

Genomic sequence of mandarin fish rhabdovirus with an unusual small non-transcriptional ORF

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Abstract

The complete genome of mandarin fish *Siniperca chuatsi* rhabdovirus (SCRV) was cloned and sequenced. It comprises 11,545 nucleotides and contains five genes encoding the nucleoprotein N, the phosphoprotein P, the matrix protein M, the glycoprotein G, and the RNA-dependent RNA polymerase protein L. At the 3' and 5' termini of SCR genome, leader and trailer sequences show inverse complementarity. The N, P, M and G proteins share the highest sequence identities (ranging from 14.8 to 41.5%) with the respective proteins of rhabdovirus 903/87, the L protein has the highest identity with those of vesiculoviruses, especially with Chandipura virus (44.7%). Phylogenetic analysis of L proteins showed that SCRV clustered with spring viremia of carp virus (SVCV) and was most closely related to viruses in the genus *Vesiculovirus*. In addition, an overlapping open reading frame (ORF) predicted to encode a protein similar to vesicular stomatitis virus C protein is present within the P gene of SCR. Furthermore, an unoverlapping small ORF downstream of M ORF within M gene is predicted (tentatively called orf4). Therefore, the genomic organization of SCR can be proposed as 3' leader-N-P/C-M-(orf4)-G-L-trailer 5'. Orf4 transcription or translation products could not be detected by northern or Western blot, respectively, though one similar mRNA band to M mRNA was found. This is the first report on one small unoverlapping ORF in M gene of a fish rhabdovirus.

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1. Introduction

Siniperca chuatsi rhabdovirus (SCRV) was isolated from the mandarin fish which is an economically important fish widely cultured in China. It had been confirmed that SCR can coinfect with *Siniperca chuatsi* spherical virus (SCSV) in cultured fish cells and cause cytopathic effect (CPE) (Zhang and Li, 1999a,b; Zhang et al., 2005). Recently, SCR was separated from the coinfecting viruses by plaque isolation and the physico-chemical characteristics were determined (Tao et al., 2007).

Rhabdoviruses are widely distributed in nature and at least 175 rhabdoviruses have been isolated from vertebrates, invertebrates and plants (Springfeld et al., 2005) till now. Rhabdoviruses are usually nonsegmented, single-strand and negative-sense RNA viruses except the Orchid fleck virus

whose genome is bipartite (Kondo et al., 2006), and are currently assigned as six genera: *Vesiculovirus*, *Lyssavirus*, *Ephemerovirus*, *Novirhabdovirus*, *Cytorhabdovirus* and *Nucleorhabdovirus* (Tordo et al., 2004). Fish rhabdoviruses are classified in the genera *Vesiculovirus* and *Novirhabdovirus*. The genome sizes of rhabdoviruses range from 11 to 15 kb which could encode five major functional structure proteins, namely the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G) and the RNA-dependent RNA polymerase protein (L) in the order 3' N-P-M-G-L 5'.

Complete genomic sequences of fish rhabdoviruses have been determined for spring viremia of carp virus (SVCV) (Hoffmann et al., 2002), infectious hematopoietic necrosis virus (IHNV) (Morzunov et al., 1995; Schutze et al., 1995), viral hemorrhagic septicemia virus (VHSV) (Schutze et al., 1999), snakehead rhabdovirus (SHRV) (GenBank Acc. No. NC_000903) and hiram rhabdovirus (HIRRV) (Kim et al., 2005). Compared with other known fish rhabdoviruses, however, the molecular characteristics of SCR are less well understood and limited to the N gene

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(Tao et al., 2007). Multiple sequence alignment has shown that the N protein of SCRV shares 6.9–41.5% identity with other rhabdoviruses and phylogenetic analysis has indicated SCRV was most closely related to Vesiculoviruses. To better investigate the genomic structure and organization of SCRV, and the relationships between SCRV and other rhabdoviruses, and possible to trace the genome evolution trends among different rhabdoviruses, it is necessary to obtain the complete genomic sequence of SCRV. In this study, we present the cloning and analysis of the complete genomic sequence of SCRV. Furthermore, one unusual ORF was identified at the mRNA and protein level by northern and Western blots, respectively.

2. Materials and methods

2.1. Virus propagation, purification and RNA preparation

Siniperca chuatsi rhabdovirus (SCRV) was plaque cloned from co-infecting virus samples and the virus was cultured in the grass carp fins (GCF) cells. The procedures of virus propagation and purification were according to previous reports (Zhang et al., 2006; Tao et al., 2007). The virus pellet was resuspended in TE buffer (10 mM Tris–Cl, 1 mM EDTA, pH 7.4) and stored at -20°C until use. SCRV RNA was extracted from purified virions by Trizol reagent (Invitrogen, USA) according to the manufacture's instructions. The resulting RNA was resuspended in DEPC-treated water and stored at -80°C until use.

2.2. Primer design and cloning strategy

For the cloning of the phosphoprotein (P), matrix protein (M) and glycoprotein (G) genes, specific primers were designed according to our own sequence determination and the conserved gene junction sequence of vesiculoviruses. Fragments L1 and L2 of the RNA-dependent RNA polymerase protein (L) gene were products of non-specific amplification of N and M genes. The primers used for amplification of L3 were designed according to the sequences of the G gene and fragment L1. The fragment L4 was amplified by primers designed according to the sequences of fragments L2 and L3. L5, L6 and L7 were amplified by 5'RACE. Primers used for cloning the complete genome are listed in Table 1 and the cloning strategy is shown in Fig. 1.

2.3. Reverse transcription and polymerase chain reaction

cDNA was synthesized in a 25- μl reaction volume containing 100 pmol forward primer, $1\times$ M-MLV RT reaction buffer (50 mM Tris pH 8.3, 75 mM KCl, 10 mM dithiothreitol, 3 mM MgCl_2), 50 μM dNTPs, 200 U M-MLV reverse transcriptase (Promega), 40 U of RNase inhibitor and 2- μl RNA extracted above. The reaction was performed at 42°C for 1 h. PCR reactions were performed using the temperature profiles within the following ranges, depending on expected product size and primer sequence: 94°C for 3 min, followed by 35 cycles of 30 s at 94°C , 30–60 s at $55\text{--}62^{\circ}\text{C}$, 30–120 s at 72°C . For final extension, tubes were incubated at 72°C for 8 min. Products were analyzed by electrophoresis on agarose gels and visualized by ethidium bromide staining.

2.4. Determination of the 5' end of the genome

The 5' end of the genome was amplified as three overlapping clones L5, L6 and L7. cDNA was synthesized as described above by using L5F1 primer (Table 1). The resulting cDNA was extracted with phenol/chloroform/isoamylalcohol, and precipitated with ethanol. The purified cDNA was poly (C) tailed by terminal deoxynucleotidyl transferase (TdT) (TaKaRa, Japan) at 37°C for 30 min and incubated at 90°C for 5 min to inactivate TdT. The first round of PCR was performed using the primers L5F1 and 5' AP. The second round of PCR was carried out using internal primer pairs L5F2 and 5' UP. Fragments L6 and L7 were amplified by the same method as used for amplification of fragment L5.

2.5. DNA sequencing and sequence analysis

The PCR products were purified using a DNA Extraction Kit (Fermantas), cloned into pMD-18T vector (TaKaRa, Japan) and transformed into *Escherichia coli* DH5 α . Positive clones were sequenced using a 377 or 3730 DNA sequencer (Bioasia Biotechnology Company, Shanghai, China).

Nucleotide sequence and deduced amino acid sequences were analyzed using the EditSeq program (DNASTAR, USA). Multiple sequence alignment was conducted using the Clustal X 1.83 program and the sequence identities were calculated using the

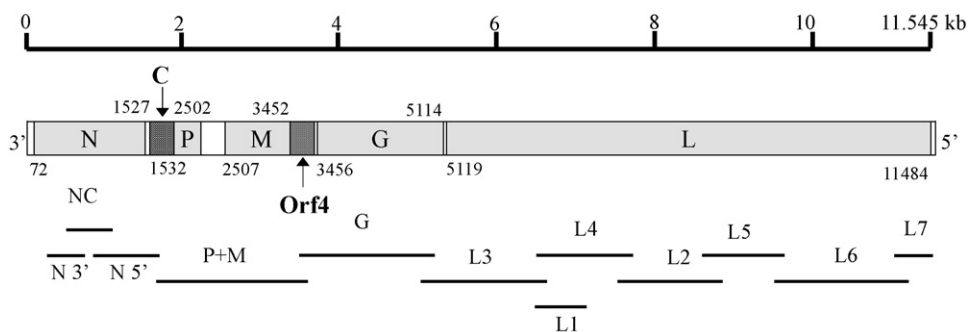


Fig. 1. Cloning strategy of SCRV genome and deduced SCRV genome structure. The location and relative size of the SCRV genes are shown. The numbers indicate the start and end of the respective genes. The short lines below show the cDNA clones used during the course of cloning the complete genome. The shadow frameworks marked by small arrows reveal the deduced C protein and orf4 position.

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