Arch Virol (2012) 157:993–998
DOI 10.1007/s00705-012-1258-2

BRIEF REPORT

Comparative efficacy of double-stranded RNAs targeting WSSV structural and nonstructural genes in controlling viral multiplication in *Penaeus monodon*

M. Sanjuktha · V. Stalin Raj · K. Aravindan · S. V. Alavandi · M. Poornima · T. C. Santiago

Received: 25 September 2011 / Accepted: 17 January 2012 / Published online: 21 February 2012
© Springer-Verlag 2012

Abstract RNA interference (RNAi) is a potential strategy to control shrimp viral diseases, including the white spot disease caused by White Spot Syndrome Virus (WSSV). Selection of genes for targeting is an important criterion. We have compared the efficacy of dsRNAs targeting structural (vp28 and vp281) and nonstructural genes (rr1 and dnapol) of WSSV in controlling viral multiplication in *Penaeus monodon*. Targeting the rr1 and vp28 genes provided better protection (93.3% and 90% survival respectively) compared to vp281 and dnapol in experimentally infected shrimp. Temporal transcriptional analysis of the corresponding genes and PCR-based diagnosis of WSSV in samples collected at different time points in the experiment supported this observation, thereby indicating that targeting a combination of rr1 and vp28 would be effective in limiting WSSV multiplication.

White spot syndrome is an economically devastating viral disease of farmed shrimp. The disease is caused by white spot syndrome virus (WSSV), which is a double-stranded DNA virus of the genus *Whispovirus*, family *Nimaviridae* [1]. WSSV outbreaks in shrimp farms result in 100% mortality within 5-10 days, causing serious economic losses, and the problem is compounded by the wide host range of this virus [2–4]. There is no established therapy available for the treatment of WSSV infection in shrimp. Administering inactivated virus [5] and WSSV envelope proteins as an oral vaccine or intramuscular injection has been reported to increase the survival of experimentally infected shrimp [6–8]; however, application of these products in field trials have not been reported so far. At present, the practical method to control this virus includes screening of brooders and shrimp seeds for WSSV prior to spawning and stocking, respectively.

RNA interference (RNAi), a natural biological phenomenon, is currently being examined as an antiviral therapeutic strategy. RNAi is the silencing of gene expression that occurs when dsRNA is introduced into a cell [9]. Long dsRNAs in the cytoplasm produced by viral infections, transposons, or cellular transcripts are processed into 21- to 23-nucleotide (nt) RNA duplexes called short interfering RNAs (siRNAs) by an RNase III nuclease called Dicer [10]. These siRNA sequences are incorporated into an RNA-induced silencing complex (RISC) and are subsequently unwound. The guide strand (complementary to the mRNA), which has a less thermodynamically stable 5′ end [11] remains with the RISC while the passenger strand is cleaved and ejected from the complex. The complex then binds to the complementary mRNA in the cytoplasm and cleaves it. This results in silencing of gene expression at the posttranscriptional level. The RNAi phenomenon has been reported in plants, fungi, nematodes, insects, crustaceans and mammals [12–14]. The use of RNAi as an antiviral agent in humans has been reported

M. Sanjuktha · V. Stalin Raj · K. Aravindan · S. V. Alavandi · M. Poornima · T. C. Santiago (✉)
Aquatic Animal Health and Environment Division, Central Institute of Brackishwater Aquaculture, 75, Santhome High Road, Chennai 600 028, India
e-mail: santiagotc1@gmail.com

Present Address:
V. Stalin Raj
Department of Virology, Room No: Ee 1720, Dr. Molenwaterplein 50, 3015 GE Rotterdam, The Netherlands

Present Address:
T. C. Santiago
Entomology Research Institute, Loyola College, Chennai 600 034, India

[15]. RNAi elicited by administering either long dsRNAs or siRNAs has been shown to induce antiviral protection in shrimp [14, 16–20].

The present investigation was undertaken to study whether targeting genes with different functions in the viral life cycle have similar or varying impact on viral multiplication in the host. Four genes– two nonstructural protein-encoding genes, viz., *rr1* (large subunit of ribonucleotide reductase, RR gene) and *dnapol* (DNA polymerase), and two genes encoding viral envelope proteins viz., *vp28* and *vp281*, were selected. These genes were targeted by administering *in vitro*-synthesized dsRNA to WSSV-infected shrimp, and the protection thus afforded was compared.

In this study, for dsRNA synthesis, sequences of the WSSV genes *vp28* (GenBank ID: DQ979320), *vp281* (GenBank ID: EF534251), *rr1* (GenBank ID: AF099142) and *dnapol* (GenBank ID: AF369029) were retrieved from GenBank. Primers designed to amplify partial sequences of these genes are as follows: vp28 (F: 5′-GCAAAATCAAG ATCCGCAAT-3′ and R: 5′-GTGAAGGAGGAGGTGTT GGA-3′), vp281 (F: 5′-GACCGAATGACACCCGTAA G-3′ and R: 5′-CCACTGTCGCTATCTGTTGC-3′), rr1 (F: 5′-CGGACTGAGGACGCTAGAAT-3′ and R: 5′-CCCT CGTCCTCAAATCTTCA-3′) and dnapol (F: 5′-TCTGC GTGGCTAGGTCTCTT-3′ and R: 5′-TGACGGCCAATC AGATTACA-3′). DNA extracted from WSSV-infected shrimp tissue was used as template. The amplified products were cloned in the vector LITMUS28i (New England Biolabs) by blunt-end cloning of the PCR products according to standard protocols [21]. The insert sequences in each clone were amplified using T7 minimal primer (New England Biolabs) corresponding to the dual T7 promoter in the vector. The amplicons were then transcribed to dsRNA *in vitro* using T7 RNA polymerase (New England Biolabs). The template DNA was removed by the addition of DNase I (New England Biolabs).

Severely infected moribund shrimps collected during a WSSV outbreak in Nagapattinam, India, and stored at −70°C were used as the source of WSSV. The shrimps were tested for the presence of WSSV and other shrimp viruses using the appropriate IQ 2000 kits and were found to be positive for only WSSV (data not shown). Challenge experiments were performed using extracts from the infected tissues, and the infected animals were found to be positive for WSSV, thereby confirming that the virus in the stored infected tissues were infectious (data not shown). For the present experiment, WSSV-infected tissue extract was prepared by homogenizing 1 g of pleopod tissue in 10 ml of phosphate-buffered saline (PBS) (pH 7.4). The homogenate was centrifuged, and the supernatant was filtered through a 0.22-μm filter. For this study, we sought to use a dose of WSSV that would give 100% mortality over an extended period. A dose producing mortality within a short span of time would not be conducive to observing the effects of dsRNA injection. Hence, the filtrate was diluted at various ratios (1:10, 1:20, 1:30, 1:40 and 1:50) and injected in shrimp. The dilution (1: 40) that produced 100% mortality within 9-14 days was chosen as viral inoculum for the present study (data not shown).

P. monodon weighing 1.5 (±0.5) g, reared from WSSV-free seeds, were procured from a farm in Mahabalipuram, India. In our laboratory, the shrimps were maintained at 28°C in 100-l tanks containing filtered seawater at a salinity of 28 g/l with continuous aeration. The shrimps were fed three times a day with commercial pellet feed. The shrimps were divided into six groups in triplicate with ten animals in each. Each group was maintained in a 50-l tank. The negative control group was injected with TN only (50 mM Tris, pH 7.5, 100 mM NaCl) buffer. The four test groups each received one type of dsRNA (and were named the dsRNAvp28, dsRNAvp281, dsRNArr1 and dsRNAdnapol groups, according to the WSSV gene targeted). Six μg/g body weight of dsRNA in TN buffer was injected intramuscularly in the third segment of the abdomen of each animal of the test groups. We had previously tested 2-10 μg/g body weight dsRNA concentrations and found that there was no significant difference in the efficacy from 5-10 μg (data not shown). Hence, we used 6 μg in the present study. After 24 h, WSSV extract was injected intramuscularly in the dilution (1:40) that produced 100% mortality in 9-14 days. The positive control group received only the WSSV inoculum injection. The dead animals were not removed from the tanks to allow a re-infection process similar to what would happen in the pond. The animals were observed for 14 days. The significance of the mortality rates observed in the different experimental groups was analyzed by one-way ANOVA followed by Turkey's multiple comparison test using GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego, California USA, http://www.graphpad.com. Another replicate of each test and control group was maintained and subjected to the same treatments described above. This replicate was not used for mortality studies. Shrimps were collected from this replicate at regular intervals (every 48 h from the time of dsRNA injection), and tissues were preserved in 95% ethanol at −20°C for RNA and DNA extraction.

The IQ2000™ WSSV Detection and Prevention System (Farming IntelliGene Tech. Corp, Taiwan; henceforth referred to as IQ2000 WSSV kit) was used to diagnose the steady-state level of WSSV infection in the experimental animals sampled at regular intervals. The kit allows for differentiation of WSSV infection as very light, light, medium and severe (by comparison with positive standards provided in the kit).

Total RNA was extracted from the gill tissues of shrimps collected at each time interval using TRIzol reagent (Invitrogen). First-strand cDNA was synthesized using the oligo-dT primer, and the cDNA was used for PCR in a 25-µl reaction mixture containing the appropriate primer pair. An 18S rDNA primer set, Deca 20A2 (5′-ACTTCCCCCGGA ACCCAAAGACT-3′) and Deca 20S9 (5′-GGGGGCATTC GTATTGCGA-3′), was used as an internal control for RNA quality and amplification efficiency. The level of transcription in each group at all time points was compared to the level of transcription of the gene in the third sample (144 h) collected from the positive control group.

Injection of dsRNA at 6 µg/g body weight was found to afford protection against WSSV in *P. monodon*, and the effectiveness of the dsRNA varied with the viral gene targeted for silencing. The positive control group injected with only WSSV showed mortality from the 4th dpi, and cumulative mortality reached 100% by the 13th dpi. The highest survival rates were observed in dsRNArr1 group at 93.3% (Fig. 1a) and dsRNAvp28 group, which had a survival rate of 90%. The mortality rates in these two groups were found to be significantly different from those in the remaining groups, including the positive control group ($P < 0.05$). However, the mortality rates of the dsRNAvp28 and dsRNArr1 groups were not significantly different from each other ($P < 0.05$), thereby indicating that both the dsRNAs gave similar levels of protection against WSSV (Fig. 1b). The dsRNAdnapol and dsRNAvp281 groups had comparatively lower survival rates of 76.6% and 66.7%, respectively (Fig. 1a). There was no mortality in the negative control group. dsRNAs targeting *rr1*, a nonstructural-protein-encoding gene, and *vp28*, a structural-protein-encoding gene, were both found to control viral multiplication with high efficiency. On the other hand, dsRNAs targeting *dnapol*, a nonstructural-protein-encoding gene, and *vp281*, a structural-protein-encoding gene, were comparatively less efficient in controlling WSSV infection. This observation indicates that the function of the protein encoded by the gene and its role in the viral life cycle should form the criteria for selection of a gene as target for RNAi.

On performing WSSV diagnostic PCR using a IQ2000 WSSV kit, all samples collected from the dsRNAvp28 group at all time points (from 48 h post-dsRNA-injection up to 336 h at 48-h intervals) were found to be very slightly positive for WSSV (Fig. 2). RNA extracted from these samples was subjected to RT-PCR with the appropriate primer pair to study the level of transcription, and the *vp28* transcript was detected up to 144 h post-dsRNA-injection, but at a low level (Fig. 3). This indicates that there had not been complete viral clearance; however, the viral load was insufficient to trigger WSD and subsequent death. We hypothesize that in this group, viral DNA replication took

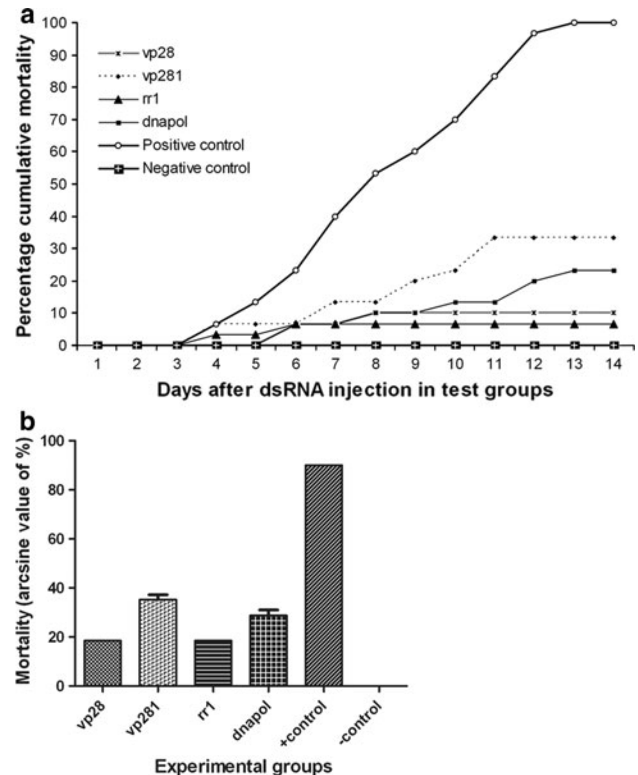

Fig. 1 (**a**) Graph showing percentage of cumulative mortality in *P. monodon* injected with dsRNA corresponding to four WSSV genes in relation to the positive control and negative control groups. Higher survival rates were found in the dsRNArr1 (93.3%) and dsRNAvp28 (90%) groups. (**b**) Analysis of the total mortality observed in the various experimental groups that received different dsRNAs in comparison with the controls. The percentage mortality values were converted to arcsine values for statistical analysis by one-way ANOVA followed by Turkey's multiple comparison test. There was significant difference in the mortality rates between the different experimental groups ($P < 0.05$). The mortality rates of dsRNAvp28 and dsRNArr1 groups were not significantly different

place initially, but as the major envelope protein was not synthesized, there was inhibition of viral assembly. This in turn could have inhibited cellular destruction and further spread of infection and mortality. The transcription analysis results suggest that viral DNA was able to support transcription of the *vp28* gene up to 144 h at a detectable level, after which this gene was not transcribed to a level detectable by the method adopted for analysis. It is likely that the incompletely assembled viral particles or viral DNA had still not been removed from the animal's system, which accounts for the detection of WSSV DNA by IQ 2000.

Administration of dsRNA targeting *vp281* gave protection against WSSV; however, the mortality rate of this group was significantly higher when compared to the other dsRNA-treated groups (Fig. 1b). Samples collected from the dsRNAvp281 group at all time points were found to be very slightly positive (Fig. 2). RT-PCR analysis also

showed that the *vp281* transcript was detected up to 240 h post-dsRNA-injection (Fig. 3). Based on these results, we suggest that WSSV replication can take place even when the expression of VP281 protein is decreased, although this can be ascertained only by further experiments.

The dsRNAdnapol group showed a survival rate of 76.6% (Fig. 1a). Samples from this group collected at 48 h and 96 h were negative for WSSV, while subsequent samples were very slightly positive for WSSV infection (Fig. 2). RT-PCR analysis showed that the *dnapol* transcript was not detected until 144 h and was detected in subsequent samples from 192 to 336 h (Fig. 3). Although, the *dnapol* gene is vital in viral replication, knockdown of this gene by RNAi did not adversely affect viral multiplication. This observation probably indicates a relationship between the host and viral replication machinery that is yet to be understood.

In the present study, the dsRNArr1 group had a high survival rate of 93.3% (Fig. 1a), and all of the samples from this group were negative for WSSV infection throughout the course of the experiment (Fig. 2). The *rr1* transcript was also not detected in the RT-PCR analysis of these samples at any of the time points (Fig. 3), thereby indicating a complete knockdown of the gene. *rr1* is an early gene of WSSV [22] and its knockdown could result in reduced or failed viral DNA replication. In the present experiment, dsRNA was administered 24 h prior to the WSSV inoculum. When viral gene transcription starts in the host cells, siRNAs targeting WSSV *rr1* are already present in the shrimp system. Therefore, the knockdown of *rr1*, which is transcribed within 4-6 h of infection could result in decreased or failed WSSV DNA synthesis. This could account for the absence of infection as detected by IQ2000 analysis and absence of any detectable transcripts in the RT-PCR analysis.

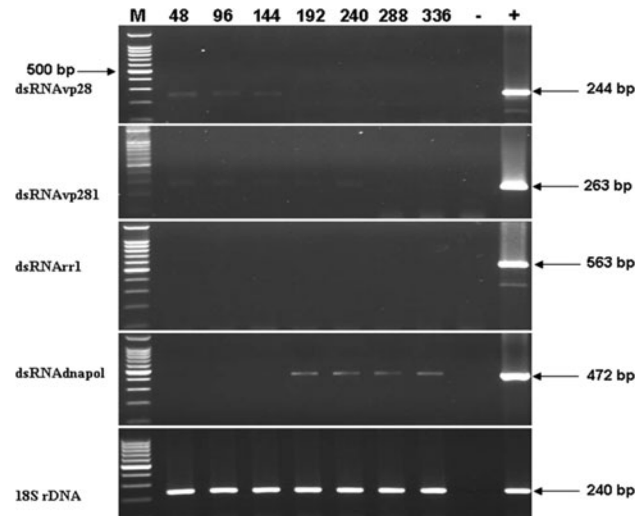

Fig. 3 Transcriptional analysis of the four WSSV genes. 18S rDNA amplified from the positive control group is shown as an internal control. – indicates the negative control, and + indicates the positive control collected at 144 h

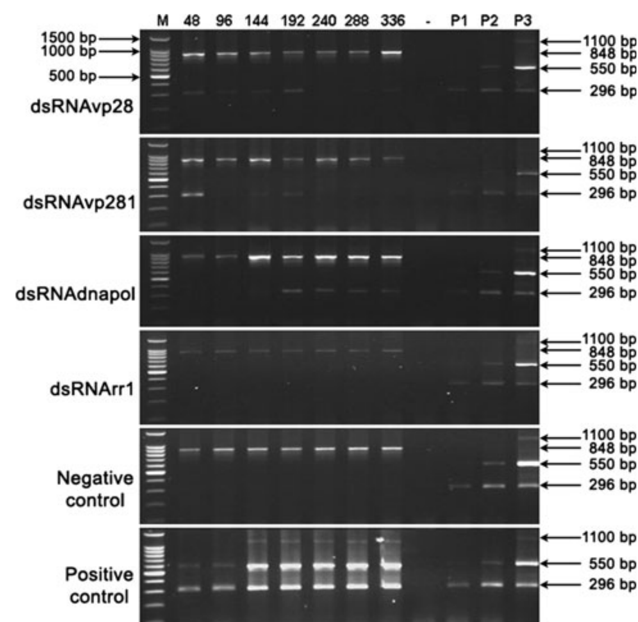

Fig. 2 Determination of the SSV infection level at various time points (48-336 h) using an IQ 2000 kit. Diagnosis of WSSV infection is based on the following band patterns, as given by the manufacturer: The presence of only one band of 848 bp, which is the product of the housekeeping gene, indicates a negative sample. The presence of bands of 848 bp and 296 bp indicates very light infection. The presence of a band of 296 bp alone indicates light infection. The presence of bands of 550 bp and 296 bp indicates moderate infection. The presence of bands of 1100 bp, 550 bp and 296 bp indicates severe infection. Lane M: 100-bp DNA ladder Lane N: Negative control without any template Lane P1: Manufacturer's standard corresponding to 20 viral copies (light positive) Lane P2: Manufacturer's standard corresponding to 200 viral copies (moderate positive) Lane P3: Manufacturer's standard corresponding to 2000 viral copies (severe positive)

The aim of this study was to ascertain whether targeting structural or nonstructural genes of WSSV by RNAi gives better protection against this virus. However, our results suggest that the function of the encoded protein forms a more important criterion for selection. To our knowledge, this is the first time the nonstructural-protein-encoding gene, the *rr1* gene of WSSV, has been targeted for silencing viral multiplication by RNAi. WSSV RR has a large (RR1) and small (RR2) subunit [22], which can be differentiated from host cellular RR [23]. WSSV RR has been reported to be functionally involved during infection [24]. RR is an important enzyme in the viral life cycle, as it catalyses the reduction of ribonucleotides into deoxyribonucleotides [25]. The activity of host RR is linked to DNA replication and is reported to be absent or inactive in resting cells [26]. Viral RRs are believed to enable the virus to replicate even in non-dividing cells. It has been

demonstrated that the WSSV RR is functionally active and enables the virus to replicate even in resting cells, thus contributing to the rapid onset and lethality of WSSV infection [24]. Hence, targeting this gene imparted the highest degree of protection against WSSV compared to all the other genes examined in this study.

Targeting the *vp28* gene gave the second-highest degree of protection against WSSV in this study. Reports on siRNA and dsRNA targeting this gene in affording antiviral response have also been promising [17, 18, 20]. Robalino et al. reported 85% survival in shrimps treated with *vp28*-specific dsRNA and challenged with WSSV [16].The V28 protein plays an important role in the systemic infection of shrimp [27]. Yi et al. have demonstrated that VP28, a major envelope protein of WSSV, is involved in attachment to shrimp cells, and the protein enters the cytoplasm at 3 h post-adsorption [28]. Tang et al. proposed that the N-terminal transmembrane region of VP28 could interact with the host receptor or fuse with host cell membrane [29]. These features of *vp28* make it an attractive target for antiviral studies.

Due to its central role in viral DNA replication, the viral DNA polymerase gene has been a target for development of antiviral therapeutics, and even RNAi has been used to target this gene of many viruses. siRNAs targeting the 3D *pol* of poliovirus have been shown to decrease the viral titer in human cells [30]. Surprisingly, dsRNA corresponding to *dnapol* gene afforded less protection, with only 76.6% survival. Similar results have been observed by Robalino et al., who reported only 44% survival in DNA polymerase dsRNA-treated groups [16] and Wu et al., who reported 50% survival in *L. vannamei* injected with *dnapol*-specific siRNA [19]. Despite being a vital enzyme in viral DNA replication, decreased expression of this protein did not adversely affect WSSV multiplication in any of these studies. Cavanaugh and Kuchta showed that in HSV-1, the host DNA polymerase α, is more efficient in extending the viral primase-synthesized RNA primers than the viral polymerase, thereby suggesting that host polymerases are also involved in herpesviral DNA replication [31]. It is possible that WSSV, and its host too, could share a similar mechanism of viral DNA replication, which could, to some extent, explain why targeting the *dnapol* gene of WSSV for knockdown does not completely inhibit viral multiplication.

The VP281 protein of WSSV is a minor envelope protein [32, 33] that is reported to be involved in infection by binding to shrimp cell membranes [34, 35]. Administration of anti-vp281 antibodies [35] and DNA vaccines encoding *vp281* [36] has been shown to give protection against WSSV. In the present study, administration of dsRNA targeting this gene gave the least protection against WSSV when compared to the other targeted genes. Similar observations were reported by Kim et al., wherein injection of dsRNA targeting vp281 afforded less protection against WSSV in *P. chinensis* than other dsRNAs tested [17]. Hence, this gene may not be a suitable candidate for developing RNAi-based antiviral strategies against WSSV.

Selection of viral genes for targeting would play an important role in determining the success of this RNAi as an antiviral therapeutic. A significant observation in this study has been that the role and importance of the protein encoded by the target gene in the viral life cycle is a central factor in determining its suitability for developing RNAi-based therapeutics. From the results of this study, a combination of dsRNA targeting *rr1* and *vp28* would be a suitable strategy to protect shrimp against WSSV infection. Under farm conditions, it would not be practical to apply this technique to the shrimps in the grow-out ponds. However, this strategy has great potential in hatcheries to produce virus-free broodstock, thereby ensuring production of WSSV-free seeds. Further research on the RNAi pathway in crustaceans will help in the development of new strategies to combat viral diseases affecting cultured crustaceans.

Acknowledgments Sanjuktha M acknowledges the financial support of the Council of Scientific and Industrial Research (CSIR) during her tenure as a CSIR Senior Research Fellow. The authors are thankful to the Director, CIBA, for his support. The authors are thankful to Dr. C. P. Balasubramanian for his help in performing the statistical analyses.

References

1. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) (2005) Virus taxonomy, VIIIth report of the ICTV. Elsevier/Academic Press, London
2. Lightner DV (1996) A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. World Aquaculture Society, Baton Rouge
3. Sánchez-Martínez JG, Aguirre-Guzmán G, Mejía-Ruíz H (2007) White spot syndrome virus in cultured shrimp: a review. Aqua Res 38:1339–1354
4. Escobedo-Bonilla CM, Alday-Sanz V, Wille M, Sorgeloos P, Pensaert MB, Nauwynck HJ (2008) A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. J Fish Dis 31:1–18
5. Bright Singh IS, Manjusha M, Pai SS, Philip R (2005) *Fenneropenaeus indicus* is protected from white spot disease by oral administration of inactivated white spot syndrome virus. Dis Aquat Organ 66:265–270
6. Caipang CM, Verjan N, Ooi EL, Kondo H, Hirono I, Aoki T, Kiyono H, Yuki Y (2008) Enhanced survival of shrimp, *Penaeus* (*Marsupenaeus*) *japonicus* from white spot syndrome disease after oral administration of recombinant VP28 expressed in *Brevibacillus brevis*. Fish Shellfish Immunol 25:315–320
7. Ha YM, Soo-Jung G, Thi-Hoai N, Ra CH, Kim KH, Nam YK, Kim SK (2008) Vaccination of shrimp (*Penaeus chinensis*) against white spot syndrome virus (WSSV). J Microbiol Biotechnol 18:964–967
8. Witteveldt J, Cifuentes CC, Vlak JM, van Hulten MCW (2004) Protection of *Penaeus monodon* against white spot syndrome virus by oral vaccination. J Virol 78:2057–2061

9. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391:806
10. Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409:363–366
11. Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD (2003) Asymmetry in the assembly of the RNAi enzyme complex. Cell 115:199–208
12. Bernstein E, Denli AM, Hannon GJ (2001) The rest is silence. RNA 7:1509–1521
13. Hannon GJ (2002) RNA interference. Nature 418:244–251
14. Robalino J, Browdy CL, Prior S, Metz A, Parnell P, Gross P, Warr G (2004) Induction of antiviral immunity by double stranded RNA in a marine invertebrate. J Virol 78:10442–10448
15. Castanotto D, Rossi JJ (2009) The promises and pitfalls of RNA-interference-based therapeutics. Nature 457:426–433
16. Robalino J, Bartlett T, Shepard E, Prior S, Jaramillo G, Scura E, Chapman RW, Gross PS, Browdy CL, Warr GW (2005) Double-starnded RNA induces sequence-specific antiviral silencing in addition to nonspecific immunity in a marine shrimp: convergence of RNA interference and innate immunity in the invertebrate antiviral response? J Virol 79:13561–13571
17. Kim CS, Kosuke Z, Nam YK, Kim SK, Kim KH (2007) Protection of shrimp (*Penaeus chinensis*) against white spot syndrome virus (WSSV) challenge by double-stranded RNA. Fish Shellfish Immunol 23:242–246
18. Westenberg M, Heinhuis B, Zuidema D, Vlak JM (2005) siRNA injection induces sequence-independent protection in *Penaeus monodon* against white spot syndrome virus. Virus Res 114: 133–139
19. Wu Y, Lü L, Yang LS, Weng SP, Chan SM, He JG (2007) Inhibition of white spot syndrome virus in *Litopenaeus vannamei* shrimp by sequence-specific siRNA. Aquaculture 271:21–30
20. Xu J, Han F, Zhang X (2007) Silencing shrimp white spot syndrome virus (WSSV) genes by siRNA. Antivir Res 73:126–131
21. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
22. Tsai MF, Lo CF, van Hulten MCW, Tzeng HF, Chou CM, Huang CJ, Wang CH, Lin JY, Vlak JM, Kou GH (2000) Transcriptional analysis of the ribonucleotide reductase genes of shrimp white spot syndrome virus. Virology 277:92–99
23. Lin ST, Tzeng HF, Lo CF, Kou GH (2004) White spot syndrome virus (WSSV) ribonucleotide reductase (RR) is differentiated from the cellular RRs by its insensitivity to allosteric inhibition by dTTP and dATP. J Fish Soc Taiwan 31:191–200
24. Lin ST, Chang YS, Wang HC, Tzeng HF, Chang ZF, Lin JY, Wang CH, Lo CF, Kou GH (2002) Ribonucleotide reductase of shrimp white spot syndrome virus (WSSV): expression and enzymatic activity in a baculovirus/insect cell system and WSSV-infected shrimp. Virology 304:282–290
25. Elledge S, Zhou Z, Allen J (1992) Ribonucleotide reductase: regulation, regulation, regulation. Trends Biochem Sci 17:119–123
26. Nordenskjold B, Skoog L, Brown N, Reichard P (1970) Deoxyribonucleotide pools and deoxyribonucleic acid synthesis in cultured mouse cells. J Biol Chem 245:5360–5368
27. van Hulten MCW, Witteveldt J, Snippe M, Vlak JM (2001) White spot syndrome virus envelope protein VP28 is involved in the systemic infection of shrimp. Virology 285:228–233
28. Yi G, Wang Z, Qi Y, Yao L, Qian J, Hu L (2004) VP28 of shrimp white spot syndrome virus is involved in the attachment and penetration into shrimp cells. J Biochem Molbiol 37:726–734
29. Tang X, Wu J, Sivaraman J, Hew CL (2007) Crystal structures of major envelope proteins VP26 and VP28 from white spot syndrome virus shed light on their evolutionary relationship. J Virol 81:6709–6717
30. Gitlin L, Karelsky S, Andino R (2002) Short interfering RNA confers intracellular antiviral immunity in human cells. Nature 418:430–434
31. Cavanaugh NA, Kuchta RD (2009) Initiation of new DNA strands by the herpes simplex virus-1 primase-helicase complex and either herpes DNA polymerase or human DNA polymerase alpha. J Biol Chem 284:1523–1532
32. Huang C, Zhang X, Lin Q, Xu X, Hew CL (2002) Characterization of a novel envelope protein (VP281) of shrimp white spot syndrome virus by mass spectrometry. J Gen Virol 83:2385–2392
33. Lin Y, Xu L, Yang F (2010) Tetramerization of white spot syndrome virus envelope protein VP33 and its interaction with VP24. Arch Virol 155:833–838
34. Liang Y, Huang J, Song XL, Zhang PJ, Xu HS (2005) Four viral proteins of white spot syndrome virus (WSSV) that attach to shrimp cell membranes. Dis Aquat Organ 66:81–85
35. Wu W, Wang L, Zhang X (2005) Identification of white spot syndrome virus (WSSV) envelope proteins involved in shrimp infection. Virology 332:578–583
36. Rout N, Kumar S, Jaganmohan S, Murugan V (2007) DNA vaccines encoding viral envelope proteins confer protective immunity against WSSV in black tiger shrimp. Vaccine 25:2778–2786