Functional characterization of putative promoter elements from infectious hypodermal and hematopoietic necrosis virus (IHHNV) in shrimp and in insect and fish cell lines

Arun K. Dhar a, *, Dilip K. Lakshman b, Savithiry Natarajan c, F.C. Thomas Allnutt a,1, Nikolai A.M. van Beeka

a Advanced BioNutrition Corporation, 7155 Columbia Gateway Drive, Suite H, Columbia, MD 21046, United States
b USDA-ARS, FNPRU, Beltsville, MD 20705, United States
c USDA-ARS, Soybean Genomics and Improvement Laboratory, PSI, Beltsville, MD 20705, United States

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Abstract

Infectious hypodermal and hematopoietic necrosis virus (IHHNV) of shrimp contains a linear single-stranded DNA genome of approximately 4.1 kb with three putative open reading frames (ORFs) on the same DNA strand designated, the Left, Middle, and Right ORFs. The Left ORF codes for non-structural protein and the Right ORF codes for capsid protein, whereas the role of the Middle ORF is still unknown. Two putative promoters, designated P2 and P61, were detected upstream of the Left ORF and Right ORF, respectively. We evaluated the activities of these two promoters with or without a transcriptional enhancer element via the use of firefly luciferase reporter constructs in insect and fish cells, and in shrimp tail muscle. In insect and fish cells, the P2 promoter was stronger than the P61 promoter. The presence of the SV40 enhancer element negatively affected P2 but not P61 promoter activity in insect cells. However, in fish cells, the SV40 enhancer element dramatically increased the activities of both promoters. In shrimp, there was no significant difference in luciferase expression driven by these two promoters. In shrimp tail muscle, the presence of SV40 enhancer element in the construct had no significant effect on the P2 promoter and a negative effect on the P61 promoter. The IHHNV P2 and P61 promoters were found to be constitutive promoters that can drive gene expression in both invertebrate and vertebrate hosts.

Keywords: IHHNV; Shrimp viral promoter; Sf9 cells; Fish cells; Luciferase assay

1. Introduction

Infectious hypodermal and hematopoietic necrosis virus (IHHNV) is one of the major viruses infecting penaeid shrimp worldwide (Bell and Lightner, 1984; Lightner et al., 1996). The virus was first detected in Hawaii in 1981 where it caused a lethal disease (up to 90% mortality) in juvenile blue shrimp (Penaeus stylirostris) (Lightner et al., 1983) and was infectious to other penaeid species (Kalagayan et al., 1991; Primavera and Quinitio, 2000). In Penaeus vannamei and Penaeus monodon, IHHNV causes “Runt Deformity Syndrome” (RDS), which is characterized by reduced growth rates and a variety of cuticular deformities of the rostrum, antenna, thorax, and abdomen (Kalagayan et al., 1991; Primavera and Quinitio, 2000). IHHNV forms as icosahedral, non-enveloped particles measuring 22 nm in diameter (Bonami et al., 1990). The IHHNV genome consists of a single-stranded linear DNA (~4.1 kb) and, analogous to other members of the densovirus group of paroviruses, positive or negative DNA strands may be encapsidated, but not in the same virus particle (Kelly et al., 1977).

The paroviruses are single-stranded DNA viruses and known to contain two large ORFs, both on the same DNA strand. The first ORF covers much of the left half of the genome and encodes two non-structural (NS) proteins, NS1 and NS2, respectively, due to alternative splicing events (Muzy and Berns, 2001). NS1 is specifically required for replication. The sec-
ond large ORF on the right half of the viral DNA codes for coat proteins. Three co-amino-terminal coat proteins have been detected in parvovirions. The promoter element upstream of the NS1 ORF has been described as P4 and the corresponding promoter element of the right-hand ORF is named P38. NS1 is required for viral replication and has a role in the transactivation of the two viral promoters (Afanasiev et al., 1991, 1994). Negative regulation of the P38 promoter, possibly by a cellular factor, has also been reported (Muzy and Berns, 2001).

Viruses belonging to the subfamily Densovirinae of the family Parvoviridae attack arthropods. The physiochemical properties of densoviruses are similar to those of vertebrate parvoviruses, although the former group shares very little sequence homology with the later. Densoviruses have single-stranded genomes of 4–6 kb. In vertebrate parvoviruses and densoviruses of the genera Iteravirus and Brevidensovirus, the coding sequences of all viral proteins are located on one strand of the viral genome, which by convention is designated the viral minus strand (Ward et al., 2001). On the other hand, genus Densovirus of Densovirinae has a unique genome organization characterized by its ambisense structure (Muzy and Berns, 2001).

Brevidensoviruses, which include IHHNV and Aedes aegypti Densonucleosis virus (AeDNV), are viruses with 4 kb monosense genomes with unmatched terminal hairpins. The AeDNV has two non-structural proteins, which are coded from the same DNA sequence utilizing two different reading frames (Afanasiev et al., 1991, 1994). The structural proteins (VP1 and VP2) of AeDNV are encoded within the same ORF. VP2 may originate as a result of proteolytic cleavage of VP1 or the result of a different translation initiation codon (Ward et al., 2001). The IHHNV genome putatively codes for three ORFs (Shike et al., 2000). The putative polypeptide (666 aa) of the Left ORF has homology with mosquito densoviruses, and contains conserved replication initiator motifs, NTP-binding, and helicase domains with NS1 proteins from other parvoviruses. The Right ORF (329 amino acids) likely encodes structural proteins (VPs) as in other parvoviruses. The two putative IHHNV promoters (P2 and P61) are located upstream of the Left and Right ORFs, respectively (Fig. 1), and are presumed to regulate the expression of NS1 and VP genes, respectively (Shike et al., 2000). The function of the Middle ORF (363 amino acids) could not be assigned at present as it lacks homology with any other viral proteins from the GenBank database. Unlike mammalian parvoviruses, however, the core promoter structure of Densovirus is not well defined.

To determine the activities of the putative IHHNV promoters P2 and P61, we cloned these two promoters upstream of the firefly luciferase (luc) coding sequence in two transcription reporter vectors, pGL3-Basic and pGL3-Enhancer. These vectors only differ in that the latter carries the SV40 enhancer sequence downstream of the reporter gene. The resulting constructs were used to transfect insect cells (Spodoptera frugiperda cell line SF9), fish cells (EPC; Fijan et al., 1983), and shrimp (P. vannamei) tail muscle tissue to determine luciferase expression. The IHHNV promoters were demonstrated herein to be pantropic in nature and capable of driving expression of a heterologous gene in both invertebrate and vertebrate hosts.

![Fig. 1. Genome and promoter organization of IHHNV.](image)

(A) A schematic representation of the genome organization of IHHNV (based on GenBank accession number AF273615). The location of the two promoters, designated as P2 and P61, in the viral genome is indicated. The numbers along the bottom scale correspond to the nucleotide numbers of the sequence ORF. (B) The nucleotide sequence of P2 and P61 promoter regions of IHHNV indicating the putative TATA-box, activator element, A-rich region, and downstream promoter element (DPE). The putative initiation of transcription (Inr, C/A) is indicated by an ‘→’. and the conserved guanosine residue (G) at nucleotide position +24 is marked and bolded. The numbers in parenthesis indicate the nucleotide positions in the GenBank accession AF273215. ➔ indicates the inverted repeat region.

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2. Materials and methods

2.1. Plasmid construction

2.1.1. IHHNV promoters

The putative IHHNV P61 promoter region (GenBank Accession no: AF273215) was amplified by PCR using the primers: IHHNP61F: 5′GGTACCTCCAGCTGATGGTAAAGCT3′ (nucleotides 2347–2371) and IHHNP61R: 5′TTCGTATTTCTTGGAGAGTGCTTCAG3′ (nucleotides 2512–2488) as forward and reverse primers, respectively. The P61 amplicon was cloned into vector PCR2.1-TOPO (Invitrogen, Carlsbad, CA), and reamplified by PCR to generate SacI and NheI endonuclease restriction sites on the 5′ and 3′ termini of the amplicon, respectively. To synthesize the putative IHHNV P2 promoter region (nucleotides 21–110) flanked by SacI and NheI sites, 108-mer sense and antisense oligonucleotides were synthesized (The Midland Certified Reagent Co., Midland, TX). The sense and antisense oligonucleotides were annealed, then digested with SacI and NheI for insertion into the luciferase reporter vectors using DNA Ligation kit “Mighty Mix” (Takara, Shiga, Japan) according to the manufacturer’s recommendations.

2.1.2. Luciferase reporter plasmid constructs

Plasmids constructed for this study were based on pGL3-Basic and pGL3-Enhancer luciferase reporter vectors (Promega, Madison, WI). These vectors contain a modified luc coding region for firefly (Photinus pyralis) luciferase that has been optimized for evaluating transcriptional activity in transfected eukaryotic cells. Vector pGL3-Enhancer differs from the pGL3-Basic in that it contains a SV40 enhancer element downstream of the luciferase coding region (Fig. 2). The P2 and P61 promoters were inserted between the SacI and NheI sites of pGL3-Basic and pGL3-Enhancer vectors upstream of the luciferase coding sequence. The resulting plasmids were sequenced and those derived from pGL3-Basic containing the P2 and P61 promoter were designated as pSTI05 and pSTF, respectively. The sequenced plasmids derived from pGL3-Enhancer containing the P2 and P61 promoter were designated as pSTI and pSTH, respectively (Fig. 2). Plasmids were maintained in Escherichia coli strain JM109 (Invitrogen).

Two known promoters were used as controls for the luciferase reporter assays. These include the SV40 early promoter and the promoter for a shrimp antimicrobial peptide penaeidin 3-1 (PEN3-1; O’leary and Gross, 2006). The SV40 early promoter in pGL3-Basic (also called pGL3-Promoter Vector; Promega Corp.) and pGL3-Enhancer backbone (also called pGL3-Control Vector; Promega Corp.) were purchased from Promega. The PEN3-1 promoter region was amplified by PCR using total DNA isolated from the hemocytes of P. vannamei shrimp and the primers that were designed based on the published sequence (O’leary and Gross, 2006): PEN3F: 5′GCAATGGTACCTGGAAAATTTTAC GTT3′ and PEN3R: 5′GCATAGCCTTCGGAGGCTCCTCTCGCA3′. The underlined sequences indicate KpnI and HindIII restriction sites, respectively, with four additional nucleotides added to facilitate the restriction digestion. The PEN3-1 amplicon (approximately 200 bp) was digested with the enzymes KpnI and HindIII, and cloned at the homologous sites in pGL3-Basic and pGL3-Enhancer vectors upstream of the luciferase coding sequence, respectively. Recombinant clones were sequenced to validate the clones before further work.

2.2. Sequence analysis

The nucleotide sequence of the promoters P2, P61, SV40, and PEN3 were analyzed using both Neural Network Promoter Prediction (PPNN: http://www.fruitfly.org/seq_tools/promoter.html) and TRANSFAC Promoter signal scan (http://bimas.dcri.nih.gov:80/molbio/signal/) programs. The promoter sequences of other Brevidensoviruses, SV40, and penaeidin 3 (PEN3) were included in the analysis for comparison.

2.3. Sample preparation

2.3.1. Insect cells

Spodoptera frugiperda Sf9 cells were grown in TnM-FH medium (Sigma–Aldrich) supplemented with 10% FBS (fetal
bovine serum; Cambrex, Walkersville, MD). Cells in log phase were subjected to transfection experiments as follows: a 24-well tissue culture plate (Falcon, San Jose, CA) was seeded with 0.5 mL Sf9 cells per well at 5 × 10⁵ cells/mL. The plate was sealed with tape and placed in a plastic bag with a moist paper towel. After approximately 20 h, the medium was removed, cells washed once with serum-free TnM-FH, and then 0.5 mL transfection suspension was added to each well. Suspensions with Cellfectin (Invitrogen) as a transfection aid were prepared as follows: 4 μg plasmid DNA and 30 μL Cellfectin were combined in 400 μL serum-free TnM-FH and then incubated at RT for 35 min. The lipid-DNA mixture was diluted to 2 mL with serum-free TnM-FH containing streptomycin at 50 μg/mL. Three replicate wells were treated with plasmids containing the P2 or P61 controlled luciferase gene with and without the SV40 enhancer sequences. Appropriate control plasmids lacking a promoter were tested in the same manner. Sf9 cells were incubated for 1.5 h at 28 °C, then the transfection medium was removed and cells covered with 0.5 mL TnM-FH containing 10% FBS. Cells were harvested at 48 h by aspiration of the medium, treated for 15 min in 100 μL Cell Culture Lysis Buffer (Promega, Madison, WI), followed by removal of cell debris by centrifugation. The cell lysates were assayed immediately for luciferase activity and an aliquot of each sample was stored at −20 °C for total protein determination. Luciferase activity was then normalized to total protein content of each sample for analysis.

2.3.2. Fish cells

*Epithelioma papulosum cyprinid* cells (EPC) were grown at 24 °C in MEM medium supplemented with l-glutamine, antibiotic–antimycotic mixture (A5955; all from Sigma–Aldrich), and 10% FBS (Cambrex). Transfection and harvesting of the fish cells was done as described above for Sf9 cells except that MEM was used instead of TnM-FH.

2.3.3. Shrimp (*Penaeus vannamei*)

Specific pathogen-free *P. vannamei* shrimp (Kona line) were purchased from Marine Resources Research Institute, South Carolina Department of Natural Resources (Charleston, SC) and kept in artificial sea water at approximately 24 °C. Shrimp, weighing approximately 1.0–1.5 g, were injected laterally in the tail muscle (4th abdominal segment) with approximately 30 μL of a freshly prepared suspension consisting of 10 μg plasmid DNA in 20% glycerol and 0.9% NaCl. Animals were sacrificed at around 72 h post-injection, immediately frozen in liquid N₂, and then stored at −80 °C until assayed. Approximately 0.25 mg tissue, around the site of injection, was cut from each treated shrimp then manually homogenized with a sterilized pestle in a microcentrifuge tube in 200 μL Cell Culture Lysis Buffer (Promega), while kept on ice. The tissue homogenate was spun down and 50 μL of the resulting shrimp extract (supernatant) was assayed immediately for luciferase activity while the remainder was stored at −20 °C for protein determination. The results of two experiments were combined for analysis and data points from shrimp that did not show luciferase activity higher than the background signal plus two standard deviations were excluded from further analysis.

2.3.4. Luciferase assay

A 50 μL sample was assayed for luciferase activity using the Bright-Glo assay system (Promega) according to the manufacturer’s protocol. Luminescence measurements were performed in white, flat-bottomed 96-well microplates (Greiner, Bio-One, Longwood, FL) on a SpectraFluor Plus plate reader in the luminescence mode (using XFLUOR4 Version 4.50 Software; Tecan US, Research Triangle Park, NC). Readings were converted into luciferase equivalents via a calibration curve prepared by serial dilution of luciferase (Quantilum recombinant luciferase, Promega) prepared in lysates from uninfected Sf9 or EPC cells, or sham-injected shrimp. Data sets were compared using a student’s t-test with two-samples assumed to have an equal variance and a normal distribution.

2.3.5. Protein determination

Total soluble protein of all samples was determined according to the method of Lowry et al. (1951) with bovine serum albumin (BSA) as the protein in the calibration series. Luciferase activity values, as determined with the Bright-Glo assay, were normalized using the total protein content per sample. The luciferase expression was presented in parts per million or parts per billion.

3. Results and discussion

Studies in shrimp virology and functional genomics have been hampered by the lack of immortalized cell lines, effective promoters for expression of homologous or heterologous gene(s), transposons, regulatory genes, enhancers, or any of the myriad other tools necessary for effective directed manipulation in whole shrimp. This study characterized two shrimp virus promoters that will enhance this meager arsenal of tools for molecular studies in shrimp. In order to characterize these shrimp viral promoters, expression in both shrimp and other eukaryotic hosts was evaluated. Such information should help in the future application of these promoters in studies involving shrimp and potentially other systems due to their pantropic nature elucidated herein.

3.1. Sequence analysis of IHHNV promoters

The Left ORF and Right ORF promoters, P2 and P61, respectively, were reported initially by Shike et al. (2000). The Left ORF’s P2 promoter region (nucleotides 20–110) possesses the canonical TATA box (TATATAA, nucleotides 69–75) as shown diagrammatically in Fig. 1B. There is a GC-rich sequence GGAGCGCT (nucleotides 23–31) and a palindromic sequence ACCTATGACGTCATAGGT (nucleotides 49–66) is located downstream of the GC-rich activator region and upstream of the TATA box. An initiation of transcription motif (Inr) CAGT (nucleotides 100–103) is located 24 nucleotides downstream of the TATA box. The Inr sequence has been found to play an important role for the expression of many mammalian and arthropod promoters of both TATA containing and TATA-less types (Blissard et al., 1992; Cherbas and Cherbas, 1993; Smale and Baltimore, 1989). The CAGT sequence motif is known to
interact with cellular transcription factors such as TFIID (Smale and Baltimore, 1989). In addition to TATA and Inr motifs, there is a G residue (nucleotide 124) at position +24 from the transcription initiation site and a putative downstream promoter-like element (DPE) ATCC (nucleotides 128–132), starting at position +28 nucleotide. The G residue at position +24 of Drosophila core promoters was reported to result in a 2–4 fold higher level of basal transcription (Kutach and Kadonaga, 2000). There is an AP1 transcription factor binding site (TGAC) four nucleotides downstream of the TATA box. In a separate transcription mapping experiment we validated the A of the CAGT Inr box as the 5′-most nucleotide of the Left ORF mRNA (unpublished data).

The P61 promoter region (nucleotides 2347–2512) does not have a canonical TATA box (Shike et al., 2000). However, several lines of evidence suggest that this region could potentially serve as the promoter for the IHHNV Right ORF. There is an AT rich site (AAATAAAA; nucleotides 2409–2416), an Inr box (CAGT; nucleotides 2441–2444) 24 nucleotides downstream of the AT rich site, a G nucleotide (nucleotide 2465) at +24 position, and a downstream promoter element (DPE) starting at the +28 position (AGATG; nucleotides 2469–2473). In three Drosophila TATA-less promoters and in human TATA-less IRF-1 promoter, the GA/TCG DPE motif that was located about thirty nucleotides downstream of the transcription start site has essential promoter function and is known to bind the cellular transcription factor TFIID (Burke and Kadonaga, 1996). Similarly, the Inr motif CAGT is known to interact with TFIID (Antonucci et al., 1989). In fact, the TATA sequence upstream of the Inr box was dispensable for efficient gene expression of Aedes aegypticus densonucleosis virus (AeDNV) while mutation in the Inr motif reduced protein expression by 93% (Ward et al., 2001). Finally, in transcription mapping conducted in our laboratory it was confirmed that the A of CAGT motif as the 5′-most nucleotide of the Right ORF mRNA (unpublished data).

Alignment of the IHHNV P2 and P61 core promoter regions with the corresponding promoter regions of Brevidensoviruses from the mosquito Aedes albopictus (Boublik et al., 1994), Aedes albopictus cell line C6/36 Densovirus (Chen et al., 2004), AaeDNV (Afanasiev et al., 1991), AalDNV (Boublik et al., 1994), the SV40 early promoter (Benoin and Chambon, 1981; Rosenthal, 1987), and the P. vannamei PEN3-1 promoter (O’leary and Gross, 2006) can be seen in Fig. 3. The relative distance between TATA box and Inr box sequences is conserved in all cases. Those observations strengthened our initial delineation of the promoter regions of the IHHNV genome (Shike et al., 2000).

3.2. Luciferase expression in insect cells

The IHHNV promoters were functional in cells derived from S. frugiperda, a lepidopteran insect species, which is not a host for IHHNV. The luciferase expression results shown in Fig. 4 were typical. Negative control vectors did not express luciferase and were not significantly different from each other (p > 0.10). The P2 promoter-driven luciferase activity is approximately 2.5–3.5 fold higher (p < 0.001) than that observed using the P61 promoter in the pGL3-Basic vector backbone. Interestingly, the P2 promoter activity was negatively affected in presence of the SV40 enhancing element (p < 0.001), whereas the P61 activity was not significantly affected (p > 0.05) (Fig. 4). The SV40 early promoter (in both pGL3-Basic and pGL3-Enhancer backbone) and Pen3-1 promoter in pGL3 Basic vector did not drive luciferase expression in Sf9 cells. In a recent paper, Pfeifer et al. (1997) noted that the mammalian cytomegalovirus (CMV)
and SV40 early promoters did not function in the Sf9 insect cell lines. Schenborn and Goiffon (1997) noted that the SV40 early promoter and enhancer containing pGL3 vectors, also used in our studies, gave rise to negligible luciferase activity in Sf9 transfected cell lines. In addition, Gray and Cotes (2004) found that SV40 enhancer element allowed relatively low levels of expression of luciferase expression in Aedes albopictus cells. Studies in shrimp molecular virology have been greatly hampered due to the lack of a shrimp immortalized cell line. This ability of the IHHNV promoters to drive a reporter gene expression in insect cells provides a rationale for using the currently available insect cell lines as a platform for the study of promoters and other regulatory elements of IHHNV and potentially other shrimp viruses.

3.3. Fish cell expression

In EPC cells, both P2 and P61-containing constructs showed low levels of luciferase expression in the pGL3-Basic vector. But the level of expression was significantly higher \( (p < 0.001) \) than background. However, there was no significant difference \( (p > 0.05) \) between P2 and P61 driven expression (Fig. 5). The presence of the SV40 enhancer element increased the expression levels approximately 20 and 4 fold for the P2 and P61 promoters, respectively (Fig. 5). The expression driven by the P2 promoter in the presence of the SV40 enhancer element was significantly higher than that from the P61 promoter \( (p < 0.001) \). It is most likely that the SV40 enhancer element, a mammalian viral enhancer element, is functional in fish but poorly or not at all in invertebrates (insects and shrimp). The SV40 and PEN3-1 control promoters also showed luciferase activities above background \( (p < 0.001 \text{ for both}) \). The PEN3-1 promoter with the SV40 enhancer element had 6-fold higher luciferase expression compared to the same promoter based on pGL3-Basic (Fig. 5). We concluded that the IHHNV promoters function in fish cells but require enhancer elements for significant activity. The shrimp peneidin promoter (PEN3-1) also functions in fish cells and is positively affected by the presence of an enhancer element.

3.4. Shrimp muscle tissue transient expression

The results of shrimp tail muscle transient expression were highly variable, probably due to the number of animals and the usual variation between animals seen in the use of whole organisms (Fig. 6). The data clearly show that both the P2 and P61 promoters were functional in shrimp however the data were noisy. The presence of the SV40 enhancer element had no significant effect on the luciferase expression driven by the P2 promoter \( (p > 0.05) \). However, the SV40 enhancer element appeared to have a negative impact on expression of luciferase as driven by the P61 promoter when compared to the activity without the enhancer element, although the difference was not significant at the 95% confidence level \( (p > 0.05) \). Although the mean luciferase expression value when driven by the P61 promoter was higher than the corresponding mean value of the P2 promoter, the difference was not significant \( (p > 0.05) \) possibly due to high variability between individual
shrimp. Nevertheless, the results demonstrated that significant numbers of shrimp exhibited luciferase activity when injected with the IHNV promoter-driven luciferase constructs. Among the two control promoters used in this assay, SV40 early promoter did not provide significant luciferase expression, whereas P61 promoter worked although the activity appeared less than that provided by the IHNV P2 and P61 promoters. However, the differences in expression between the P2, P61, and PEN3 promoters were not significant at the 95% confidence level ($p > 0.05$). The PEN3 gene is known to be constitutively expressed in shrimp (Destoumieux et al., 2000).

Thus, our data showing a trend toward higher expression of luciferase driven by P2 and P61 promoters compared to the PEN3-1 promoter (Fig. 6) could be interpreted that the two viral promoters perform better than the PEN3-1 promoter in shrimp tissues. Further study will have to be done to confirm this finding.

The shrimp viral promoter-driven luciferase expression was less than 10,000 fold compared to the luciferase expression in insect cells and fish cells even though insects and fish are not hosts for this virus. This observation is most likely due to the differences in the efficiency of transfection of cultured cells (Sf9 and EPC) compared to in vivo transfection (shrimp). In shrimp, we have observed using the water-soluble dye C-phycoerythin (Martek Biosciences, Columbia, MD) that on injection in the tail segment muscle the dye is rapidly distributed throughout the shrimp body (data not shown). Therefore, it is reasonable to assume that part of the transfection suspension spreads rapidly throughout the shrimp body to diffuse the plasmid DNA from the site of DNA injection, thereby lowering the effective dose of DNA delivered to the cells being sampled in the shrimp tail muscle. Additionally, as we collected the tissue surrounding the site of injection to be used for the luciferase assay, probably not all transfected shrimp cells were included. Therefore, the amount of vector actually used for transfection of the shrimp tissue at the sampling site was, on a target tissue weight basis, much less than that used for the other comparable host systems. Additionally, the methods for in vivo shrimp cell transfection are not yet optimized. Therefore, we expect that the transfection efficiency was extremely low when compared to transfection in the well-defined Sf9 and EPC cell systems. Overall, the differences in promoter activity among the host systems recorded in this study might be caused by differences in efficiency of transfection, transcription, or a combination of the two.

In conclusion, our data indicate that both P2 and P61 are constitutive promoters that can drive gene expression in both invertebrate and vertebrate hosts. The differences in expression between these two promoters in each host system might be due to the difference in recognition of these promoter elements by the cellular transcriptional machinery of the host in addition to the differences in the transfection efficiency. In IHNV, the P2 promoter drives the expression of the non-structural gene (NS-1), whereas P61 drives the expression of the structural gene (Shike et al., 2000). Promoters for viral capsid genes are generally stronger than those for non-structural genes. However, in insect and fish cells the luciferase expression was higher when the gene was under the control of the non-structural gene promoter (P2) compared to its expression under the structural gene promoter (P61). It is possible that during IHNV replication the P2 promoter transcribes the NS-1 gene first and then the P61 promoter transactivates by the NS-1 protein. In mammalian parvoviruses, such as the minute virus of mice and the rodent parvovirus H-1, there is a temporal order of expression from the structural and non-structural gene promoters. In these viruses, the non-structural protein is expressed first, and this, in turn, transactivates the promoter for the structural gene (Doerig et al., 1988; Rhode, 1985). Transactivation of structural gene promoter by the viral non-structural protein has also been reported in the Aedes aegypti and Junonia coenia Dengue virus (Afanasiev et al., 1994; Giraud et al., 1992). These newly characterized shrimp viral promoters provide a sorely needed additional tool for the study of shrimp viruses and shrimp molecular biology and may even have broader applicability.

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