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Up-regulation of *Ribophorin I* after yellow head virus (YHV) challenge in black tiger shrimp *Penaeus monodon*☆

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Abstract This work constitutes the second report from a continuing investigation of shrimp genes that may be involved in apoptosis associated death resulting from yellow head virus (YHV) infection. Here, we describe from the black tiger shrimp *Penaeus monodon*, a *ribophorin I*-like gene that is probably a subunit of the oligosaccharyltransferase complex (OST), a key enzyme in *N*-linked glycosylation that occurs in the endoplasmic reticulum. The OST complex also contains DAD1 (defender against apoptotic death 1) that has been reported to control apoptosis and that we have previously reported from *P. monodon*. The full length *ribophorin I* of *P. monodon* comprised 2157 bp with the ORF of 1806 bp corresponding to 601 deduced amino acids and three putative *N*-linked glycosylation sites. Analysis revealed hydrophobic properties implying that it could be a membrane protein. Tissue distribution analysis using real-time RT–PCR with SYBR Green revealed that *ribophorin I* was endogenously expressed in all examined tissues of normal shrimp. However, unlike *DAD1* that was down-regulated after YHV challenge, *ribophorin I* expression was up-regulated and remained high until the moribund stage.

Introduction

The first oligosaccharyltransferase (OST) complex was isolated from the dog pancreas by sucrose density gradient separation and ion exchange chromatography [1]. The

☆ The GenBank accession number for the sequence reported in this paper is EF581987.

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complex is comprised of three integral membrane proteins as follows: ribophorin I [2,3], ribophorin II [2,4] and OST48 [5]. The OST complex was later purified from other organisms including other mammals [6,7], yeast [8], and fungi [9]. Studies on the yeast OST complex led to the identification of DAD1 (defender against apoptotic death 1) as an additional OST component [5].

The OST complex is reported to catalyze the linkage of preassembled high mannose oligosaccharides to asparagine residues (i.e., *N*-linked glycosylation) [10]. It has also been suggested that the complex is involved in apoptosis, although the mechanism remains unclear [11]. During the onset of DNA fragmentation, a hallmark of apoptosis, *DAD1* has been reported to decrease [12, 13]. In addition, it has been reported that *ribophorin I* expression is up-regulated before the onset of DNA fragmentation during apoptosis [11]. *DAD1* from the black tiger shrimp is described in a companion report [14].

Yellow head virus (YHV) is one of the most lethal viral pathogens for cultivated shrimp [15,16]. Mortality occurs within 2 to 4 days after challenge with YHV and can cause massive losses in production [17]. YHV-infected cells exhibit nuclear pyknosis and karyorrhexis that are signs of viral triggered apoptosis [18]. If YHV triggers apoptosis in shrimp, then one might expect that the expression of *ribophorin I* would be increased with disease progression, as has been previously reported [11].

Here we describe the isolation and characterization of shrimp (*P. monodon*) *ribophorin I* and its expression pattern as determined by real-time RT—PCR analysis with SYBR Green in various normal tissues and in hemocytes after YHV challenge.

Materials and methods

Construction of a normal cDNA library and EST analysis

We subjected 5 μg of mRNA from ovaries of *P. monodon* to cDNA synthesis and cloning using a ZAP-cDNA Synthesis and Cloning Kit (BD Biosciences Clontech) [19]. Size-selected cDNAs (>500 bp) were cloned into dephosphorylated EcoRI/XhoI-digested Uni-ZAP® XR and transfected into *Escherichia coli* XL1-Blue MRF′. The lambda library was converted into the pBluescript library by *in vivo* excision. Recombinant clones were selected by a lacZ′ system following standard protocols [20]. Recombinant clones were randomly selected from those carrying insert sizes greater than 500 bp. Plasmid DNA was extracted and unidirectional sequenced on a MegaBase 1000 automated DNA sequencer (Amersham Biosciences, presently called GE Healthcare).

Rapid amplification of cDNA ends—polymerase chain reaction (RACE—PCR)

Gene-specific primers (5′-RACE; RB-1; 5′-AGT GGG TCT TCC ATC CTC CAA ACA-3′ and 3′-RACE; RB-2: 5′-GTG TCT CAC TGG GGC AAT ATT GCC GT-3′) were designed from sequences of a *ribophorin I* homologue from an EST library [21]. RACE—PCR was carried out using a SMART RACE cDNA Amplification Kit (BD Biosciences Clontech) following the protocol recommended by the manufacturer. The resulting products were size-fractionated, eluted from the gel and subjected to nested PCR using nRB-1 (5′-GGA TGA TGG TCT TGA AGG ATT TGA CAC-3′) and nRB-2 (5′-GAT ACG ACT TCC AGA GAG AGC ACA-3′) and nested UPM (5′-AAG CAG TGG TAT CAA CGC AGA GT-3′) primers. The resulting products were size-fractionated, eluted from the gel, cloned into pGEM-T Easy vector and sequenced.

Table 1 Key to ribophorin I obtained from various organisms and used in comparisons

Species	Abbreviation	Accession no.
Aedes aegypti	Aaeg	EAT34721
Anopheles gambiae str. PEST	Agam	EAA13877
Arabidopsis thaliana	Atha	AAD12699
Aspergillus oryzae	Aory	BAE57188
Chaetomium globosum CBS 148.51	Cglo	EAQ92849
Danio rerio	Drer	AAN15068
Drosophila melanogaster	Dmel	NP_995680
Homo sapiens	Hsap	BAD97315
Hordeum vulgare	Hvul	CAA74910
Medicago trunatula	Mtru	ABE83243
Mus musculus	Mmus	AAH85483
Neurospora crassa OR74A	Ncra	XP_965672
Oxyuranus scutellatus	Oscu	AAU25924
Penaeus monodon	Pmon	EF581987
Rattus norvegicus	Rnor	NP_037199
Strongylocentrotus purpuratus	Spur	XP_782614
Sus scrofa	Sscr	NP_999498
Xenopus lavis	Xlae	AAH45212
Yarowia lipolytica	Ylip	XP_505429

The *P. monodon* protein sequence was deduced from combined RACE—PCR sequences herein and EST [21].

Sequence analysis and phylogenetic tree analysis

For the generation of multiple alignments and phylogenetic trees, conserved motifs of ribophorin I from several organisms were used (Table 1). The programs and protocols used for sequence alignment and phylogenetic tree construction are described in a companion manuscript [14]. *N*-linked glycosylation sites were predicted according to

Table 2 List of primers used for quantitative real-time PCR analysis

Target	Primer sequence
Elongation factor-*1α (EF-1α)*	5′-GAACTGCTGACCAAGATCGACAGG-3′ 5′-GAGCATACTGTTGGAAGGTCTCCA-3′
Yellow head virus (YHV)	5′-ATCGGCACAGGAGCAGACA-3′ 5′-GTAACCCCGGCCATGACTT-3′
Ribophorin I	5′-ACTTCCAGAGAGAGCACAATACTTACT-3′ 5′-TGTGAGGTGGAGATATTTCCGATCTCA-3′

The specific primers for YHV amplification were derived from a previous publication [34].