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Identification and characterization of a novel FstK-like protein from spotted knifejaw iridovirus (genus *Megalocytivirus*)

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ABSTRACT

Prokaryotes contain many DNA binding proteins with large molecular weights and multiple domains. DNA binding proteins are involved in DNA replication, transcription, and other physiological processes. In this study, a DNA binding protein, containing an Ftsk-like protein (FLP) domain, was cloned and characterized from SKIV-ZJ07, a member of the RSIV-type megalocytivirus, using bioinformatics and molecular biology approaches. SKIV-FLP is 3762 base pairs long, encodes a viral protein of 1253 amino acid residuals, and contains an Ftsk (or EBV-NA3) and a Grx-2 domain. Virion localization indicated that SKIV-FLP is a major viral structural protein located below the major capsid protein. Laser confocal microscopy showed that SKIV-FLP is a cytoplasm-/nuclear-localized protein. However, the reconstruction experiments demonstrated that SKIV-FLP may contain three nuclear localization signals, each present in FLP-NT (1–380 aa), FtsK domain (380–880 aa), and Grx-2 domain (880–1253 aa). When SKIV-FLP was fused to the Gal-4 DNA-binding domain and co-transfected with L8G5-Luc, SKIV-FLP suppressed L8G5-Luc transcription. As a transcription inhibitor, SKIV-FLP also inhibited the transcription of NF-κB and IFN-γ (a type II IFN) promoter in HEK293T cells, suggesting that SKIV-FLP has a role in evading host immunity.

1. Introduction

Iridoviruses are large double-stranded DNA viruses that infect poikilotherms, including fish, amphibians, reptiles, and insects (Jancovich et al., 2012; Williams et al., 2005). The viruses that infect vertebrates are collectively called piscine iridoviruses, which include lymphocytivirus, ranavirus, and megalocytivirus (Chinchar et al., 2005). In the past decade, megalocytivirus has become one of the most alarming disease causative agents in the bony fish aquaculture industry worldwide (Kurita and Nakajima, 2012; Marcos-López et al., 2011; Subramaniam et al., 2012; Waltzek et al., 2012). Megalocytivirus-associated disease outbreaks have resulted in significant economic losses in public aquaria, food fish, and ornamental fish industries, as well as wild fish stock endangerment (Chinchar et al., 2009; Kurita and Nakajima, 2012). In the past several years, great advancements have been achieved in understanding the epidemiology, pathology, and genomics of megalocytiviruses; however, knowledge of their viral protein profiles is still very limited (Shuang et al., 2013; Xu et al., 2010). Recently, structural proteins of two megalocytiviral strains have been determined using comprehensive proteomic approaches (Dong et al., 2011; Shuang et al., 2013). Both viral proteomic approaches have shown that the mature megalocytiviral virions contain 38 to 49 viral proteins in purified viral particles, which include approximately 20 highly abundant viral structural proteins. Among these viral proteins, a rock bream iridovirus ORF058L (RBIV-ORF058L) homologous protein has been identified as the largest abundant viral structural protein (Dong et al., 2011; Shuang et al., 2013).

In RBIV, 118 potential non-overlapping open reading frames with coding capacities for polypeptides ranging from 50 to 1253 amino acids have been identified through computer-assisted analysis of its complete genomic DNA sequence; among which, RBIV-ORF058L encodes the largest viral protein, with 1253 aa length, which may act as a DNA-binding protein, according to a bioinformatics analysis (Do et al., 2004). The Blast program from the NCBI Conserved Domain Database indicates that RBIV-ORF058L contains an FtsK-like domain (380–496AA) or an EBNA-3 domain (397–561AA) at a similar site. Furthermore, according to a domain forecast program (http://myhits.isb-sib.ch), RBIV-ORF058L also contains a Grx-2 domain (880–980AA). Given that RBIV-ORF058L and its homologs in other megalocytiviruses contain an FtsK-like domain, these viral proteins are designated as FtsK-like proteins (FLP) in this study.

Abbreviations: aa, amino acid; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; CPE, cytopathic effects; DBP, DNA binding protein; DMEM, Dulbecco's modified Eagle's medium; EGFP, enhanced green fluorescent protein; FLP, FtsK-like proteins; hpi, hour post infection; IFA, immunofluorescence assay; ISKNV, infectious spleen and kidney iridovirus; MCP, major capsid protein; MMP, myristylated membrane protein; NBT, nitroblue tetrazolium; RBIV, rock bream iridovirus; RSIV, red seabream iridovirus; SGIV, Singapore grouper iridovirus; TRBIV, Turbot reddish body iridovirus.

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Proteins with FtsK domains have multiple biological functions, such as the implementation of bacterial chromosome separation, cell division, viral packaging, and separation of particles (Capiaux et al., 2002; Goldberg et al., 2003; Wang et al., 2006). In phages and herpesviruses, Ftsk domain-containing proteins are evolutionarily related and belong to an FtsK-HerA superfamily of ATPases (Iyer et al., 2004; Przech et al., 2003). The advent of the direct testing of translocation models helps provide mechanistic insight into these systems and other systems of nucleic acid translocation (Maluf and Feiss, 2006). The EB-NA3 domain has a structure similar to that of EBNA-3 proteins in the Epstein–Barr virus. The protein families are nuclear antigens, including EBNA-3A, -3B, and -3C, and EBNA-3C, which have been reported to exhibit transcriptional regulation activity (Arad et al., 2011; Hickabottom et al., 2002). Studies have indicated that the proteins are essential for evading host immunity by sustaining proliferation and through the immunoblastic transformation of B-lymphocytes (Hickabottom et al., 2002). Glutaredoxin protein contains a Grx domain and functions as an electron carrier in the glutathione-dependent synthesis of deoxyribonucleotides through the enzyme ribonucleotide reductase. These proteins have been widely studied in prokaryotic and eukaryotic organisms (Guagliardi et al., 1995; McFarlan et al., 1992; Minakuchi et al., 1994; Morell et al., 1995). In the vaccinia virus, a glutaredoxin homolog participates in the form of a viral structure (Ahn and Moss, 1992). Although RBIV-ORF058L has been previously predicted to be a DNA-binding protein in megalocytiviral genome (Do et al., 2004), it is still poorly understood despite further studies on its protein property. In the current study, an RBIV-ORF058L homologous protein was cloned and characterized from a virulent megalocytiviral strain, SKIV-ZJ07, and was designed as SKIV-FLP. The virion and subcellular localizations of SKIV-FLP and its mutants were determined. The functions of SKIV-FLP were also primarily analyzed. This study provides information for further understanding viral protein characteristics and their functions in megalocytiviruses and other iridoviruses.

2. Materials and method

2.1. Cell lines, virus and antibodies

Mandarin fish fry (MFF-1) cells were developed and characterized in our laboratory, and were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS, Gibco) at 25 °C (Dong et al., 2008). HeLa cells and HEK293T cells were cultured in DMEM with 10% FBS in an incubator at 37 °C, 95% humidity, and 5% CO_2. Megalocytiviral strain SKIV-ZJ07 was isolated from diseased cage-cultured spotted knifejaw (*Oplegnathus punctatus*) using MFF-1 cells, and then characterized and kept in our laboratory (Dong et al., 2010; Shuang et al., 2013). Rabbit anti-ISKNV-rMCP and -rMMP sera were prepared and kept in our laboratory (Dong et al., 2011).

2.2. Cloning and phylogenetic analysis of SKIV-FLP

Total genomic DNA from SKIV-ZJ07 infected MFF-1 cells was prepared as template for RBIV-ORF058L homolog amplification using a primer set of FLP-NP-F and FLP-NP-R (Table 1). The PCR products were cloned into pGEM-T easy vector (Promega, USA) for sequencing using an ABI 3770 sequencer (Applied Biosystems, USA). Comparison and phylogenetic analysis of FLP were performed using the MegAlign program of the DNASTAR software. The Clustal method was used to correct the distances for multiple substitutions at a single site. Some megalocytiviruses whose whole genomes are known were selected for phylogenetic analysis.

2.3. Expression and antibody preparation of SKIV-FtsK (380–880 aa) fragment

According to the obtained full length of SKIV-FLP (accession number: HM246185), a primer set (Ftsk-L & pET-FLP-R, Table 1) was designed to clone and express SKIV-FtsK (380–880 aa) in the pET32a expression vector, as previously described (Dong et al., 2011). The recombinant His-FtsK was subsequently purified through affinity chromatography on Ni-NTA Superflow resin (Qiagen, Germany), according to the instructions of the manufacturer. Protein concentration was determined using the Bradford assay; the purified proteins were stored at −80 °C until use. For the antibody preparation, 0.5 mg of purified recombinant His-SKIV-FtsK was emulsified with an equal volume of Freund's complete adjuvant for the first immunization and Freund's incomplete adjuvant (FIA) for the three following immunizations. A rabbit received the three subcutaneous immunizations at 2-week intervals. Blood samples were obtained on the tenth day after the last immunization for sera collection.

2.4. Purification and fractionation of SKIV-ZJ07

SKIV-ZJ07 was purified from infected-MFF-1 cells through gradient sucrose density centrifugation, as described previously (Shuang et al., 2013). The purified virus was resolved in TMN buffer [150 mM NaCl, 2 mM $MgCl_2$, and 20 mM Tris–HCL (pH 7.5)], and then stored at −80 °C until use. The fractionation of the purified virions was prepared according to a previous description on the treatment of ISKNV (Dong et al., 2011). In brief, 100 μl of 2% Triton X-100 was added to an equal volume of purified SKIV-ZJ07 suspension, and was mixed thoroughly. After incubation for 3 min at room temperature, the mixture was centrifuged at 20,000 ×*g* for 30 min at 4 °C. The supernatant was removed and kept until use. The pellet was resolved in 200 μl of TMN buffer.

Table 1
Primer sets used in this work.

Primer	Orientation	Nucleotide sequences	Target[a]
FLP-NP-F	Sense	5′-ATGGCAGAAGGTGGAATGAAGCCT-3′	Whole length of SKIV-FLP
FLP-NP-R	Antisense	5′-CTACGACTCTTCGGCTGAGCTTTTAG-3′	
FLP-QT-F	Sense	5′-AGCCAGGGACATACGACCACA-3′	Q-PCR for SKIV-FLP
FLP-QT-R	Antisense	5′-CTCTGCCGCATAATCCTTCACA-3′	
Actin-QT-F	Sense	5′-GTACGTCGCCCTGGACTTCG-3′	Q-PCR for actin
Actin-QT-R	Antisense	5′-CTGTTGTAGGTGGTCTCGTGGATT-3′	
MCP-QT-F	Sense	5′-GGTATCACCAACGGTCAGACTATGC-3′	Q-PCR for SKIV-MCP
MCP-QT-R	Antisense	5′-GCTGGGTGCTCTGGCTGATGA-3′	
FLP-NT-L	Sense	5′-AAAGAATTCATGTTACGGGGCCTGC-3′	F: pEGFP-N1, pCMV-Flag, pCMV-BD (1-1253 aa) and pEGFP-N1 (1-380 aa).
pET-FLP-R	Antisense	5′-TTTCTCGAGTAGGTCCCGCTTGTTG-3′	R: pET32a (380-880 aa)
Grx-R	Antisense	5′-AAAGTCGACATGGTATTATCAAACCCATT-3′	R: pEGFP-N1, pCMV-Flag, pCMV-BD (1-1253 aa) and pEGFP-N1 (880-1253 aa).
Ftsk-L	Sense	5′-AATGAATTCATGTGCAAGCGTCTGCT-3′	F: pEGFP-N1 (380-880 aa) and pET32a (380-880 aa).
FLP-NT-R	Antisense	5′-TTTGTCGACAGGTCCCGCTTGTT-3′	R: pEGFP-N1 (1-380 aa)
Ftsk-R	Antisense	5′-AAAGTCGACATGGTATTATCAAACCCATT-3′	R: pEGFP-N1 (380-880 aa)

[a] F: forward primer; R: reverse primer.