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Variable region of betanodavirus RNA2 is sufficient to determine host specificity

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ABSTRACT: Betanodaviruses, the causative agents of viral nervous necrosis in marine fish, have bipartite positive-sense RNA genomes. The viruses have been classified into 4 distinct types based on nucleotide sequence similarities in the variable region (the so-called T4 region) of the smaller genomic segment RNA2 (1.4 kb). Betanodaviruses have marked host specificity, although the primary structures of the viral RNAs and encoded proteins are similar among the viruses. We have previously demonstrated, using reassortants between striped jack nervous necrosis virus (SJNNV) and redspotted grouper nervous necrosis virus (RGNNV), that RNA2, which encodes the coat protein, strictly controls host specificity. However, because RNA2 is large, we were unable to propose a mechanism underlying this RNA2-based host specificity. To identify the RNA2 region that controls host specificity, we constructed RNA2 chimeric viruses from SJNNV and RGNNV and tested their infectivity in the original host fish, striped jack *Pseudocaranx dentex* and sevenband grouper *Epinephelus septemfasciatus*. Among these chimeric viruses, SJNNV mutants containing the variable region of RGNNV RNA2 infected sevenband grouper larvae in a manner similar to RGNNV, while RGNNV mutants containing the variable region of SJNNV RNA2 infected striped jack larvae in a manner similar to SJNNV. Immunofluorescence microscopic studies using anti-SJNNV polyclonal antibodies revealed that these chimeric viruses multiplied in the brains, spinal cords and retinas of the infected fish, as in infections by the parental viruses. These results indicate that the variable region of RNA2 is sufficient to control host specificity in SJNNV and RGNNV.

KEY WORDS: Betanodavirus · Host specificity · Viral nervous necrosis · Chimeric virus · RNA2 · T4 region · SJNNV · RGNNV

INTRODUCTION

Betanodaviruses, members of the family *Nodaviridae*, are the causative agents of a highly destructive disease of hatchery-reared larvae and juveniles of several varieties of marine fish. The disease, designated viral nervous necrosis (VNN) when it was first described in 1990 (Yoshikoshi & Inoue 1990), is also known as viral encephalopathy and retinopathy (Office International des Epizooties 2006). VNN has spread to more than 30 marine fish species from 14 families in the Indo-Pacific and Mediterranean regions, Scandinavia and North America. Adult fish have also been reported to suffer from the disease in some species (Munday et al. 2002). The viruses localize in the brain, spinal cord and retina of the affected fish. Affected fish exhibit erratic swimming patterns and a range of neurological abnormalities, including vacuolization and cellular necrosis in the central nervous system and retina.

Betanodaviruses are nonenveloped, spherical viruses with a bipartite positive-sense RNA genome. The larger genomic segment, RNA1 (3.1 kb), encodes an

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RNA-dependent RNA polymerase (protein A). The smaller genomic segment, RNA2 (1.4 kb), encodes the coat protein (CP). A subgenomic RNA (RNA3) transcribed from RNA1 encodes protein B2, which has a suppressor function in posttranscriptional gene silencing (Iwamoto et al. 2005, Fenner et al. 2006). A genetic engineering system of betanodaviruses has already been established based on a cDNA-mediated infectious RNA transcription strategy (Iwamoto et al. 2001, 2004). Furthermore, qualitative and quantitative analysis of virus multiplication is possible with betanodaviruses, using the E-11 cell line (Iwamoto et al. 2000) cloned from SSN-1 cells (Frerichs et al. 1996). Based on nucleotide sequence similarities in the variable RNA2 region (the so-called T4 region; Nishizawa et al. 1995), which encodes the C-terminal half of the CP, betanodaviruses have been classified into 4 distinct types: striped jack nervous necrosis (SJNNV), redspotted grouper nervous necrosis virus (RGNNV), barfin flounder nervous necrosis virus (BFNNV) and tiger puffer nervous necrosis virus (TPNNV) (Nishizawa et al. 1997). The host ranges of SJNNV and TPNNV are limited to striped jack *Pseudocaranx dentex* and tiger puffer *Takifugu rubripes*, respectively. However, recently, SJNNV was found in European sea bass *Dicentrarchus labrax*, sea bream *Sparus aurata* and Senegalese sole *Solea senegalensis* farmed in the Iberian Peninsula, though the samples used were not specified as diseased fish (Thiéry et al. 2004, Cutrín et al. 2007). BFNNV has been isolated from some coldwater species, such as halibut *Hippoglossus hippoglossus* and turbot *Scophthalmus maximus*. RGNNV has a broad host range and causes disease among a variety of warm-water fish, particularly groupers and sea bass (Munday et al. 2002). Taken together, these data suggest that the variable region includes the host-specificity determinants of betanodaviruses.

Our experimental infections of sevenband grouper *Epinephelus septemfasciatus* and striped jack larvae with RGNNV and SJNNV revealed that the viruses have strict host specificity (Iwamoto et al. 2004). Furthermore, similar infection experiments using reassortants constructed from SJNNV and RGNNV demonstrated that RNA2 and/or the encoded CP controls host specificity in these viruses (Iwamoto et al. 2004). However, we did not identify the RNA2 regions that were sufficient to determine host specificity in betanodaviruses. The necessary RNA2 regions may differ among the 4 types of viruses because the viruses have different host ranges. In this study, to identify the host-specificity-determining region in RNA2, we constructed several chimeric viruses, the RNA2 segments of which consisted of both SJNNV and RGNNV sequences, and tested their infectivity in striped jack and sevenband grouper larvae.

MATERIALS AND METHODS

Plasmids. The plasmids used or constructed in this study are listed in Table 1. These plasmids contain the cDNAs of SJNNV (SJNag93 strain), RGNNV (SGWak97 strain) (Iwamoto et al. 1999) and the chimeric viruses constructed from SJNNV and RGNNV. SJNag93 and SGWak97 were isolated from striped jack and sevenband grouper, respectively. For the construction of pSG2TK13B, a nucleotide substitution of A for T was made by replacing a portion of the original cDNA with a mutated sequence produced using a polymerase chain reaction (PCR)-based method. Similarly, a series of chimeric viral cDNAs (Table 1, Fig. 1) was constructed by replacing the original cDNA sequences with corresponding heterologous viral cDNAs synthesized by PCR. All the recombinant DNA

Table 1. Plasmids used in this study

Plasmid	Characteristics	Source
Wild type viral cDNA		
pSJ1TK19	Full-length cDNA of SJNNV RNA1	Iwamoto et al. (2001)
pSJ2TK30	Full-length cDNA of SJNNV RNA2	Iwamoto et al. (2001)
pSG1TK5	Full-length cDNA of RGNNV RNA1	Iwamoto et al. (2004)
pSG2TK13	Full-length cDNA of RGNNV RNA2	Iwamoto et al. (2004)
Mutated or chimeric viral cDNA		
pSG2TK13B	1057th T in pSG2TK13 is replaced by A	This study
pRGSJ2Afl	ca. 440 bp *Afl*III–*Bsp*EI fragment in pSG2TK13B is replaced by that from pSJ2TK30	This study
pRGSJ2Bsp	ca. 360 bp *Bsp*HI–*Bsp*EI fragment in pSG2TK13B is replaced by that from pSJ2TK30	This study
pRGSJ2Bam	ca. 300 bp *Bam*HI–*Bsp*EI fragment in pSG2TK13B is replaced by that from pSJ2TK30	This study
pRGSJ2Pvu	ca. 260 bp *Pvu*II–*Bsp*EI fragment in pSG2TK13B is replaced by that from pSJ2TK30	This study
pSJRG2Afl	ca. 440 bp *Afl*III–*Bsp*EI fragment in pSJ2TK30 is replaced by that from pSG2TK13B	This study
pSJRG2Bsp	ca. 360 bp *Bsp*HI–*Bsp*EI fragment in pSJ2TK30 is replaced by that from pSG2TK13B	This study
pSJRG2Bam	ca. 300 bp *Bam*HI–*Bsp*EI fragment in pSJ2TK30 is replaced by that from pSG2TK13B	This study

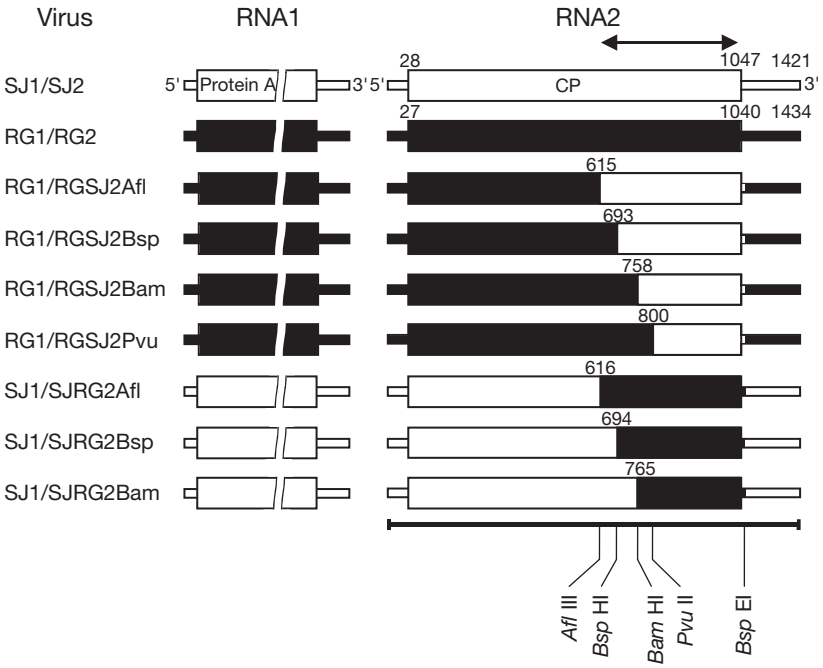

Fig. 1. Genome structures of chimeric viruses. Open boxes and white lines represent ORFs and noncoding RNAs of SJNNV, respectively. Similarly, filled boxes and black lines indicate ORFs and noncoding RNAs of RGNNV, respectively. The line at the bottom represents the physical map of viral cDNAs. Nucleotide positions of start codons, stop codons, 3′ ends, and chimeric junctions are shown on the diagrams. The nucleotide positions of *Bsp* EI restriction sites are 1061 (SJ1/SJ2), 1054 (RG1/RG2), 1060 (RG1/RGSJ2Afl), 1060 (RG1/RGSJ2Bsp), 1054 (RG1/RGSJ2Bam), 1054 (RG1/RGSJ2Pvu), 1055 (SJ1/SJRG2Afl), 1055 (SJ1/SJRG2Bsp) and 1061 (SJ1/SJRG2Bam). Two-headed arrow directs the variable region that has been used for the classification of betanodaviruses (Nishizawa et al. 1997)

techniques used in this study are described in Sambrook & Russell (2001). The nucleotide sequences of the constructed cDNAs were verified by DNA sequencing.

RNA transfection and virus inoculation of cultured fish cells. The synthesis of infectious transcripts from the viral cDNAs (Table 1) and the transfection of E-11 cells with these transcripts were performed to produce mature viruses (Table 2), as described previously (Iwamoto et al. 2001), with some exceptions. After *in vitro* transcription, RNA was purified using the RNeasy Mini Kit (Qiagen) and was transfected into E-11 cells using Lipofectamine 2000 (Invitrogen). Virus multiplication was then confirmed by the appearance of cytopathic effects. The culture supernatants were stored at −80°C before use. For the virus multiplication assay, E-11 cells were inoculated with each of the viruses (Table 2) at a multiplicity of infection of 1.0 and incubated at 23°C. The culture supernatants were collected periodically and their viral titers were measured by determining the 50% tissue culture infectious dose ($TCID_{50}$) ml^{-1} (Reed & Muench 1938).

Virulence assay in fish larvae. One day old striped jack and sevenband grouper larvae, reared at Kamiura Station of National Research Institute of Aquaculture, Fisheries Research Agency, were used for the virulence assays of parental and chimeric viruses, as described previously (Iwamoto et al. 2004). Approximately 50 striped jack and 250 sevenband grouper larvae were immersed with $10^{4.0}$ and $10^{4.7}$ $TCID_{50}$ ml^{-1} of viruses, respectively, in each treatment. Throughout the experiments, the larvae were kept at 23°C in glass beakers containing 1 l of seawater supplemented with 5 µg ml^{-1} kanamycin sulfate. Control fish were exposed under the same conditions to 100 µl of cell culture supernatant obtained from uninoculated E-11 cells. After exposure, dead or moribund fish were collected, fixed with 10% formalin and embedded in paraffin. Sections were subjected to immunofluorescence staining, as described previously (Iwamoto et al. 2004).

Table 2. Viruses used in this study

Virus	Viral cDNA clone[a] RNA1	RNA2	Source
SJ1/SJ2	pSJ1TK19	pSJ2TK30	Iwamoto et al. (2001)
RG1/RG2[b]	pSG1TK5	pSG2TK13	Iwamoto et al. (2004)
RG1/RGSJ2Afl	pSG1TK5	pRGSJ2Afl	This study
RG1/RGSJ2Bsp	pSG1TK5	pRGSJ2Bsp	This study
RG1/RGSJ2Bam	pSG1TK5	pRGSJ2Bam	This study
RG1/RGSJ2Pvu	pSG1TK5	pRGSJ2Pvu	This study
SJ1/SJRG2Afl	pSJ1TK19	pSJRG2Afl	This study
SJ1/SJRG2Bsp	pSJ1TK19	pSJRG2Bsp	This study
SJ1/SJRG2Bam	pSJ1TK19	pSJRG2Bam	This study

[a]*In vitro* transcripts from the indicated viral cDNAs were co-transfected into E-11 cells to obtain the viruses
[b]RG1/RG2 is described as SG1/SG2 in Iwamoto et al. (2004)

Verification of progeny viruses. Total RNA was extracted from dead or moribund fish collected after exposure to virus and used as the template for reverse transcription-PCR. For infections with SJ1/SJ2, SJ1/SJRG2Afl, or SJ1/SJRG2Bsp, first-strand cDNA was synthesized using the SJ2Rid primer (5′-ACAGGTT CGGCGAGGTAAGC-3′), followed by PCR amplification using the SJRGF1 (5′-GCAACTCGTGGTGCAGTC-3′) and SJ2R6 (5′-CCTTGGGTGCTTTGTCGTT-3′) primers. Similarly, for infections with RG1/RG2, RG1/RGSJ2Afl, or RG1/RGSJ2Bsp, first-strand cDNA synthesis was performed using the RG2Rid primer (5′-CGCTTCCAGC-CGTGTATAGG-3′) and PCR amplification was performed with SJRGF1 and RG2R3 (5′-TAGCGAGTGATT CAATTTCGC-3′). The amplified DNA was sequenced using the same PCR primers.

RESULTS

Construction of chimeric viral cDNAs

To test the hypothesis that the variable RNA2 region may be involved in determining host specificity in betanodaviruses, we produced a series of chimeric viruses, in which a part of the RNA2 sequence was replaced with the corresponding sequence of another virus. RG1/RGSJ2Afl, RG1/RGSJ2Bsp, RG1/RGSJ2Bam and RG1/RGSJ2Puv are RGNNV mutants in which RNA2 was replaced, in part, with that of SJNNV (Fig. 1). Similarly, SJ1/SJRG2Afl, SJ1/SJRG2Bsp and SJ1/SJRG2Bam are SJNNV mutants containing parts of the RGNNV RNA2 sequence. As indicated by the names of the chimeric RNA2 segments, RGSJ2Afl and SJRG2Afl, RGSJ2Bsp and SJRG2Bsp, and RGSJ2Bam and SJRG2Bam, were constructed symmetrically (Fig. 1).

Table 3. Multiplication of the parental and chimeric viruses in E-11 cells. E-11 cells were inoculated with each of the parental and chimeric viruses. Viral titers of the culture supernatants were measured at the given time points. Data are means from 2 independent experiments

Virus	Viral titer (log $TCID_{50}$ ml^{-1})		
	Day 0	Day 4	Day 6
SJ1/SJ2	3.6	7.5	8.8
RG1/RG2	3.6	8.5	8.8
RG1/RGSJ2Afl	2.6	6.7	8.0
RG1/RGSJ2Bsp	3.5	6.6	8.0
RG1/RGSJ2Bam	3.7	6.0	7.5
RG1/RGSJ2Pvu	3.2	6.2	7.3
SJ1/SJRG2Afl	3.6	8.5	8.4
SJ1/SJRG2Bsp	2.8	7.4	8.0
SJ1/SJRG2Bam	1.8	6.3	7.7

Multiplicative competence of the chimeric viruses

To evaluate multiplicative competence of the chimeric viruses, they were inoculated into E-11 cells and the viral titers of the culture supernatants were measured periodically. The titers of the chimeric viruses ranged from $10^{6.0}$ to $10^{8.5}$ $TCID_{50}$ ml^{-1} 4 d after inoculation and from $10^{7.3}$ to $10^{8.4}$ $TCID_{50}$ ml^{-1} 6 d after inoculation (Table 3). These titers were the same or slightly lower than those of the parental viruses. These experiments were carried out at 23°C, which was also used for the immersion assay of fish larvae described below.

Infectivity of the chimeric viruses in host fish

The infectivity of the chimeric viruses was examined by challenging striped jack or sevenband grouper larvae with each of the viruses. All of the striped jack larvae challenged with RG1/RGSJ2Afl, RG1/RGSJ2Bsp, or SJ1/SJ2 died within 4 d of inoculation (Fig. 2). The viruses had multiplied in the brains, spinal cords and retinas of the dead striped jack larvae (Fig. 3). In contrast, the numbers of dead fish after challenge with RG1/RGSJ2Bam, RG1/RGSJ2Pvu or RG1/RG2 were similar to the numbers seen in the controls (Fig. 2). There was no evidence of virus multiplication in these dead fish (Fig. 3). Challenge of sevenband grouper larvae with SJ1/SJRG2Afl and RG1/RG2 resulted in 100% mortality within 4 d (Fig. 4). While challenge with SJ1/SJRG2Bsp produced a mean cumulative mortality of 76% 5 d after inoculation. These 3 viruses had multiplied in the brains, spinal cords and retinas of the dead sevenband grouper larvae (Fig. 5). Conversely, challenge with SJ1/SJRG2Bam, RG1/RGSJ2Bsp and SJ1/SJ2 resulted in low levels of mortality similar to the levels seen in the control groups. No multiplication of these viruses was observed in any of the dead fish (Fig. 5). Collectively, these results demonstrate that RG1/RGSJ2Bsp and SJ1/SJRG2Bsp retain the complete host-specificity determinants for striped jack and sevenband grouper, respectively. RG1/RGSJ2Bam and SJ1/SJRG2Bam must lack some RNA2 sequences important for host recognition because these 2 chimeric viruses did not infect their corresponding host fish. This important RNA2 region in SJNNV (nucleotides [nt] 695–765) and RGNNV (nt 694–758) showed 60% homology at the nucleotide level and 55% homology at the amino acid level (data not shown). Verification of the viral RNA purified from the dead or moribund fish showed that all of the replaced RNA2 regions (Fig. 1) were completely conserved in the progeny viruses (data not shown).

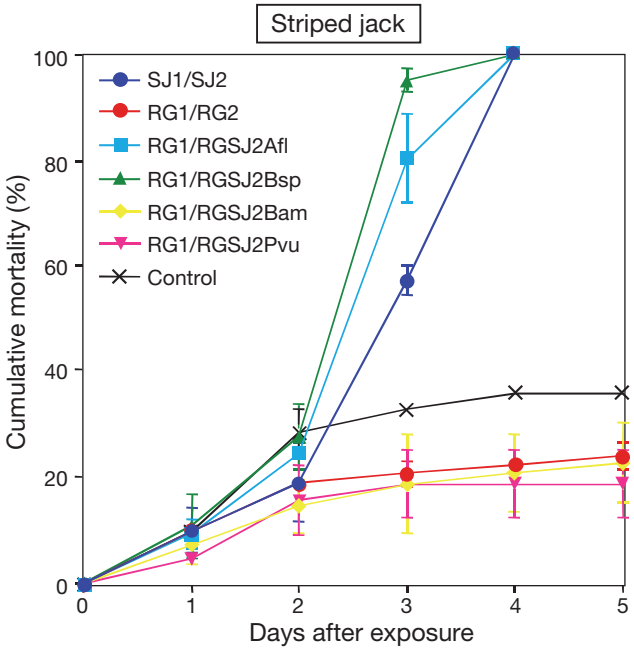

Fig. 2. *Pseudocaranx dentex*. Cumulative mortality of striped jack larvae exposed to parental or chimeric viruses. Striped jack larvae were bath challenged with each of the viruses at $10^{4.0}$ $TCID_{50}$ ml^{-1}. Control fish were exposed to tissue culture supernatant from uninfected E-11 cells. Data are means ± SD of 2 independent experiments

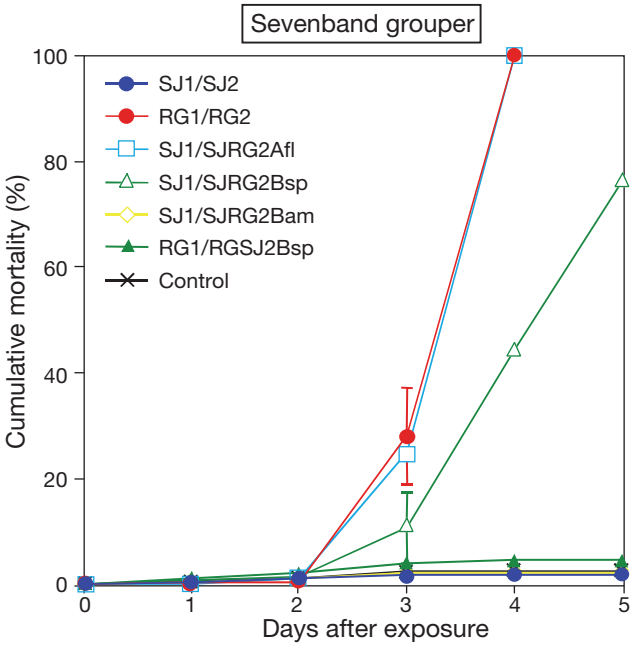

Fig. 4. *Epinephelus septemfasciatus*. Cumulative mortality of sevenband grouper larvae exposed to parental or chimeric viruses. Sevenband grouper larvae were bath challenged with each of the viruses at $10^{4.7}$ $TCID_{50}$ ml^{-1}. Control fish were exposed to tissue culture supernatant from uninfected E-11 cells. Data are means ± SD of 2 independent experiments

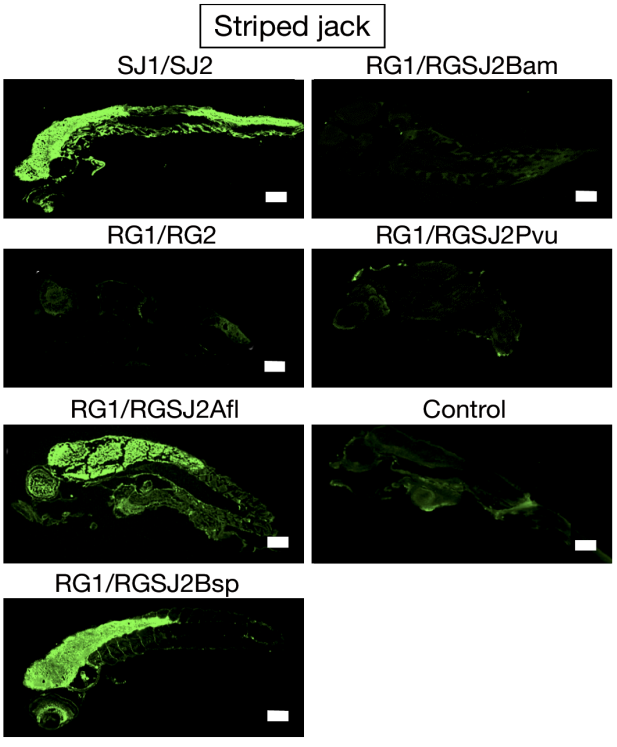

Fig. 3. *Pseudocaranx dentex*. Immunofluorescence staining of viral antigens in striped jack larvae inoculated with parental or chimeric viruses. Dead or moribund fish were collected and sectioned for immunofluorescence staining. Scale bars = 100 µm

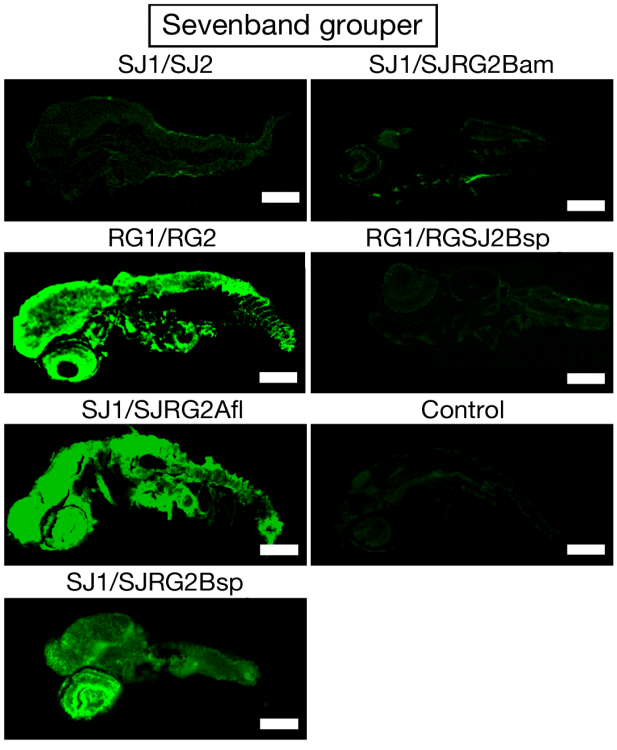

Fig. 5. *Epinephelus septemfasciatus*. Immunofluorescence staining of viral antigens in sevenband grouper larvae inoculated with parental or chimeric viruses. Dead or moribund fish were collected and sectionedfor immunofluorescence staining. Scale bars = 100 µm

DISCUSSION

We constructed chimeric viruses from SJNNV and RGNNV to test their infectivity in their original hosts. We demonstrated that, of these chimeric viruses, SJNNV mutants retaining the variable region of RGNNV RNA2 (nucleotides [nt] 694–1054) infected sevenband grouper larvae showing neurotropism, as does RGNNV (Figs. 1, 4 & 5). In contrast, RGNNV mutants containing the variable region of SJNNV RNA2 (nt 695–1061) infected striped jack larvae, similar to infection by SJNNV (Figs. 1, 2 & 3). Interestingly, these 2 regions are almost the same as the T4 sequence (Fig. 1) (Nishizawa et al. 1997) and are located in the same RNA2 position when the SJNNV and RGNNV RNA2 sequences are aligned (authors' unpubl. data). These results indicate that SJNNV and RGNNV use the same RNA2 and/or encoded CP regions to recognize their own hosts, although the 2 viruses have different host ranges. As mentioned earlier, betanodaviruses have been classified into the 4 types according to similarities in the T4 nucleotide sequences: SJNNV, RGNNV, BFNNV and TPNNV (Nishizawa et al. 1997). These types of viruses seem to have different host ranges according to their originated fish species, though the borders among their host ranges are not very clear. For example, both RGNNV and SJNNV are isolated from Japanese flounder *Paralichthys oliveatus* (Munday et al. 2002) and SJNNV was found in European sea bass, sea bream and Senegalese sole (Thiéry et al. 2004, Cutrín et al. 2007). Therefore, the RNA2 and/or CP regions may function as one of the major host-specificity determinants among all the 4 types of viruses. Our data do not eliminate the possibility that RNA1 as well as RNA2 control host specificity of SJNNV and RGNNV at a high or low temperature because RNA1 controls predominantly the temperature sensitivity of these viruses (authors' unpubl. data).

Cryo-electron microscopy analysis and folding motif analysis of an RGNNV isolate, malabaricus grouper nervous necrosis virus, suggested that the C-terminal region of CP is presented on the surfaces of virions, as well as on CP monomers (Tang et al. 2002). The N-terminal region of the betanodavirus CP, which contains many positively charged amino acid residues, may play a role in neutralizing the negative charge of viral RNA in virions and also could be involved in the encapsidation process (Marshall & Schneemann 2001, Tang et al. 2001). Furthermore, the pairwise surface probability plot for SJNNV and RGNNV CPs showed some significant differences in the values within the host-specificity-determining region observed in this study, which suggests structural differences between SJNNV and RGNNV CPs (Iwamoto et al. 2004). Therefore, those structurally unique sites are good candidates for host-specificity determinants, although there is no evidence that the CP, rather than the viral RNA, controls host specificity. In this study, we did not define the structural differences that determine host specificity in SJNNV and RGNNV. Nevertheless, our inoculation experiments revealed that at least 1 determining site was encoded by nt 694–758 in RGNNV RNA2 and nt 695–765 in SJNNV RNA2 (Fig. 1) because the chimeric viruses required these regions to infect their appropriate hosts (Figs. 2 to 5).

In many viruses, host specificity and tissue tropism are controlled by the interactions of viral surface proteins with related receptors that are displayed on the surfaces of target cells (Baranowski et al. 2001, Smith & Helenius 2004). Such a receptor binds to a specific attachment site(s) on a virus particle and facilitates viral entry into the cell. For example, the receptors of nonenveloped poliovirus and coxsackievirus bind to the clefts of the capsid surface (Rossmann et al. 2002). Even a small number of amino acid substitutions in their CPs dramatically change the host specificity and tissue tropism of these viruses (Baranowski et al. 2001). Similarly, in enveloped viruses, surface viral proteins serve as interacting factors for related receptors and control cell specificity (Baranowski et al. 2001). Recently, SSN-1 fish cells (Frerichs et al. 1996) were shown to have receptor-like molecules for RGNNV on their surfaces (Lu et al. 2003). The sialic acid moiety on the receptor-like molecule is required for the binding of SSN-1 cells to virus particles (Liu et al. 2005). Because SSN-1 cells are susceptible to all the 4 types of betanodaviruses (Iwamoto et al. 1999), the putative receptor should be able to bind the 4 kinds of virus particles and induce viral entry into the cells. Thus, betanodaviruses presumably infect SSN-1 cells through a putative nonspecific sialic acid receptor on the cell surface. Therefore, the host specificity of SJNNV and RGNNV observed in this study should be controlled by different specific receptors, each of which occurs exclusively in striped jack or sevenband grouper, if this specificity is based on virion-receptor interactions. To evaluate the plausibility of the virus-receptor interaction model in determining host specificity in betanodaviruses, in our next study we may prepare primary cultured cells from striped jack and sevenband grouper to test their bindings with parental and chimeric virus particles. Alternatively, host specificity may be directed by intracellular host factors that are expressed only in the susceptible hosts and support virus multiplication. Such specific factors might be involved in many virus multiplication processes, including uncoating, genome replication, viral protein translation, viral protein processing, encapsidation and viral diffusion.

Generally, the attenuation of host defense mechanisms, including immune systems and posttranscriptional gene silencing, by viral factors is important for virus multiplication in target host cells (Alcami & Koszinowski 2000, Weber et al. 2004, Buchon & Vaury 2006). Thus far, nothing is known about the role of betanodavirus CP in the inhibition of host defense systems, although CP molecules are translated in the cell cytoplasm and transferred, in part, into the nuclei of infected cultured cells (Guo et al. 2003). Nevertheless, we cannot rule out the possibility that CP monomers or virions interact with putative host-specific factors, leading to the inhibition of the defense apparatus in fish, thus determining host specificity.

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LITERATURE CITED

Alcami A, Koszinowski UH (2000) Viral mechanisms of immune evasion. Trends Microbiol 8:410–418

Baranowski E, Ruiz-Jarabo CM, Domingo E (2001) Evolution of cell recognition by viruses. Science 292:1102–1105

Buchon N, Vaury C (2006) RNAi: a defensive RNA-silencing against viruses and transposable elements. Heredity 96: 195–202

Cutrín JM, Dopazo CP, Thiéry R, Leao P, Olveira JG, Barja JL, Bandín I (2007) Emergence of pathogenic betanodaviruses belonging to the SJNNV genogroup in farmed fish species from the Iberian Peninsula. J Fish Dis 30:225–232

Fenner BJ, Thiagarajan R, Chua HK, Kwang J (2006) Betanodavirus B2 is an RNA interference antagonist that facilitates intracellular viral RNA accumulation. J Virol 80: 85–94

Frerichs GN, Rodger HD, Peric Z (1996) Cell culture isolation of piscine neuropathy nodavirus from juvenile sea bass, *Dicentrarchus labrax.* J Gen Virol 77:2067–2071

Guo YX, Dallmann K, Kwang J (2003) Identification of nucleolus localization signal of betanodavirus GGNNV protein alpha. Virology 306:225–235

Iwamoto T, Mori K, Arimoto M, Nakai T (1999) High permissivity of the fish cell line SSN-1 for piscine nodaviruses. Dis Aquat Org 39:37–47

Iwamoto T, Nakai T, Mori K, Arimoto M, Furusawa I (2000) Cloning of the fish cell line SSN-1 for piscine nodaviruses. Dis Aquat Org 43:81–89

Iwamoto T, Mise K, Mori K, Arimoto M, Nakai T, Okuno T (2001) Establishment of an infectious RNA transcription system for striped jack nervous necrosis virus, the type species of the betanodaviruses. J Gen Virol 82: 2653–2662

Iwamoto T, Okinaka Y, Mise K, Mori K, Arimoto M, Okuno T, Nakai T (2004) Identification of host-specificity determinants in betanodaviruses by using reassortants between striped jack nervous necrosis virus and sevenband grouper nervous necrosis virus. J Virol 78:1256–1262

Iwamoto T, Mise K, Takeda A, Okinaka Y and others (2005) Characterization of striped jack nervous necrosis virus subgenomic RNA3 and biological activities of its encoded protein B2. J Gen Virol 86:2807–2816

Liu W, Hsu CH, Hong YR, Wu SC and others (2005) Early endocytosis pathways in SSN-1 cells infected by dragon grouper nervous necrosis virus. J Gen Virol 86:2553–2561

Lu MW, Liu W, Lin CS (2003) Infection competition against grouper nervous necrosis virus by virus-like particles produced in *Escherichia coli.* J Gen Virol 84:1577–1582

Marshall D, Schneemann A (2001) Specific packaging of nodaviral RNA2 requires the N-terminus of the capsid protein. Virology 285:165–175

Munday BL, Kwang J, Moody N (2002) Betanodavirus infections of teleost fish: a review. J Fish Dis 25:127–142

Nishizawa T, Mori K, Furuhashi M, Nakai T, Furusawa I, Muroga K (1995) Comparison of the coat protein genes of five fish nodaviruses, the causative agents of viral nervous necrosis in marine fish. J Gen Virol 76:1563–1569

Nishizawa T, Furuhashi M, Nagai T, Nakai T, Muroga K (1997) Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. Appl Environ Microbiol 63:1633–1636

Office International des Epizooties (2006) Viral encephalopathy and retinopathy. In: Manual of diagnostic tests for aquatic animals. Office International des Epizooties, Paris, p 169–175

Reed LJ, Muench H (1938) A simple method of estimating fifty percent end points. Am J Hyg 27:493–497

Rossmann MG, He Y, Kuhn RJ (2002) Picornavirus-receptor interactions. Trends Microbiol 10:324–331

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Smith AE, Helenius A (2004) How viruses enter animal cells. Science 304:237–242

Tang L, Johnson KN, Ball LA, Lin T, Yeager M, Johnson JE (2001) The structure of pariacoto virus reveals a dodecahedral cage of duplex RNA. Nat Struct Biol 8:77–83

Tang L, Lin CS, Krishna NK, Yeager M, Schneemann A, Johnson JE (2002) Virus-like particles of a fish nodavirus display a capsid subunit domain organization different from that of insect nodaviruses. J Virol 76:6370–6375

Thiéry R, Cozien J, de Boisséson C, Kerbart-Boscher S, Névarez L (2004) Genomic classification of new betanodavirus isolates by phylogenetic analysis of the coat protein gene suggests a low host-fish species specificity. J Gen Virol 85:3079–3087

Weber F, Kochs G, Haller O (2004) Inverse interference: how viruses fight the interferon system. Viral Immunol 17: 498–515

Yoshikoshi K, Inoue K (1990) Viral nervous necrosis in hatchery-reared larvae and juveniles of Japanese parrotfish, *Oplegnathus fasciatus* (Temminck & Schlegel). J Fish Dis 13:69–77

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