Arch Virol (2011) 156:1117–1123
DOI 10.1007/s00705-011-0952-9

ORIGINAL ARTICLE

Inhibition of Taura syndrome virus replication in *Litopenaeus vannamei* through silencing the LvRab7 gene using double-stranded RNA

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Received: 30 July 2010 / Accepted: 10 February 2011 / Published online: 24 February 2011

Abstract Taura syndrome virus (TSV) is a major cause of high mortality in Pacific white shrimp (*Litopenaeus vannamei, Lv)*. Previously, silencing of *Penaeus monodon* Rab7 (PmRab7) by injecting double-stranded RNA corresponding to PmRab7 (dsRNA-PmRab7) prevented white spot syndrome virus or yellow head virus infection. Rab7 is proposed to be involved in intracellular trafficking of the viruses. This study aimed to investigate whether knockdown of Rab7 in *L. vannamei* by dsRNA-PmRab7 could inhibit replication of TSV. RNA interference (RNAi) technology using dsRNA targeting the LvRab7 gene was used to silence the mRNA expression of LvRab7. The silencing of the LvRab7 gene inhibited TSV replication dramatically when compared to groups receiving dsRNA-GFP or NaCl. This is the first demonstration that dsRNA targeting the endogenous shrimp gene LvRab7 strongly reduces TSV replication. It provides further evidence that LvRab7 is involved in the endosomal trafficking pathway of viruses infecting penaeid shrimp.

Introduction

The Pacific white shrimp, *Litopenaeus vannamei,* has increased in demand in Thailand since 2001 due to a slow growth syndrome in *Penaeus monodon*. The importation of stocks to Thailand has introduced a new viral pathogen, Taura syndrome virus (TSV), to the shrimp farming industry [12]. Taura syndrome (TS) was first discovered in Ecuador in 1992 [10]. It is caused by TSV, which is a cytoplasmic, non-enveloped icosahedral virus of 32 nm in diameter [4]. The TSV genome is a positive-sense single-stranded RNA of 10.2 kb containing two open reading frames (ORFs). ORF1 encodes non-structural proteins including helicase, protease, and RNA-dependent RNA polymerase. ORF2 encodes three major capsid proteins: CP1, CP2, and CP3 [13]. TS is a major cause of shrimp mortality in cultured *Litopenaeus vannamei* in the Americas and Asia. TSV can infect a number of penaeid shrimp species [11]. Symptoms include high mortality, general reddening or darkening of the body due to chromatophore expansion, flaccid musculature, soft cuticle, lethargy, and anorexia in the acute phase and grossly visible multifocal melanized cuticular lesions during the subsequent transition phase [8, 9, 11].To date, there is no effective method to prevent infection or cure TSV-infected shrimp.

RNA interference (RNAi) is a promising method to prevent or cure viral diseases in shrimp. The RNAi mechanism is triggered by double-stranded RNA (dsRNA), which leads to sequence-specific mRNA degradation of its homologous mRNA [6]. In shrimp, dsRNA corresponding to a viral gene such as the protease gene of yellow head virus (YHV) has been shown to prevent or cure YHV infection in *P. monodon* [26, 34]. In addition, dsRNA or small interfering RNA (siRNA) corresponding to the white spot syndrome virus (WSSV) gene has been used to suppress viral replication [3, 22, 31, 33]. On the other hand, an alternative approach is to use dsRNA corresponding to an endogenous gene required for viral entry. One such target is the intracellular trafficking pathway used during viral

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replication. Prevention of YHV infection in primary lymphoid cells was observed by using dsRNA corresponding to a putative endogenous YHV receptor [2]. Silencing of the endogenous gene *Penaeus monodon* Rab7, which was first identified as one of the WSSV binding proteins [24], was shown to prevent not only WSSV but also YHV infection [16] and Laem-Singh virus (LSNV) replication [17]. This suggests that PmRab7 functions as an important regulator of intracellular trafficking that is used by several viruses. The Rab proteins belong to members of the Ras superfamily of small GTPases that act as a molecular switch between the GTP-bound active and GDP-bound inactive states. Mammalian Rab7 localizes in the early and late endosome and plays an essential role in endocytic trafficking and lysosomal degradation [5, 23, 29]. Similarly, Rab7 in shrimp may play an important role in the endosomal trafficking processes used by several viruses. Knockdown of PmRab7 inhibited both DNA (WSSV) and RNA (YHV, LSNV) viral infections [16, 17]. Whether Rab7 is involved in the intracellular transport process of other shrimp viruses remains to be elucidated. In this study, RNAi technology using dsRNA-PmRab7 was employed to investigate whether knockdown of the Rab7 gene resulted in inhibition of TSV replication in *Litopenaeus vannamei*. The results showed an antiviral effect of knockdown of the LvRab7 gene in reduction of TSV replication.

Materials and methods

Shrimp culture

L. Vannamei juveniles (1–2 g) were obtained from shrimp farms in Nakhon Pathom and Cha-Cheung-Sao provinces, Thailand. Shrimp (10 shrimp per tank) were maintained in eight 50-liter tanks containing 30 liters of continuously aerated artificial saltwater at 10 ppt salinity. Shrimp were acclimatized for 2–3 days before dsRNA injection and fed with commercial feed ad libitum twice a day. Half of the saltwater was exchanged each day during this 7-day study.

Virus stock

TSV stock (Thai isolate) was kindly provided by Dr. Witoon Tirasophon, Mahidol University. The TSV inoculum was prepared from TSV-infected gill tissues according to a method described earlier [33] and was stored at −80°C. The viral nucleic acid was purified from the crude TSV stock lysate using a High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany) and subjected to reverse transcription (RT) and PCR to determine the viral titer using primers TSVF: 5′ GTA GTA TGG CAG CGA TTG GTG 3′ and TSVR: 5′ TTA AGC CAA TTC GGC AGG TCC 3′ designed from GenBank accession no. AY997025.1. The viral titer of TSV that caused 100% detection of TSV genome expression in shrimp within 2 days (10^6 copies) was used in this study.

Cloning of LvRab7 cDNA

If PmRab7 and LvRab7 share high nucleotide sequence identity, the plasmid pET17b-stRab7, which contains an inverted repeat sequence of PmRab7 [17], could be used to express dsRNA-PmRab7 to knock down either PmRab7 or LvRab7 mRNA. To determine whether the nucleotide sequence of LvRab7 is similar to PmRab7 or not, amplification of LvRab7 cDNA was performed using Rab7 gene-specific primers (GenBank accession number DQ231062) (5′ ATG GCA TCT CGC AAG AAG ATT 3′ and 5′ TTA GCA AGA GCA TGC ATC CTG 3′). The PCR product was gel-purified using Wizard® SV Gel and PCR clean-up system (Promega), cloned into pGEM-T Easy Vector and subjected to automated DNA sequencing (Macrogen, Korea).

Production of dsRNA-PmRab7

Double-stranded RNA prepared by *in vitro* transcription or by *in vivo* bacterial expression showed no difference in inhibition of gene expression [15]. The stem region of dsRNA-PmRab7, prepared by *in vivo* bacterial expression in this study, was at nucleotide position 246-639 of PmRab7. This corresponds to the same nucleotide position as dsRNA-PmRab7 produced by *in vitro* transcription in a previous study by Ongvarrasopone et al. [16]. In this study dsRNA-PmRab7 was expressed from *E. coli* HT115 transformed with plasmid pET-17b-StRab7 [17] and was purified according to the method described earlier [15]. Similarly, an unrelated dsRNA-GFP was also produced using an *in vivo* bacterial expression system [15]. The amounts of the dsRNAs were estimated by comparison with the standard DNA marker in agarose gel electrophoresis. The integrity of dsRNA-PmRab7 and dsRNA-GFP were analyzed by ribonuclease III and ribonuclease A digestion assay.

Injection of dsRNA-PmRab7 and virus

Shrimp (1–2 g, 10–20 shrimp per group) were injected with 30 µl dsRNAs into hemolymph using a 0.5-ml insulin syringe with a 29G x1/2" needle. A high (2.5 µg/g shrimp) or low dose (0.625 µg/g shrimp) of dsRNA-PmRab7 or dsRNA-GFP (an unrelated gene) was used. dsRNA was injected 48 h prior to TSV (10^6 copies) challenge. Injection of 150 mM NaCl and TSV inoculum alone were used as controls. Gill tissues from individual shrimp were collected

48 h after TSV challenge and stored at −80°C for subsequent analysis.

Total RNA extraction and RT-PCR analysis

Total RNA was extracted from gill tissues using Tri Reagents (Molecular Research Center) according to the manufacturer's instructions. The RNA concentration was determined by spectrophotometry at 260 nm. The absorbance ratio of A260 nm/A280 nm was between 1.8 and 2.0, indicating that the RNA samples were relatively pure. Total RNA (2.5 μg) extracted from individual gill sample was used to synthesize the first-strand cDNA using Impromp-II™ reverse transcriptase (Promega) for RT-PCR analysis [16], oligo-dT_{12} and TSV reverse primers (5′ TTA AGC CAA TTC GGC AGG TCC 3′). To determine the expression levels of LvRab7 and TSV, primer pairs for the LvRab7 (GenBank accession no. FJ811529) (5′ ATG GCA TCT CGC AAG AAG ATT 3′ and 5′ TTA GCA AGA GCA TGC ATC CTG 3′) and the TSV (920 bp) genes (primers TSVF and TSVR) were used. Actin (550 bp) primers: 5′ GAC TCG TAC GTC GGG CGA CGA GG 3′ and 5′ AGC AGC GGT GGT CAT CAC CTG CTC 3′ were used as an internal control to normalize for RNA loading. Multiplex PCR for TSV and actin genes and for LvRab7 was performed [16].

Quantitation of RT-PCR products

The resulting LvRab7 (618 bp), TSV (920 bp) and actin (550 bp) PCR products were analyzed by gel electrophoresis using a 1.5% agarose gel. The gel was stained with ethidium bromide and photographed using a short enough exposure time that the band intensities were not saturated. Densitometric scanning of the photograph was performed, and the band intensities were quantified using the Scion image analysis program. The signal was corrected for the background. The band intensity of TSV or LvRab7 of each sample was normalized against the band intensity of the actin of that sample to give the arbitrary units of the relative expression of TSV genome replication and LvRab7.

Statistical analysis

The relative expression of TSV or LvRab7 in each group was expressed as mean ± standard error of the mean (SEM) and plotted in the histogram. The mean of each group was used to calculate percent change when compared to the control group. Statistical analysis was performed using the ANOVA test in the Sigma Stat program. A statistically significant difference was measured at $p < 0.05$.

Results

Cloning of the coding region of LvRab7

The nucleotide sequences of the LvRab7 coding region were amplified from *L. vannamei* hemocyte cDNA, and the resulting amplicons sequenced and assigned GenBank accession number FJ811529. Comparison between LvRab7 and PmRab7 (GenBank accession number DQ231062) showed 96.3% and 100% nucleotide and amino acid sequence identity, respectively. There were 16 nucleotide differences between PmRab7 and LvRab7 in the stem region (corresponding to nucleotide position 185–579 of LvRab7, GenBank accession number FJ811529) that was used to produce dsRNA-PmRab7, and none of these changes affected the stem-loop structure.

Production of dsRNA-PmRab7

The plasmid pET17b-stRab7 was used to express dsRNA-PmRab7 to knock down LvRab7 mRNA. Double-stranded RNA of PmRab7 is about 400 bp long. In addition, two minor bands were observed at 300 bp and 200 bp. All of these bands can be cleaved by RNase III but cannot be cleaved by RNase A, suggesting that the dsRNA obtained for this study was of reasonable quality (Fig. 1). The two minor bands were due to the mismatched sequence between the sense and antisense stem region of the plasmid used to express dsRNA-PmRab7. In addition, an unrelated dsRNA, dsRNA-GFP, was used as a control. dsRNA-GFP is about 400 bp long. The quality of dsRNA-GFP is shown in Fig. 1.

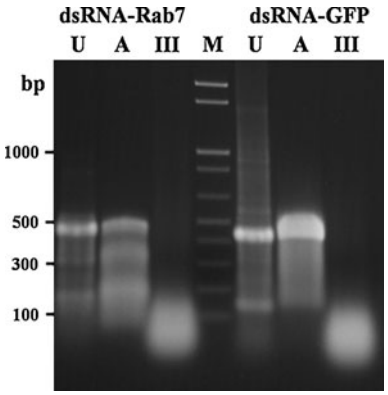

Fig. 1 Ribonuclease digestion assay of dsRNA-PmRab7 (left panel) and dsRNA-GFP (right panel). Undigested dsRNAs (3 μg) (lane U) were subjected to RNaseA (lane A) and RNase III (lane III) digestion and separated by 1.5 % agarose gel electrophoresis. Lane M is 1 kb plus DNA ladder (200 ng)

Suppression of TSV replication in LvRab7-knockdown shrimp

To investigate whether LvRab7 is essential for the TSV trafficking pathway, dsRNA-PmRab7 was introduced 48 h before TSV challenge. Its silencing effect was determined 48 h after TSV challenge. Large differences in TSV genome expression could be observed in the NaCl->TSV-challenged group (Figs. 2a, 3a). This may be because some of the *L. vannamei* imported into Thailand were of the TSV-resistant strain [1, 32]. The results demonstrated that silencing of the endogenous gene, LvRab7, using a high dose (Fig. 2a) of dsRNA-PmRab7 (2.5 μg/g shrimp) resulted in a dramatic and significant ($p < 0.05$) reduction in TSV genome expression by 92% when compared to the group injected with TSV alone (Fig. 3a). Injection of dsRNA-GFP, an unrelated gene, showed a significant ($p < 0.05$) decrease in TSV genome expression of 73% (Fig. 3a). Administering a high dose of dsRNA-PmRab7 caused a significant ($p < 0.05$) reduction in LvRab7 expression by 90% in gill tissues (Figs. 2a, 3b). In addition, shrimp injected with a high dose of dsRNA-GFP showed a significant reduction ($p < 0.05$) in LvRab7 expression of approximately 58% when compared to the NaCl group and a 50% reduction when compared to the NaCl->TSV group. Expression of LvRab7 was not significantly different between the NaCl->TSV and NaCl groups (Fig. 3b). No TSV genome expression was detected in the group injected with 150 mM NaCl alone, suggesting that shrimp in this study were virus-free.

RNAi technology in invertebrates using a high dose of dsRNA-PmRab7 or an unrelated gene, dsRNA-GFP, may result in an off-target effect and nonspecific antiviral immunity. To test whether silencing of TSV by dsRNA-PmRab7 is specific and is not due to nonspecific antiviral immunity, shrimp injected with a low dose (Fig. 2b) of dsRNA-PmRab7 (0.625 μg/g shrimp) were included in the same set in the high-dose experiment. Therefore, the results could be compared with those from the NaCl- or TSV-challenged groups. The result (Figs. 2b, 3a) showed an effect on silencing of TSV genome expression similar to that obtained using a high dose of dsRNA-PmRab7. Interestingly, a low dose of dsRNA-PmRab7 can inhibit TSV genome expression approximately 90%, whereas, a low dose of dsRNA-GFP has no inhibitory effect (Figs. 2b, 3a) when compared to the group injected with TSV alone. Injection with a low dose of dsRNA-Rab7 resulted in a significant reduction (84%) of LvRab7 mRNA expression when compared with the NaCl-injected group (Figs. 2b, 3b). In contrast, the expression of LvRab7 in shrimp injected with a low dose of dsRNA-GFP was not different from that of the TSV- or NaCl-injected shrimp (Fig. 3b). Taken together, the

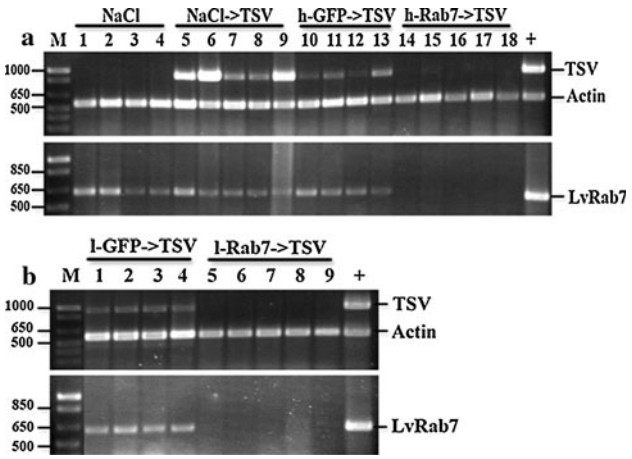

Fig. 2 Silencing of LvRab7 by dsRNA-PmRab7 inhibits TSV replication. A representative gel showing randomly selected RT-PCR products of TSV and actin (top panel) and LvRab7 (lower panel) mRNAs from gills of shrimp injected with a high (a) and low (b) dose of dsRNA. In panel a, shrimps were injected with 150 mM NaCl alone (lane 1–4), or NaCl followed by TSV injection (lane 5–9), a high (h) dose (2.5 μg/g shrimp) of dsRNA-GFP (lane 10–13) or dsRNA-PmRab7 (lane 14–18) 48 h before TSV challenge. In panel b, shrimps were injected with a low (l) dose (0.625 μg/g shrimp) of dsRNA-GFP (lane 1–4) or dsRNA-PmRab7 (lane 5–9) 48 h before TSV challenge. Each lane represents an individual shrimp. + represents the positive control for PCR. M is a 1 kb Plus DNA Ladder

results suggest that LvRab7 is involved in TSV replication in shrimp.

Discussion

Double-stranded RNA is a potent trigger in the RNAi pathway and is involved in posttranscriptional gene silencing. RNAi plays an important role in antiviral defense. In shrimp, dsRNA or siRNA corresponding to viral genes such as YHV protease and WSSV VP28, have been extensively studied as an antiviral defense to inhibit viral gene expression [19, 20, 25, 26, 31, 34]. Interestingly, dsRNA corresponding to PmRab7 inhibits endogenous PmRab7 and can prevent both DNA (WSSV) and RNA (YHV, LSNV) virus infection [16, 17], suggesting that PmRab7 may act as a common regulator required for virus replication in shrimp.

In this study, we have shown that suppression of LvRab7 by dsRNA-Rab7 can inhibit TSV replication. In order to demonstrate that inhibition of TSV genome expression is not due to an off-target effect of dsRNA, both high and low doses of dsRNA-Rab7 were used. The results showed that both high and low doses of dsRNA-Rab7 can specifically inhibit LvRab7 by approximately 90% and also inhibit TSV replication by 90%. These findings are not due to nonspecific antiviral effects resulting from the

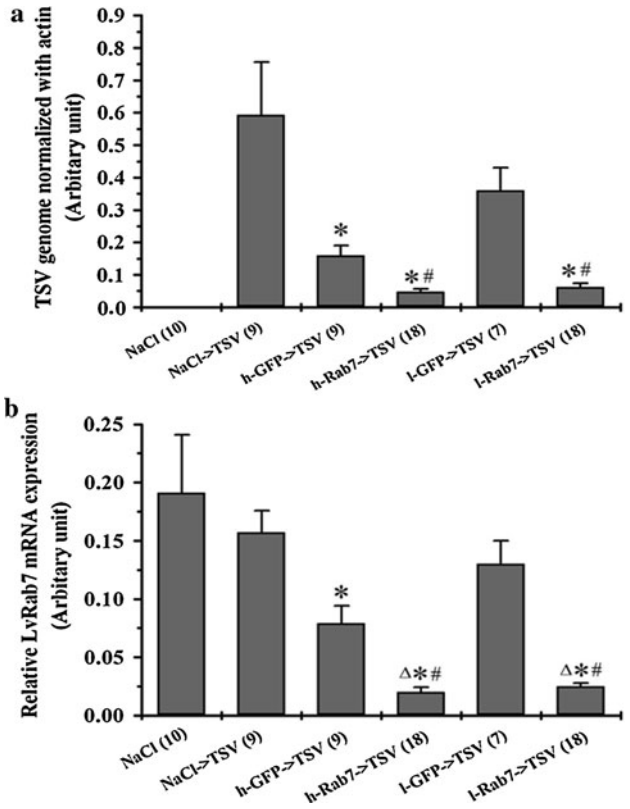

Fig. 3 Quantitation of the silencing effects of LvRab7 on the inhibition of TSV replication. TSV genome expression (a) and the relative LvRab7 mRNA expression (b) were normalized with actin and expressed as mean ± SEM. The number of shrimps used in each group is labeled in parentheses. Δ indicates a statistically significant difference at $p < 0.05$ between the NaCl group and the GFP->TSV or Rab7->TSV group. *Indicates a statistically significant difference at $p < 0.05$ between the NaCl->TSV group and the GFP->TSV or Rab7->TSV group. #Indicates a statistically significant difference at $p < 0.05$ between the GFP->TSV and Rab7->TSV groups

introduction of any dsRNA into shrimp. Injection of a high dose of dsRNA-GFP could also inhibit LvRab7 and TSV replication, whereas a low-dose injection of dsRNA-GFP did not provide protection against infection (Figs. 2, 3). It is known that long dsRNA can induce both sequence-dependent and sequence-independent antiviral immunity in marine shrimps [19, 34]. Inhibition of TSV genome replication by a high dosage of an unrelated dsRNA, like dsRNA-GFP, can be explained by the nonspecific antiviral defense in shrimp [18, 34]. In addition, nonspecific suppression of LvRab7 occurred by using a high dose of a non-homologous dsRNA such as dsRNA-GFP. Similarly, non-sequence-specific effects of dsRNA on gene expression have also been observed in insects, including silkworm cell lines [21]. The precise mechanism underlying this phenomenon remains unclear.

The sequence-specific inhibition of PmRab7 by dsRNA-PmRab7 in the current study resulted in inhibition of TSV replication, as was the case for WSSV, YHV and LSNV in previous infectivity studies. This suggests that PmRab7 is an important host-cell factor required for the replication of several viruses. Mammalian Rab7 plays a role in transporting the vesicle from an early to a late endosome and from a late endosome to a lysosome [5, 14, 27]. Rab7 is also important for lysosome biogenesis [27]. In addition, Rab7 is involved in sorting of virus and in the formation of transport vesicles. Expression of a dominant negative mutant of Rab7 caused virus accumulation in the early endosome [28, 30]. Similarly, knock-down of Rab7 in both *P. monodon* and *L. vannamei* possibly blocked the exit of several viruses that employed the endocytic trafficking pathway from early to late endosome or from late endosome to lysosome. In this scenario, the viral genomes (TSV, LSNV, YHV and WSSV) cannot be released from the endosome and replicate in the cytoplasm. Whether PmRab7 is involved in the endosomal trafficking pathway of viruses, similar to mammalian Rab7, remains to be elucidated.

Previous studies demonstrated that long-term suppression of PmRab7 by dsRNA–PmRab7 in *P. monodon* for 9 days had no effect on shrimp survival [16]. The half-life of Rab7 protein in shrimp cells has not been studied; however, the half-life of Rab7 in Hela cells is approximately 28 hours [7]. Therefore, it is most likely that Rab7 protein turns over rapidly in the cell. It is possible that LvRab7 protein levels are affected by the knockdown effect. Determination of LvRab7 protein expression cannot be performed due to the lack of an LvRab7 antibody. Knockdown of Rab7 by dsRNA-Rab7 resulted in a significant reduction in TSV replication (Fig. 3a). The onset of the TSVdisease depended on the TSV viral load. Therefore, the onset of disease is absent in the Rab7 knockdown shrimps. Reduced levels of PmRab7 expression in shrimp did not cause any morphological changes or deformities in test shrimp. Whether such therapy causes physiological disturbances in shrimp remains to be investigated. However, in vertebrates, mutation or dysfunction of Rab7 results in an endosomal trafficking disorder and causes several diseases [35]. Complete inhibition of PmRab7 expression in a long-term study may not be accomplished by using a single-injection approach. As an endogenous gene, PmRab7 can be recovered. The limitation of injecting dsRNA into shrimp is the longevity of dsRNA. The knockdown effect of dsRNA corresponding to the YHV protease gene prevents YHV infection for at least 5 days [34]. Therefore, multiple injections of dsRNA may be required to maintain the silencing effect. In addition, it would be interesting to treat shrimp with dsRNA-Rab7 in combination with other dsRNAs that target specific viral genes in order to completely inhibit viral gene expression and block the viral trafficking pathway. This could

potentially lead to the development of a sustainable virus therapy for shrimp. However, before this can occur, an efficient dsRNA delivery method for farmed shrimp needs to be developed. Delivery of dsRNA into shrimp through pelleted feed that contains dsRNA-producing bacteria or with a coating for improved dsRNA stability is one possibility. Oral delivery of a chitosan-nanoparticle-encapsulated, bacterially expressed dsRNA [22] has a potential use in farmed shrimp. Taken together, RNAi using dsRNA corresponding to the viral genes and the endogenous gene, Rab7, is a promising method that can be employed to effectively prevent or cure viral diseases in penaeid shrimp. This study supports the involvement of Rab7 in the replication process of several viruses that infect penaeid shrimp.

Acknowledgments The authors thank Asst. Prof. Dr. Witoon Tirasophon for the viral stock, Assoc. Prof. Dr. Albert Ketterman for critical reading of the manuscript, and Ms. Chawewan Chimawai and Ms. Pensri Hongthong for technical assistance. This work is supported by a Thailand Research Fund (TRF), Commission on Higher Education (CHE) and Mahidol University research grant. C.O. is the recipient of a TRF-CHE research career development grant (RMU4980004).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Argue B, Aree S, Lotz J, Moss S (2002) Selective breeding of pacific white shrimp (*Litopenaeus vannamei*) for growth and resistance to taura syndrome virus. Aquaculture 204:447–460
2. Assavalapsakul W, Smith DR, Panyim S (2006) Identification and characterization of a *Penaeus monodon* lymphoid cell-expressed receptor for the yellow head virus. J Virol 80:262–269
3. Attasart P, Kaewkhaw R, Chimwai C, Kongphom U, Namramoon O, Panyim S (2009) Inhibition of white spot syndrome virus replication in *Penaeus monodon* by combined silencing of viral rr2 and shrimp PmRab7. Virus Res 145:127–133
4. Bonami JR, Hasson KW, Mari J, Poulos BT, Lightner DV (1997) Taura syndrome of marine penaeid shrimp: characterization of the viral agent. J Gen Virol 78(Pt 2):313–319
5. Feng Y, Press B, Wandinger-Ness A (1995) Rab 7: an important regulator of late endocytic membrane traffic. J Cell Biol 131:1435–1452
6. 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 391:806–811
7. Ganley IG, Carroll K, Bittova L, Pfeffer S (2004) Rab9 GTPase regulates late endosome size and requires effector interaction for its stability. Mol Biol Cell 15:5420–5430
8. Hasson KW, Lightner DV, Poulos BT, Redman RM, White BL, Brock JA, Bonami JR (1995) Taura syndrome in *Penaeus vannamei*: demonstration of a viral etiology. Dis Aquat Org 23:115–126
9. Hasson KW, Lightner DV, Mohney LL, Redman RM, Poulos BT, White BM (1999) Taura syndrome virus (TSV) lesion development and the disease cycle in the Pacific white shrimp *Penaeus vannamei*. Dis Aquat Org 36:81–93
10. Jimenez R (1992) Sindrome de Taura (Resumen). Acuaculture del Ecuador. In: Jimenez R (ed) Revista Especializada De La Camara Nacianal De Acua Cultura, Guayquil, pp 1–16
11. Lightner DV (1996) A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp, Ringbound edition. World Aquaculture Society, Baton Rouge
12. Linda N, Wiwat S-o, Supapon C, Timothy WF (2005) Taura syndrome virus (TSV) in Thailand and its relationship to TSV in China and the Americas. Dis Aquat Org 63:101–106
13. Mari J, Poulos BT, Lightner DV, Bonami JR (2002) Shrimp Taura syndrome virus: genomic characterization and similarity with members of the genus Cricket paralysis-like viruses. J Gen Virol 83:915–926
14. Meresse S, Gorvel JP, Chavrier P (1995) The rab7 GTPase resides on a vesicular compartment connected to lysosomes. J Cell Sci 108(Pt 11):3349–3358
15. Ongvarrasopone C, Roshorm Y, Panyim S (2007) A simple and cost effective method to generate dsRNA for RNAi studies in invertebrates. ScienceAsia 33:35–39
16. Ongvarrasopone C, Chanasakulniyom M, Sritunyalucksana K, Panyim S (2008) Suppression of PmRab7 by dsRNA inhibits WSSV or YHV infection in shrimp. Mar Biotechnol (NY) 10:374–381
17. Ongvarrasopone C, Chomchay E, Panyim S (2010) Antiviral effect of PmRab7 knock-down on inhibition of Laem-Singh virus replication in black tiger shrimp. Antiviral Res 88:116–118
18. 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
19. Robalino J, Bartlett T, Shepard E, Prior S, Jaramillo G, Scura E, Chapman RW, Gross PS, Browdy CL, Warr GW (2005) Double-stranded 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
20. Robalino J, Bartlett TC, Chapman RW, Gross PS, Browdy CL, Warr GW (2007) Double-stranded RNA and antiviral immunity in marine shrimp: inducible host mechanisms and evidence for the evolution of viral counter-responses. Dev Comp Immunol 31:539–547
21. Sakashita K, Tatsuke T, Lee J, Kawaguchi Y, Kusakabe T (2009) Sequence-nonspecific suppression of gene expression by double-stranded RNA in silkworm cultured cells. J Insect Biotechnol Sericol 78:33–37
22. Sarathi M, Simon MC, Ahmed VP, Kumar SR, Hameed AS (2008) Silencing VP28 gene of white spot syndrome virus of shrimp by bacterially expressed dsRNA. Mar Biotechnol (NY) 10:198–206
23. Seabra MC, Mules EH, Hume AN (2002) Rab GTPases, intracellular traffic and disease. Trends Mol Med 8:23–30
24. Sritunyalucksana K, Wannapapho W, Lo CF, Flegel TW (2006) PmRab7 is a VP28-binding protein involved in white spot syndrome virus infection in shrimp. J Virol 80:10734–10742
25. Tirasophon W, Roshorm Y, Panyim S (2005) Silencing of yellow head virus replication in penaeid shrimp cells by dsRNA. Biochem Biophys Res Commun 334:102–107
26. Tirasophon W, Yodmuang S, Chinnirunvong W, Plongthongkum N, Panyim S (2007) Therapeutic inhibition of yellow head virus multiplication in infected shrimps by YHV-protease dsRNA. Antiviral Res 74:150–155
27. Vanlandingham PA, Ceresa BP (2009) Rab7 regulates late endocytic trafficking downstream of multivesicular body biogenesis and cargo sequestration. J Biol Chem 284:12110–12124
28. Vidricaire G, Tremblay MJ (2005) Rab5 and Rab7, but not ARF6, govern the early events of HIV-1 infection in polarized human placental cells. J Immunol 175:6517–6530

29. Vitelli R, Chiariello M, Bruni CB, Bucci C (1995) Cloning and expression analysis of the murine Rab7 cDNA. Biochim Biophys Acta 1264:268–270
30. Vonderheit A, Helenius A (2005) Rab7 associates with early endosomes to mediate sorting and transport of Semliki forest virus to late endosomes. PLoS Biol 3:e233
31. 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
32. Wyban J, Swingles J, Sweeney J, Pruder G (1993) Specific pathogen free *Penaeus vannamei*. World Aquaculture 24:39–45
33. Xu J, Han F, Zhang X (2007) Silencing shrimp white spot syndrome virus (WSSV) genes by siRNA. Antiviral Res 73:126–131
34. Yodmuang S, Tirasophon W, Roshorm Y, Chinnirunvong W, Panyim S (2006) YHV-protease dsRNA inhibits YHV replication in *Penaeus monodon* and prevents mortality. Biochem Biophys Res Commun 341:351–356
35. Zhang M, Chen L, Wang S, Wang T (2009) Rab7: roles in membrane trafficking and disease. Biosci Rep 29:193–209