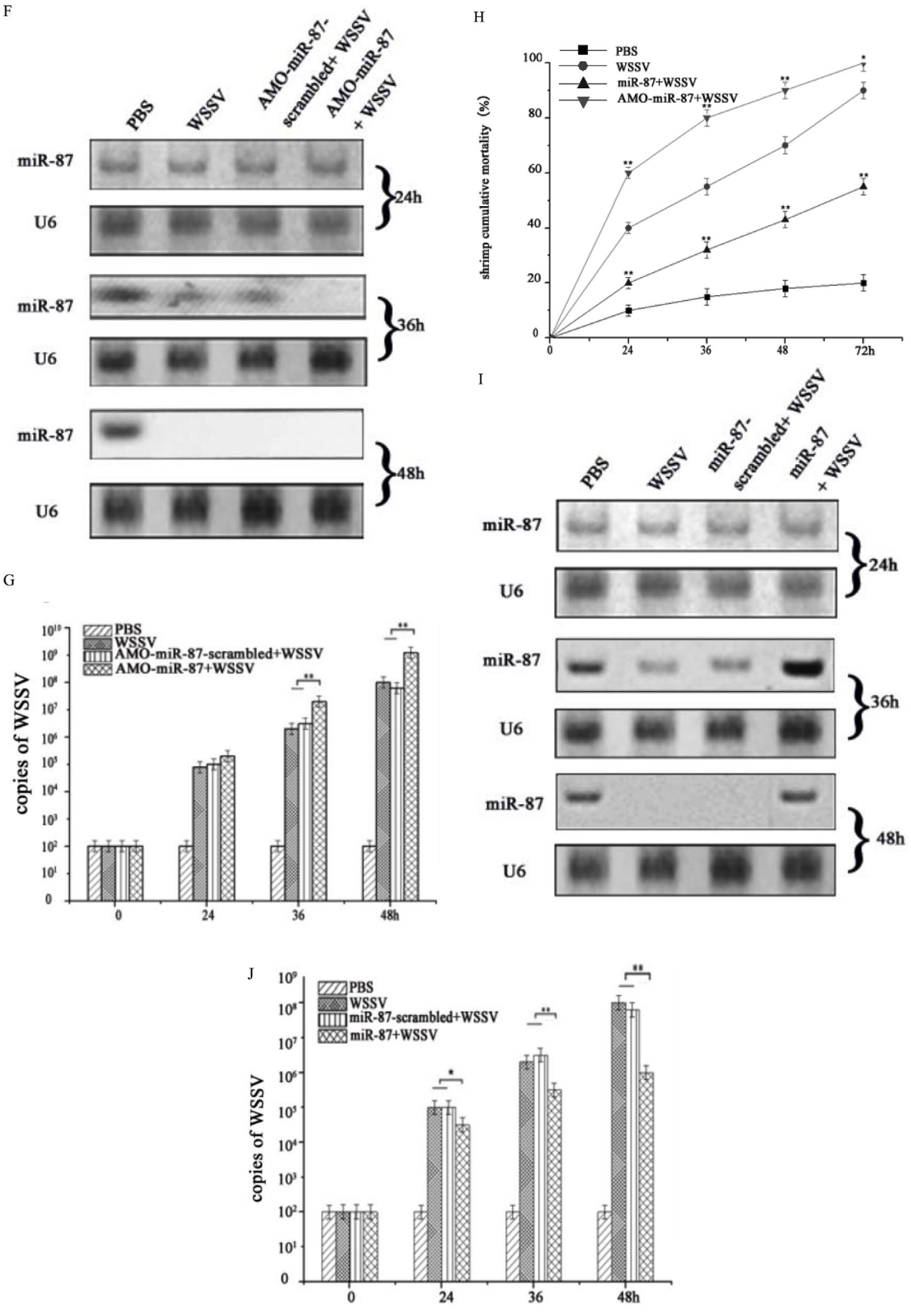

Fig. 4. (*continued*)

antiviral immunity of invertebrates and plants that rely solely on innate mechanisms to combat viral infection [30,34,37]. Up to date, however, little is known about the role of the DCP1-DCP2 complex in DNA virus infection. In the present study, the findings indicated that the silencing of shrimp DCP1 or DCP2 of the DCP1-DCP2 complex inhibited the infection of WSSV, a DNA virus of shrimp, suggesting that the DCP1-DCP2 complex facilitated DNA virus infection. Due to the suppressive role of the DCP1-DCP2 complex in animal RNAi pathway against virus infection [35,36], the DCP1-DCP2 complex could promote WSSV infection in shrimp. In this context, our study contributed a novel aspect

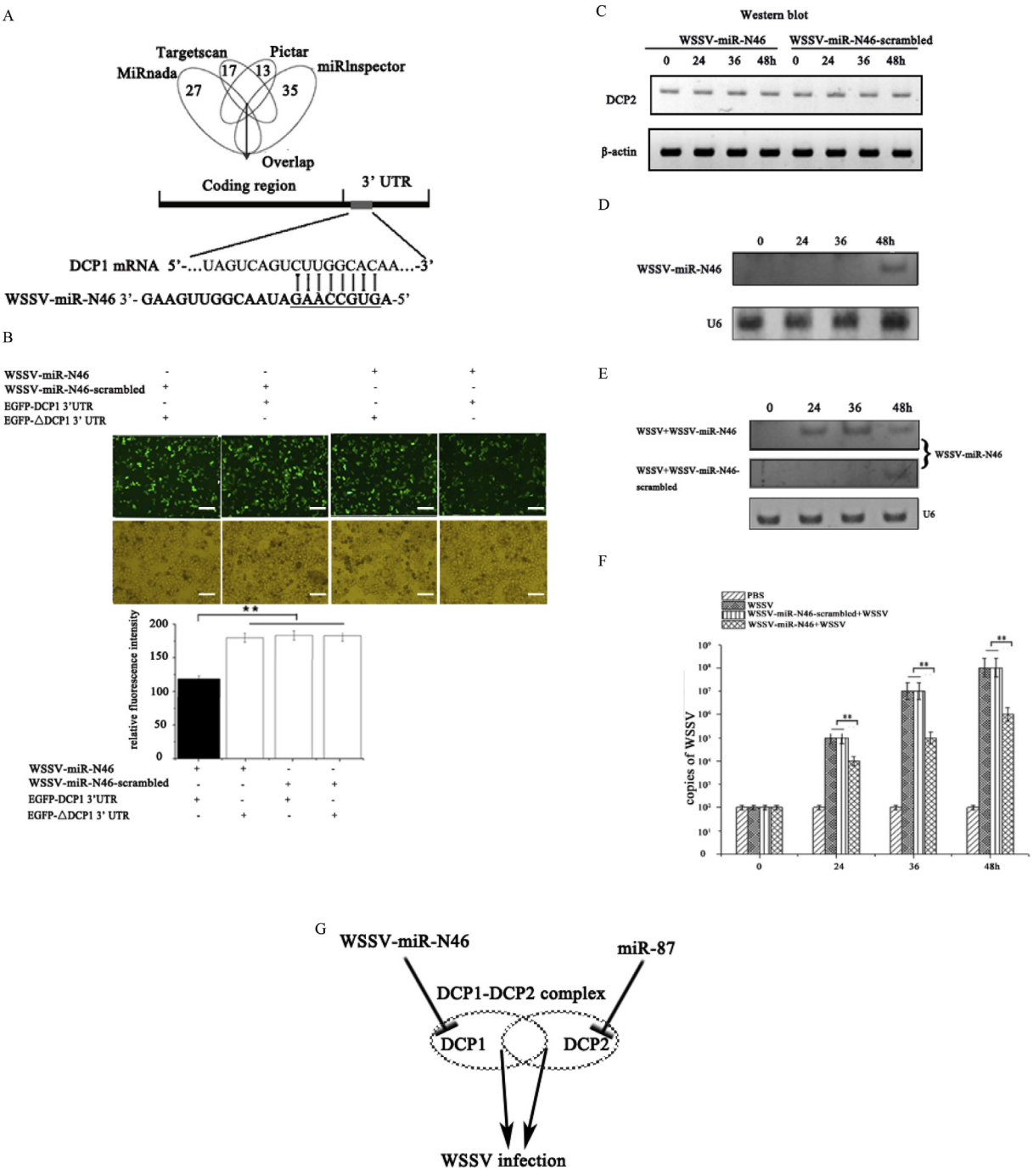

Fig. 5. Influence of viral WSSV-miR-N46 targeting *DCP1* on virus infection. (A) The prediction of viral miRNA targeting *DCP1*. As predicted, the 3′ UTR of *DCP1* was targeted by WSSV-miR-N46, a WSSV-encoded viral miRNA. The seed sequence was underlined. (B) The direct interaction between WSSV-miR-N46 and *DCP1* gene in insect cells. Insect High Five cells were co-transfected with WSSV-miR-N46 or WSSV-miR-N46-scrambled and EGFP, EGFP-DCP13′ UTR or EGFP-ΔDCP13′ UTR. At 48 h after co-transfection, the fluorescence of cells was examined (**, $p < 0.01$). Scale bar, 50 μm. (C) Influence of WSSV-miR-N46 overexpression on the DCP2 expression in WSSV-infected shrimp. WSSV-miR-N46 was overexpressed in WSSV-infected shrimp. At different time after WSSV-miR-N46 overexpression, the DCP2 protein level was examined by Western blot. As a control, WSSV-miR-N46-scrambled was included in the assays. Data were representatives of three independent experiments. The numbers showed the time post-infection. The antibodies were indicated on the left. (D) The expression pattern of WSSV-miR-N46 in shrimp in response to virus infection. Shrimp were challenged with WSSV. At different time post-infection, WSSV-miR-N46 was detected by Northern blotting. U6 was used as a control. The number indicated the time points post-infection. Probes were indicated on the left. (E) The overexpression of WSSV-miR-N46 in shrimp. Shrimp were simultaneously injected with WSSV and WSSV-miR-N46. As a control, WSSV-miR-N46-scrambled was included in the injection. At different time post-infection, shrimp hemolymph was subjected to Northern blotting. U6 was used as a control. The probes were shown on the right. (F) The influence of WSSV-miR-N46 overexpression on WSSV infection. Shrimp were simultaneously injected with WSSV-miR-N46 and WSSV. As a control, WSSV-miR-N46-scrambled was included in the injection. At different time post-infection, the WSSV copies were examined with quantitative real-time PCR (**, $p < 0.01$). (G) Mode for the miRNA-mediated signaling pathway in virus infection.

of the DCP1-DCP2 complex in virus-host interactions.

In the present investigation, the results showed that the host and viral miRNAs could inhibit the expressions of DCP1 and DCP2 during DNA virus infection. MiRNAs, a large class of small noncoding RNAs in diverse eukaryotic organisms, are sequentially processed by two RNase III proteins, Drosha and Dicer from the stem regions of long hairpin transcripts [28,37]. The mature miRNA strand is liberated from the miRNA:miRNA* duplex and integrated into the RNA induced silencing complex (RISC), and inhibits the expression of cognate mRNA through degradation or translation repression in the RISC [18]. As reported, the host miRNAs or/and viral miRNAs can regulate virus infection by targeting viral or/and host genes during the process of virus infection [2,17,23,24,27–29,31,32,38]. It has been well characterized that the virus-encoded miRNAs (viral mRNAs) can target virus and/or host genes, leading to virus infection or virus latency [29,32,38,39]. In shrimp, a viral miRNA WSSV-miR-N12 targets the viral wsv399 gene, resulting in virus latency [32]. The viral miRNA-mediated regulation of virus infection or virus latency is an efficient strategy for virus to escape its host immune responses. However, the involvement of miRNA in the degradation of cellular mRNAs mediated by DCP1-DCP2 complex has not been explored. Our study revealed that the host shrimp and viral miRNAs could regulate the DCP1-DCP2 complex to affect virus infection. Therefore, our study provided novel insights into the regulatory mechanism of DCP1-DCP2 complex in virus-host interactions and that the miRNA-mediated regulation of DCP1-DCP2 complex took great effects on RNAi immunity of invertebrates against virus infection.

Acknowledgements

This work was supported by National Natural Science Foundation of China (31430089) and National Program on Key Basic Research Project (973 Program) (2015CB755903). We thank bioRxiv to accept this work for preprint to facilitate the rapid availability of life sciences research [40].

References

[1] S. Bhattacharjee, S. Chattaraj, Entry, infection, replication, and egress of human polyomaviruses: an update, Can. J. Microbiol. 63 (2017) 193–211.
[2] Y. Cui, X. Huang, X. Wang, Y. Li, C. Tang, H. Wang, et al., Correlation between infection of herpes virus family and liver function parameters: a population-based cross-sectional study, J. Infect. Dev. Ctries 11 (2017) 320–325.
[3] J.J. Rossi, RNAi and the P-body connection, Nat. Cell Biol. 7 (2005) 643–644.
[4] C.Y. Chen, D. Zheng, Z. Xia, A.B. Shyu, Ago-TNRC6 triggers microRNA-mediated decay by promoting two deadenylation steps, Nat. Struct. Mol. Biol. 16 (2009) 1160–1166.
[5] D. Schaeffer, A. van Hoof, Different nuclease requirements for exosome-mediated degradation of normal and nonstop mRNAs, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 2366–2371.
[6] S.E. Munchel, R.K. Shultzaberger, N. Takizawa, K. Weis, Dynamic profiling of mRNA turnover reveals gene-specific and system-wide regulation of mRNA decay, Mol. Biol. Cell 22 (2011) 2787–2795.
[7] C.M. Gallo, E. Munro, D. Rasoloson, C. Merritt, G. Seydoux, Processing bodies and germ granules are distinct RNA granules that interact in C. elegans embryos, Dev. Biol. 323 (2008) 76–87.
[8] A. Eulalio, I. Behm-Ansmant, D. Schweizer, E. Izaurralde, P-body formation is a consequence, not the cause, of RNA-mediated gene silencing, Mol. Cell. Biol. 27 (2007) 3970–3981.
[9] N. Scheller, P. Resa-Infante, S. de la Luna, R.P. Galao, M. Albrecht, L. Kaestner, et al., Identification of PatL1, a human homolog to yeast P body component Pat1, Biochim. Biophys. Acta 1773 (2007) 1786–1792.
[10] U. Sheth, R. Parker, Decapping and decay of messenger RNA occur in cytoplasmic processing bodies, Science 300 (2003) 805–808.
[11] S.A. Barbee, P.S. Estes, A.M. Cziko, J. Hillebrand, R.A. Luedeman, J.M. Coller, et al., Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies, Neuron 52 (2006) 997–1009.
[12] R.L. Gallo, V. Nizet, Innate barriers against infection and associated disorders, Drug Discov. Today Dis. Mech. 5 (2008) 145–152.
[13] U. Wilczynska, W. Szymczak, N. Szeszenia-Dabrowska, Mortality from malignant neoplasms among workers of an asbestos processing plant in Poland: results of prolonged observation, Int. J. Occup. Med. Environ. Health 18 (2005) 313–326.
[14] G. Stoecklin, T. Mayo, P. Anderson, ARE-mRNA degradation requires the 5'-3' decay pathway, EMBO Rep. 7 (2006) 72–77.
[15] R.J. Golden, B. Chen, T. Li, J. Braun, H. Manjunath, X. Chen, et al., An Argonaute phosphorylation cycle promotes microRNA-mediated silencing, Nature 542 (2017) 197–202.
[16] M.H. Jo, S. Shin, S.R. Jung, E. Kim, J.J. Song, S. Hohng, Human argonaute 2 has diverse reaction pathways on target RNAs, Mol. Cell 59 (2015) 117–124.
[17] T. Huang, X. Zhang, Functional analysis of a crustacean microRNA in host-virus interactions, J. Virol. 86 (2012) 12997–13004.
[18] S. Jonas, E. Izaurralde, Towards a molecular understanding of microRNA-mediated gene silencing, Nat. Rev. Genet. 16 (2015) 421–433.
[19] J.A. Vidigal, A. Ventura, The biological functions of miRNAs: lessons from in vivo studies, Trends Cell Biol. 25 (2015) 137–147.
[20] G. Yang, L. Yang, Z. Zhao, J. Wang, X. Zhang, Signature miRNAs involved in the innate immunity of invertebrates, PLoS One 7 (2012) e39015.
[21] J.T. Mendell, E.N. Olson, MicroRNAs in stress signaling and human disease, Cell 148 (2012) 1172–1187.
[22] Y. Zhou, Y. He, C. Wang, X. Zhang, Characterization of miRNAs from hydrothermal vent shrimp Rimicaris exoculata, Mar Genomics 24 (Pt 3) (2015) 371–378.
[23] Y. He, X. Zhang, Comprehensive characterization of viral miRNAs involved in white spot syndrome virus (WSSV) infection, RNA Biol. 9 (2012) 1019–1029.
[24] T. Huang, Y. Cui, X. Zhang, Correction for Huang et al., "Involvement of Viral MicroRNA in the Regulation of Antiviral Apoptosis in Shrimp, J. Virol. (2018) 92.
[25] Z. Wang, F. Zhu, MicroRNA-100 is involved in shrimp immune response to white spot syndrome virus (WSSV) and Vibrio alginolyticus infection, Sci. Rep. 7 (2017) 42334.
[26] H. Ingle, S. Kumar, A.A. Raut, A. Mishra, D.D. Kulkarni, T. Kameyama, et al., The microRNA miR-485 targets host and influenza virus transcripts to regulate antiviral immunity and restrict viral replication, Sci. Signal. 8 (2015) a126.
[27] L. Shu, C. Li, X. Zhang, The role of shrimp miR-965 in virus infection, Fish Shellfish Immunol. 54 (2016) 427–434.
[28] Q. Ren, X. Huang, Y. Cui, J. Sun, W. Wang, X. Zhang, Two white spot syndrome virus MicroRNAs target the dorsal gene to promote virus infection in Marsupenaeus japonicus shrimp, J. Virol. (2017) 91.
[29] Y. He, K. Yang, X. Zhang, Viral microRNAs targeting virus genes promote virus infection in shrimp in vivo, J. Virol. 88 (2014) 1104–1112.
[30] T. Huang, D. Xu, X. Zhang, Characterization of host microRNAs that respond to DNA virus infection in a crustacean, BMC Genomics 13 (2012) 159.
[31] Y. Cui, X. Yang, X. Zhang, Shrimp mir-34 from shrimp stress response to virus infection suppresses tumorigenesis of breast cancer, Mol. Ther. Nucleic Acids 9 (2017) 387–398.
[32] Y. Cui, T. Huang, X. Zhang, RNA editing of microRNA prevents RNA-induced silencing complex recognition of target mRNA, Open Biol. 5 (2015) 150126.
[33] M.M. Gaglia, B.A. Glaunsinger, Viruses and the cellular RNA decay machinery, Wiley Interdiscip. Rev. RNA 1 (2010) 47–59.
[34] K.C. Hopkins, L.M. McLane, T. Maqbool, D. Panda, B. Gordesky-Gold, S. Cherry, A genome-wide RNAi screen reveals that mRNA decapping restricts bunyaviral replication by limiting the pools of Dcp2-accessible targets for cap-snatching, Genes Dev. 27 (2013) 1511–1525.
[35] K. Hopkins, S. Cherry, Bunyaviral cap-snatching vs. decapping: recycling cell cycle mRNAs, Cell Cycle 12 (2013) 3711–3712.
[36] U. Sheth, R. Parker, Targeting of aberrant mRNAs to cytoplasmic processing bodies, Cell 125 (2006) 1095–1109.
[37] T. Huang, X. Zhang, Contribution of the argonaute-1 isoforms to invertebrate antiviral defense, PLoS One 7 (2012) e50581.
[38] Y. He, T. Ma, X. Zhang, The mechanism of synchronous precise regulation of two shrimp white spot syndrome virus targets by a viral MicroRNA, Front. Immunol. 8 (2017) 1546.
[39] Y. He, T. Ma, X. Zhang, The mechanism of synchronous precise regulation of two shrimp white spot syndrome virus targets by a viral MicroRNA, Front. Immunol. 8 (2017) 1546.
[40] S. Yuec, et al., Role of DCP1-DCP2 complex regulated by viral and host microRNAs in DNA virus infection, BioRxiv (2018) 341362https://doi.org/10.1101/341362.