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ORIGINAL ARTICLE

Deciphering the DNA repair protein, Rad23 from kuruma shrimp *Marsupenaeus japonicus*: full-length cDNA cloning and characterization

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Abstract

Aims: Lesions of DNA are removed by nucleotide excision repair (NER) process in the living systems. NER process-related host factors are believed to aid recovery steps during viral integration. Here, we report identification and characterization of a DNA repair molecule Rad23 from kuruma shrimp *Marsupenaeus japonicus*.

Methods and Results: The full-length cDNA of *M. japonicus* Rad23 gene (MjRad23) has 1149 bp coding for a putative protein of 382 amino acids with a 5′ untranslated region (UTR) of 92 bp and 3′ UTR region of 1116 bp. Quantitative expression analysis revealed MjRad23 is constitutively expressed in all the organs of healthy shrimp, whereas with high level in muscle tissue. Although MjRad23 expression is observed in every haemolymph samplings to post-white spot syndrome virus infection, high expression is recorded at 2 h post infection (h.p.i.). MjRad23 consists of putative functional domains including one ubiquitin domain (UBQ), two ubiquitin-associated domains (UBA) and one heat-shock chaperonin-binding motif (STI1). Multiple alignment of MjRad23 with Rad23 of other species showed highly significant identity ranging from 37 to 53%; however, high homology is observed with Rad23 of *Bombyx mori* (BmRad23). UBQ domain region alignment revealed maximum of 66% homology with Rad23 of *Apis melifera* (AmRad23). MjRad23 clustered with invertebrate sector along with insect species in evolution analysis. Three-dimensional structural analyses demonstrated the highest identity between MjRad23 and human Rad23A (hHR23A).

Conclusions: The present work revealed the presence of MjRad23 gene, which is essential in DNA repair process. Further studies are required to clarify the involvement of MjRad23 in NER process.

Significance and Impact of the Study: This is the first report on identification and characterization of DNA repair protein in crustaceans, which will lead to further investigation to explore the molecular mechanisms behind the NER process.

Introduction

A family of protein containing ubiquitin-like domains is shown to play an important role in proteolysis (Funakoshi *et al.* 1999, 2002; Hiyama *et al.* 1999). Rad23 is an evolutionarily conserved protein that is important for nucleotide excision repair (NER) (Watkins *et al.* 1993; Masutani *et al.* 1994; van der Spek *et al.* 1996). Biochemical characteristics

are similar to the best in the case of Rad23 of *Saccharomyces cerevisiae* and humans, which are involved in proteolysis process (Ortolan *et al.* 2000). Rad23 interacts with DNA repair factors playing significant role in NER (Prakash and Prakash 2000) and reported to promote the targeting of proteolytic substrates to the proteasome in yeast (Chen and Madura 2002). DNA frequently employs repair mechanism to nucleotides because of damage that occurs from a wide variety of sources including chemicals and ultraviolet (UV) light from the sun. Each single cell prevents the unwanted mutations by removing vast majority of DNA damage-induced cytotoxicity, mutagenesis and carcinogenesis during the NER process. The significance of NER-based DNA repair was evidenced by severe human hereditary diseases such as *Xeroderma pigmentosum* and Cockayne's syndrome leading to increased cellular sensitivity to DNA-damaging agents, increased mutation frequency and high risk of carcinogenesis (Cleaver and Kraemer 1989).

In a nutshell, NER process can be divided into five different steps as damage recognition, incision, excision, repair synthesis and ligation (Xie *et al.* 2004). Cellular NER consists of two sub-pathways: global genome repair and transcription-coupled repair (de Laat *et al.* 1999). Global genomic repair is on both transcribed and untranscribed DNA strands in active and inactive genes throughout the genome, whereas transcription-coupled NER is specific to the repair of actively transcribed genes. Basically, two categories of NER proteins are studied. Most of the NER proteins are essential for repair corresponding to human XP. Another category of NER proteins play accessory roles in repair, without the cells that exhibit moderate rather than severe sensitivity to DNA-damaging agents (Xie *et al.* 2004). Rad23 is also found to be in yeast with such activity having homologues with mammalian HR23A and HR23B (Masutani *et al.* 1994). The mammalian homologue HR23 strongly binds to Rad4 (Guzder *et al.* 1998). Recent reports suggest that XP/HR23B complex functions in the damage recognition step of NER; however, its role is unexplored yet (Batty *et al.* 2000). It has been reported that DNA repair and DNA metabolism are involved in RNA viral infections during retroviral integration (Brin *et al.* 2000; Yoder and Bushman 2000). Rad18, a DNA repair protein, is found to be involved in post-DNA repair process during HIV-1 infection (Mulder *et al.* 2002).

Shrimp farming has continued to expand and become a major source of income in many countries. Obstacles to the future of the shrimp industry are because of outbreaks of infectious diseases and abnormal environmental conditions. Defence mechanisms and disease prevention in farmed marine invertebrates such as shrimp are poorly understood when compared with insects and mammals (Roch 1999). On the other hand, demand in understanding molecular functions has increased to analyse the culture losses because of secondary factors with reference to pathogen invasion. Several attempts are made by various researchers to identify and characterize many defence-related host genes in shrimp such as Dicer-1 (Yao *et al.* 2010), anti-LPS (Mekata *et al.* 2010a), nitric oxide synthase (Inada *et al.* 2010) and tumour necrosis factor (Mekata *et al.* 2010b) to discover the immune functions. Lack of shrimp-specific probes is still a hurdle to predict molecular mechanisms, which gives interest to the shrimp biotechnology researchers to identify new host genes involved in essential functions. Information on DNA repair protein of shrimp would be a greater advantage to understand the repair functions in DNA metabolism during diseased conditions. In this study, the full length of a novel rad23 gene from kuruma shrimp *Marsupenaeus japonicus* denoted as MjRad23 was identified and characterized as a first report from crustacean sector. We describe the molecular cloning and complete sequencing of a cDNA encoding MjRad23 (NER protein) and structural parameters involved in DNA repair process.

Materials and methods

Shrimp samples

Healthy *M. japonicus* juveniles (12 g) were obtained from commercial shrimp farms in Miyazaki, Japan. They were maintained in 80-l tank containing 60 l of artificial sea water at 35 ppt salinity with aeration. Before starting the challenging experiments, the shrimp were tested for white spot syndrome virus (WSSV) negative by PCR and acclimated for 3 days at 23°C and fed with commercial pellet. The shrimp were screened for viral contamination using standard PCR and RT-PCR methods (Flegel 2006).

Preparation of the viral inoculum

Naturally, WSSV-infected adult shrimp (*M. japonicus*) with prominent signs of disease with white spots in the carapace were collected from shrimp farms, Japan. After confirmation with PCR, they were used as the viral source for infection studies. The moribund animal was transported on dry ice to the laboratory and stored at −80°C. Frozen infected samples were thawed, and the gill tissues were homogenized in a sterile homogenizer using 10% (w/v) NTE buffer (0·2 mol l^{-1} NaCl, 0·02 mol l^{-1} Tris–HCl and 0·02 mol l^{-1} EDTA, pH 7·4). The homogenate was centrifuged at 4000 ***g*** for 20 min at 4°C; the supernatant was again clarified at 10 000 ***g*** for 10 min at 4°C, and then the final supernatant was filtered through a 0·45-μm membrane. The filtrate was then stored at −20°C

to use for challenge experiments. The quantity of the WSS viral particles in the inoculum prepared was measured as 2×10^6 copies per ml using VP28 TaqMan probe real-time PCR (Sudhakaran *et al.* 2009).

RNA extraction and cDNA synthesis

Total RNA was extracted from the lymphoid organs of kuruma shrimp using ISOGEN (Nippon Gene, Japan) according to the manufacturer's instructions. The amount of nucleic acid in the total RNA was quantified by measuring the absorbance at 260 nm using NanoDrop spectrophotometer ND-1000 (NanoDrop Technologies Inc., Wilmington, PA). Synthesis of cDNA was performed from 1·0 μg of total RNA using a ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan) and used as a template for polymerase chain reaction (PCR).

Cloning of MjRad23 complete cDNA fragment

Initially, a partial middle fragment of MjRad23 cDNA was predicted by performing PCR using a set of degenerate primers (Table 1), Rad23F and Rad23R designed from the conserved nucleotide sequences of EST database between *Litopenaeus vannamei* and *Penaeus monodon* (GenBank accession no.: FE099033.1, GO067550.1). Having isolated the partial MjRad23 gene of kuruma shrimp, full-length sequences were obtained by performing 5′ and 3′ RACE-PCR, using gene-specific primers (Table 1). The PCRs were performed in 50 μl using 2× GoTaq Green Master Mix (Promega K.K., Tokyo, Japan) containing the reaction buffer (pH 8·5), 400 μmol l^{-1} dNTP and 3 mmol l^{-1} $MgCl_2$ with preloaded dyes. The amplified products (10 μl) were analysed by electrophoresis on a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet transillumination. The target PCR products were purified with GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare, UK) and cloned into the pGEM-T Easy Vector (Promega, USA), further transformed into DH5α (Promega, USA). The transformants were identified through red–white colour selection when grown on MacConkey agar (Sigma-Aldrich, Japan). Plasmid DNA from at least three independent clones was recovered using a QIAprep Spin Miniprep kit (Qiagen, Japan) and sequenced using CEQ 8000 Automated Sequencer (Beckman Coulter Inc., Brea, CA, USA).

Table 1 Oligonucleotide primers used in the cloning and mRNA expression study of MjRad23 gene

Primers	Sequence (5′–3′)
Partial cDNA cloning	
Rad23F	ACCTCGCTGCAGAGTTTCTC
Rad23R	TTGTTTGATGGAATCCCTGT
5′-RACE	
MjRad23F1	GCTTCAAACACCTATGCTGCCACTCC
MjRad23F2	GTGCACTGTCCAGTGATAGTGGGAAA
3′-RACE	
MjRad23R1	GGAGTGGCAGCATAGGTGTTTGAAGC
MjRad23R2	ACCTGCACCAAACACAGGGTCTCCT
MjRad23R3	CTGCATGAAAGCGATCTCGCACAC
RT-PCR analysis	
Rad23 Ex-F	CGACCCCACAATTGTAGAAA
Rad23Ex-R	TTTGTCGCATCTGCTCAAAC
EF1-α exF	GTCTTCCCCTTCAGGACGTA
EF1-α exR	GAACTTGCAGGCAATGTGAG

Sequence analysis of MjRad23 cDNA

The resulting cDNA and predicted amino acid sequences were analysed for similarity with other known sequences registered in the GenBank using basic local alignment search tool (BLAST) (Altschul *et al.* 1990). The structural domains in MjRad23 amino acid sequences were predicted using simple modular architecture research tool (SMART) version 4.0 (http://smart.embl-heidelberg.de/). Direct comparison between amino acid sequences was performed using the CLUSTALW sequence alignment tool (version 1.74) (Thompson *et al.* 1994). Percent identity with a selected reference sequence was assessed with Matrix Global Alignment Tool (MATGAT) (Campanella *et al.* 2003). The phylogenetic analysis was performed with the full-length reference amino acid sequences by the neighbour-joining method (Saitou and Nei 1987), and the phylogenetic tree was conducted using MEGA4 (Tamura *et al.* 2007) with prediction of confidence limits (Felsenstein 1985).

Structure prediction of MjRad23

The 3-D structure of MjRad23 was predicted theoretically using the deduced amino acids of MjRad23 using SWISS-MODEL (http://swissmodel.expasy.org/) (Guex and Peitsch 1997; Schwede *et al.* 2003; Arnold *et al.* 2006). A comparison was made between the protein structures of MjRad23 to hHR23A (Human Rad23A; GenBank accession no.: NM_005053.2) (Masutani *et al.* 1994) using Swiss PDB DEEPVIEW 4.0.1 software (SPDBDV; Swiss Institute of Bioinformatics, Lausanne, Switzerland).

Analysis of MjRad23 expression in different tissues

The SYBR green real-time RT-PCR was performed to analyse the expression pattern of MjRad23 genes in different tissues of healthy kuruma shrimp to compare and understand the function of MjRad23 during the DNA repair processes. Total RNA was extracted from various tissues of healthy kuruma shrimp such as brain, gills,

heart, haemolymph, hepatopancreas, intestine, lymphoid organ, muscle, nerve and stomach using ISOGEN reagent (Nippon Gene) according to the manufacturer's instructions. RNA extraction and cDNA synthesized as shown in section RNA extraction and cDNA synthesis. Gene-specific primers MjRad23-F/-R for MjRad23 amplification were designed and amplified with the specific product size of 527 bp. Simultaneously, EF1-α gene (Table 1) served as an internal control for the quantity and quality of cDNA templates obtained.

Quantification of expressed MjRad23 gene in hemolymph of WSSV-infected *Marsupenaeus japonicus* shrimp

To observe the transcriptional changes of MjRad23 in experimentally WSSV-infected *M. japonicus* shrimp, a SYBR green real-time RT-PCR was performed using the hemolymph collected from different time periods post infection. Approximately, 7×10^3 copies of WSS virions diluted to 100 μl using NTE buffer and injected in second abdominal segment of shrimp using 1-ml syringe with 29 gauge needles. The hemolymph samples were collected at 0, 1, 2, 4, 6, 8, 12 and 24 h post infection of WSSV. The SYBR green real-time RT-PCR amplifications were carried out in triplicates with a total volume of 50 μl containing 25 μl of THUNDERBIRD™ SYBR qPCR Mix (Toyobo) according to manufacturer's instructions. The comparative cycle threshold (C_t) method was used to analyse the expression level of each gene. The C_t for the target amplification of each gene and the C_t for the internal control were determined for each sample. The average C_t measurement for the three determinations were used in calculations of relative expression using EF1-α as the internal control. The data obtained from RT-PCR analysis were subjected to one-way analysis of variance (one-way ANOVA) followed by an unpaired, two-tailed *t*-test. Differences were considered significant at $P < 0{\cdot}01$.

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GGACGTCACGGCTCATGGCTGCTGTTATTCAGCAGGAGTGAAACAAACAAGGCTGTGTCAAGGGGGCATTATCAGAGCAACACAGGGCGAAAATGGTGGT  100
                                                                                                  M  V  V
TCTCACGCTGAAGAACTTGCAACAGCAGACCTTCACTGTAGAAATCGAGCTGTCTGCCACGGTGAAAGCTCTAAAAGAGAAGGTTGAGAAGGAGAAAGGT  200
 L  T  L  K  N  L  Q  Q  Q  T  F  T  V  E  I  E  L  S  A  T  V  K  A  L  K  E  K  V  E  K  E  K  G
GGTGACTATCCAGCAGTAGGCCAGAAGTTAATTTATGCAGGTAAAATCCCTCAAGATGACACAACTCTTGAATCATATAACATTGATGACAAGAAGTTTT  300
G  D  Y  P  A  V  G  Q  K  L  I  Y  A  G  K  I  P  Q  D  D  T  T  L  E  S  Y  N  I  D  D  K  K  F  L
TAGTTATTATGGTAACAAAGCCCAAGGCACCTCCTCCGCCTGTTGGCCCAAGCGACCCCACAATTGTAGAAACTGAGGAGACTCCGGCAGAGGAACCAGC  400
  V  I  M  V  T  K  P  K  A  P  P  P  P  V  G  P  S  D  P  T  I  V  E  T  E  E  T  P  A  E  E  P  A
TGCCACCACTGAATCTTCCTCACAGAGTACCAGTACACCTTCCACAGAGTCAGCAGCTGCAGCAGGTACCAACACCACAACTACCACCACCACTACTTCC  500
 A  T  T  E  S  S  S  Q  S  T  S  T  P  S  T  E  S  A  A  A  A  G  T  N  T  T  T  T  T  T  T  T  S
AGCAGCCAATCTTCAGCAGCTTCCAACACTCAGTCCACACCAGCCACGCAGGCAGCACCAGGGGGGTCAGTGGGAAGTGCAGAATCTTTGCTGGTGATGG  600
S  S  Q  S  S  A  A  S  N  T  Q  S  T  P  A  T  Q  A  A  P  G  G  S  V  G  S  A  E  S  L  L  V  M  G
GTGAAGAATTTAACAGGATGGTAGAGAACATTATGGAAATGGGATATGAAAGGTCGCAGGTTGAACGTGCATTGAGGGCAAGCTTCAACAATCCTTACAC  700
  E  E  F  N  R  M  V  E  N  I  M  E  M  G  Y  E  R  S  Q  V  E  R  A  L  R  A  S  F  N  N  P  Y  T
GGCTGTTCAGTACCTAGTGGACGGGATCCCCCCCCAATCTTGAGGAGCCAGCCGCGCAGCCGGCGCAGGGTGGTGACGGGGGAGGAGAGGAACAAGTGGTT  800
 A  V  Q  Y  L  V  D  G  I  P  P  N  L  E  E  P  A  A  Q  P  A  Q  G  G  D  G  G  G  E  E  Q  V  V
GCAGAGGGAGAGGCAGACCCTGATGAGGATCCTTTAAACTTCCTTCGGTCGCAGCCTCAGTTTGAGCAGATGCGACAAATGATTAGGTCAAATCCATCAT  900
A  E  G  E  A  D  P  D  E  D  P  L  N  F  L  R  S  Q  P  Q  F  E  Q  M  R  Q  M  I  R  S  N  P  S  L
TGCTGGATGCATTCATCCGGCAGATTGGCCAAACAAATCCGCAGCTACTTCAGGTGATCCAGCAAAATCAGGAGGCCTTTGTGCGGATGACTGAACGAAG 1000
  L  D  A  F  I  R  Q  I  G  Q  T  N  P  Q  L  L  Q  V  I  Q  Q  N  Q  E  A  F  V  R  M  T  E  R  R
GGGGCAGTTCTGGAGGTGGAAGCACAGTGGCGGTAGCGGTAATCAGGGTGGAGATGGCAGTGGTGGCCGAGCTGCTCCAGGACAAAATGCAATCCTTGTC 1100
 G  Q  F  W  R  W  K  H  S  G  G  S  G  N  Q  G  G  D  G  S  G  G  R  A  A  P  G  Q  N  A  I  L  V
TCTCCTCAGGATAGAGATGCCATTGAAAGGCTGAAAGCTTTAGGTAATTTCCCTGAAGATGTGGTAATCCAAGCTTACTTTGCCTGTGAGAAGAATGAGA 1200
S  P  Q  D  R  D  A  I  E  R  L  K  A  L  G  N  F  P  E  D  V  V  I  Q  A  Y  F  A  C  E  K  N  E  N
ACCTCGCTGCAGAGTTTCTTTTCAGTCAGACTTGGGATTAGGTTATACTTGTACCCTCTCTTTAACAAATTATAAGTTAATTTAGAATATGAAATCTCTT 1300
   L  A  A  E  F  L  F  S  Q  T  W  D  *
GTTAAGTGCACAGCTAAGCTATGGGCTGATTTCCAGTGGCTTGTTATCTCGAAATTGTCCAAAGAAGGTCAATTATACAGATTTTCATATATCTAAAACC 1400
TGAGTTCATACATGTTAGGAGACCCTGTGTTTGGTGCAGGTAACTGACCAGCACTGACTATTATGTGGTTGTATGGCTTCAAACACCTATGCTGCCACTC 1500
CTTTGTGTATATAATGTGTGCGAGATCGCTTTCATGCAGATTCAATATGTTACTATTAAGAGGTAAAAAGAATTTGAGTTTTAATTACCTTGTCCTATGC 1600
TACTGACATGACACTGGTATCAGCTGGTAGTGCACTGTCCAGTGATAGTGGGAAAGTTTTATCTGAACTATTTACAGGGATTCCATCAAACAATATTTTC 1700
TACTGTTTGCGTTCCCCATTTATGCCTTTTCAGTCGGGATTAACCTTCATGATTTTCGAAAGAAAGTGATTTAAGTTGCATCTTGTGAGGTAATTTCAGC 1800
CATACATCATAGTCTTCATAGGTGATATCCATTGTGACATAGAAAAGATCAGACAAATACAAGACTCAGGTTTGTGAAGGCAGTGACATCATATAACTAT 1900
GTATTCTATCTTTTGCTTTACACACATGTATGGCGTAGCAGCTGGCTTGCCCTCTGAGTAAAGAAAATTGCAGCAAAAGGCTTCTCTGTGAGAAACAAGA 2000
TTTGTATAGTATTACTTGCTCAATTTTCATAATGTTTTGCTGTCATTTGATATTTGTTATAAAAGTTTCTTGAAAGTTTCATCCTTTAAATTGGAAATAAG 2100
TTATCAAATGTGGGACTGCTATATTGTTTGCTTTCGTTGGTAAGAATTTATGGTAAGCAGTTTTCCTTAAAGACTGCCAGCAGTTTCCTAACAAGGTATT 2200
CTTGTCCTGTCTGAAGCTTTTACAGCACATTAGCAGCACACTTATGAGTGAAACTGAGAATTCATGTTCATTATTAAGTTTTTGTTAGAGCTAGTTTTTT 2300
TCCCCCCCACATCTGTCCTTTATGCATGGAATGTTGCTTGAGGCATGCAGTTTTTTT  2357
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Figure 1 Complete nucleotide and deduced amino acid sequence of MjRad23 from *Marsupenaeus japonicus* (GenBank accession no.: AB563728). Translation start and termination codon are markets as M and an asterisk (*), respectively. Heat-shock chaperonin-binding motif (STI1 domain) is shown in bold underlined text. TATA box are indicated in bold red letters. The polyadenylation signal (AATAA) is shown in bold violet letters at the end of 3′ untranslated region.

Results

Cloning and characterization of MjRad23

Lymphoid organs of kuruma shrimp were sourced to obtained MjRad23 using the degenerate primers designed based on EST database of *L. vannamei* and *P. monodon*. Partial nucleotide sequences were obtained; 5′ and 3′ RACE-PCR was performed to obtain full length of MjRad23 cDNA. The cDNA sequence of the MjRad23 gene was submitted to GenBank of NCBI (accession no.: AB563728). The full-length cDNA of MjRad23 consisted of 2357 bp comprising a 5′ untranslated region (UTR) of 92 bp, an open reading frame of 1149 bp and a 3′ UTR of 1116 bp (Fig. 1). The putative MjRad23 open reading frame encoded a protein consisting of 382 amino acid residues, and TATA box sequences were observed in the full-length cDNA of MjRad23 (Fig. 1). Typical functional domains such as one ubiquitin domain (UBQ), two ubiquitin-associated domains (UBA) and one heat-shock chaperonin-binding motif were found in MjRad23 (Fig. 2).

NCBI BLASTP program revealed that the predicted amino acid sequence showed homology match with variety of Rad23s previously registered in GenBank. Homogeneity analysis was performed based on the full-length amino acid sequences of MjRad23, *Homo sapiens* Rad23 (human), *Bombyx mori* Rad23 (insect), *Bos Taurus* Rad23 (cattle), *Monodelphis domestica* Rad23 (mouse), *Canis familiaris* Rad23 (dog), *Taeniopygia guttata* Rad23 (bird) and *Danio rerio* Rad23 (fish). MjRad23 showed highest identity ranging from 44·8 to 48·4% homology when compared with reference to the above-mentioned sequences (Table 2).

Multiple alignments of MjRad23 with Rad23 of other species revealed homology from 37 to 53% (Fig. 3). Whereas alignment of UBQ domain amino acid sequences of MjRad23 with those of other species revealed identity from 35 to 66% (data not shown).

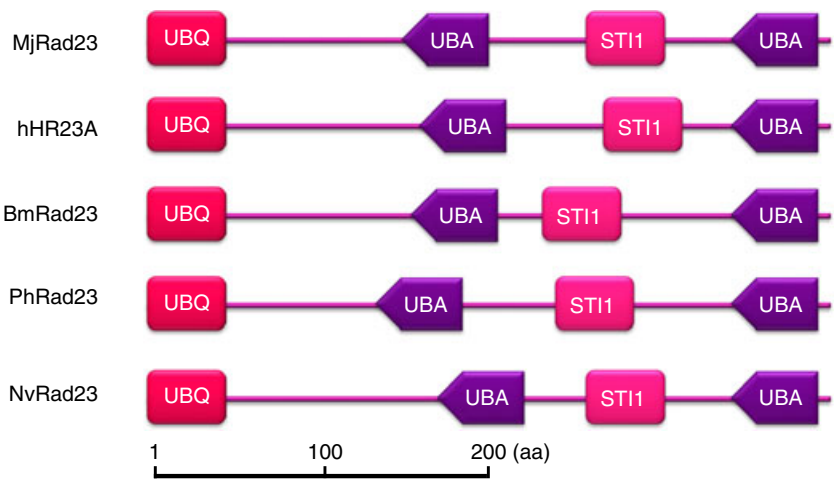

Figure 2 Comparison of protein secondary structures of MjRad23 to the DNA repair proteins of *Homo sapiens* (hH23A), *Bombyx mori* (BmRad23), *Pediculus humanus corporis* (PhRad23) and *Nasonia vitripennis* (NvRad23). UBQ and UBA are abbreviations of ubiquitin and ubiquitin-associated domains, respectively. Heat-shock chaperonin-binding motif is denoted as STI1. UBQ, ubiquitin domain; UBA, ubiquitin-associated domain.

Table 2 Amino acid identity (upper triangle) and similarity (lower triangle) of MjRad23 gene with known Rad23 genes

	Species	1	2	3	4	5	6	7	8
1	MjRad23	Shrimp	48·2	46·9	46·7	47·4	48·4	44·7	44·8
2	HsRad23	60·6	Human	48·9	92·9	59·2	92·4	78·8	56·8
3	BmRad23	57·9	57·0	Insect	47·8	50·9	47·5	47·9	49·7
4	BtRad23	59·1	95·1	56·9	Cattle	58·6	93·4	78·6	57·4
5	MdRad23	62·3	68·2	62·0	67·9	Mouse	58·9	57·9	62·9
6	CfRad23	61·1	94·4	56·2	95·1	68·2	Dog	78·7	57·6
7	TgRad23	57·0	83·9	58·3	84·3	70·5	85·0	Bird	58·2
8	DrRad23	61·8	67·7	61·9	69·1	76·5	70·4	71·0	Fish

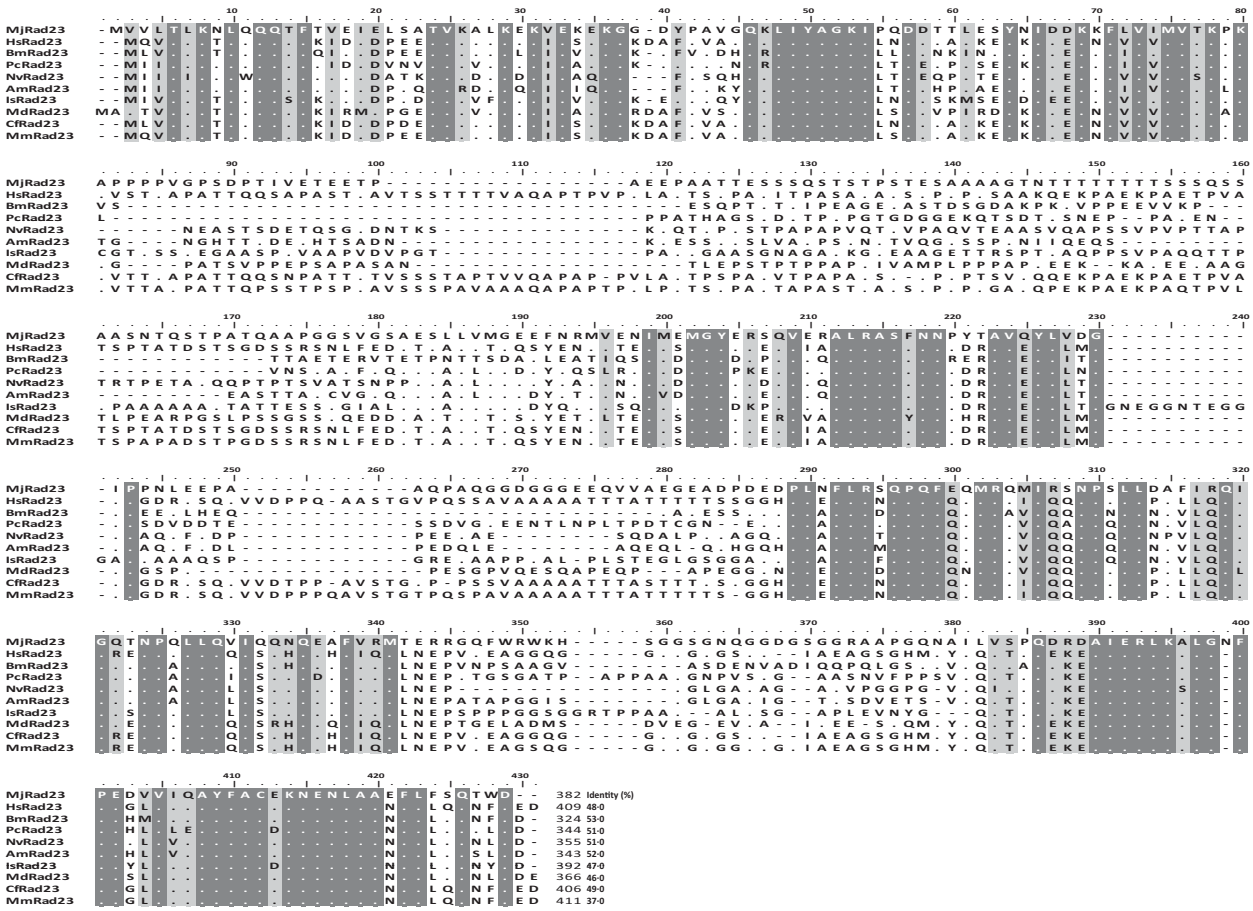

Figure 3 Multiple alignment of amino acid sequences of MjRad23 with those of other species Rad23 from HsRad23 (BAF82597.1), BmRad23 (NP_001164652.1), PcRad23 (XP_002432231.1), NvRad23 (XP_001608155.1), AmRad23 (XP_623093.1), IsRad23, CfRad23 (XP_538778.1) and MmRad23 (BAE25006.1). The accession numbers are given the brackets. Identical or highly conserved residues are shaded in black, whilst similar residues are shaded in grey. The identity in percentage with MjRad23 was showed at the end of each sequence.

A phylogenetic tree was constructed by comparing with known Rad23 sequences available in GenBank, and the results showed that MjRad23 clustered with invertebrate sector along with insect species (Fig. 4). The clusters for vertebrate and invertebrate Rad23 gene are observed in separate. The 3-D structural analysis revealed that MjRad23 is highly similar to human homologue Rad23A (hHR23A). MjRad23 revealed maximum structural identity when aligned with hHR23A (data not shown). An asterisk or pipe symbol is used to show the identity between two columns; other less common symbols include a colon for conservative substitutions and a period for semi-conservative substitutions.

MjRad23 expression in different tissues of kuruma shrimp

The SYBR green real-time RT-PCR was performed to analyse the expression pattern of MjRad23 in different organs of healthy kuruma shrimp (Fig. 5). The results observed as MjRad23 mRNA constitutively expressed in brain, heart, hemolymph and muscle when compared with other organs. The expression of MjRad23 in muscle is about tenfold higher when compared with gill, hepatopancreas and stomach.

Experimental injection of WSSV and MjRad23 expression

Expression of MjRad23 was analysed in hemolymph samples of kuruma shrimp experimentally injected with WSSV at different time intervals post injection. SYBR real-time RT-PCR was performed to examine the copy numbers obtained. It has been observed that MjRad23 is not stable, and significant changes in expression are observed in each sampling. Marked increase is observed at 2 h post-WSSV injection amongst all durations (Fig. 6).

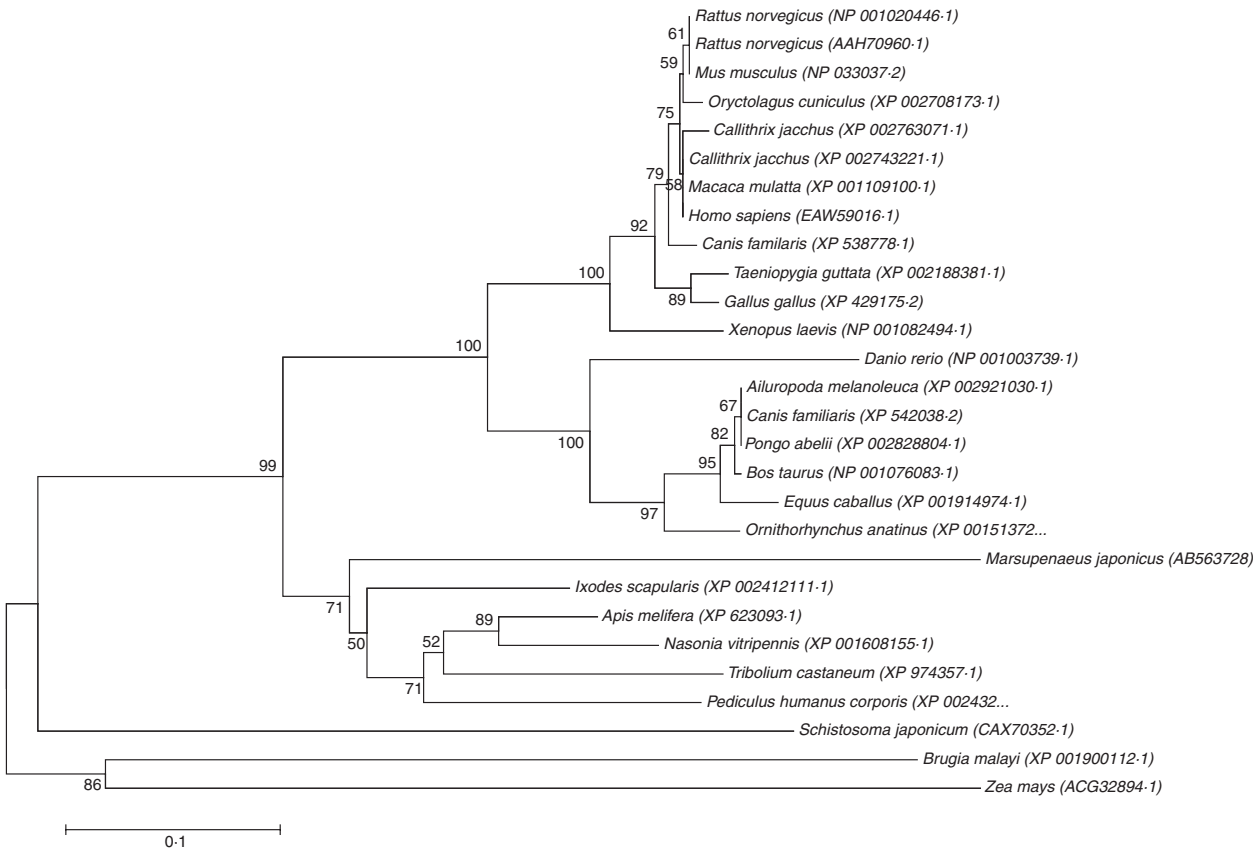

Figure 4 A boot-strapped neighbour-joining tree summarized relationships of the known RAD23 genes from various organisms. The scale bar indicates a branch length of 0·1. Only confidence probability values >50% are listed. GenBank accession numbers of each species has been given the brackets, respectively.

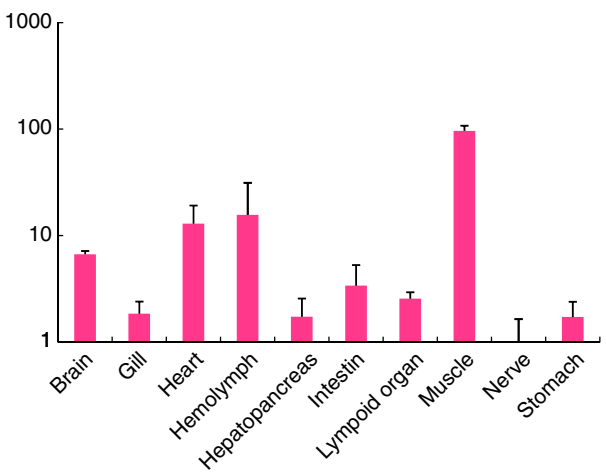

Figure 5 Transcriptional analysis of MjRad23 gene in different tissues of healthy kuruma shrimp. A SYBR green real-time RT-PCR was performed using primers specific for MjRad23 and EF1-α.

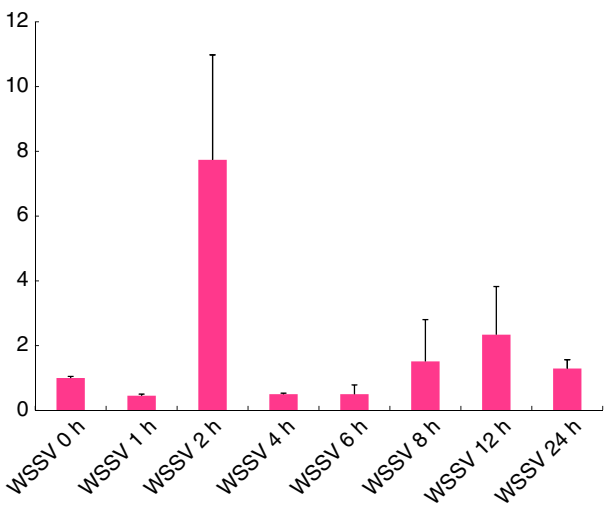

Figure 6 Transcriptional analysis of MjRad23 gene in hemolymph infected with white spot syndrome virus at different time internals (0, 5, 10, 24, 48, 72, 96 and 120 h). A SYBR green real-time RT-PCR was performed for MjRad23 gene with the cDNA obtained. Data are presented as MjRad23 expression after normalizing against EF1-α.

Discussion

NER is an essential process in detoxification of DNA damages caused by environmental factors, which includes UV irradiation and chemical modifications (Masutani *et al.* 1994; Batty *et al.* 2000). Rad23 was initially identified as a DNA repair protein interacting with Human Rad3 (Guzder *et al.* 1995b; Varshavsky 1997) to promote the assembly of repair proteins at DNA lesion sites (Guzder *et al.* 1998; Jansen *et al.* 1998). This stimulatory activity might be responsible for the direct role for Rad23 in NER. Two Rad23 homologues, namely hHR23A and hHR23B, in human are observed to be involved in forming a complex with *X. pigmentosum* group C protein (XPC) (Masutani *et al.* 1994; Batty *et al.* 2000).

DNA repair protein in higher organisms suppresses the viral infection instead of leading to establishment is partially observed. It has been demonstrated that DNA repair molecules inhibit and down-regulate hepatitis B virus replication in mammals (Zhao *et al.* 2008). Same kind of effect is expected in shrimp system when getting infected with viral pathogens. In fact, studies in yeast have reported that the molecules involved in DNA repair mechanism have an inhibitory effect on retrotransposition (Scholes *et al.* 2001; Irwin *et al.* 2005). Studies on mammalian cells suggested significant role for DNA repair molecules against viruses; for example, Rad52, a DNA repair molecule, has been reported to suppress HIV-1 infection in human (Yoder *et al.* 2006). It can be considered that DNA molecules are also meant to develop immunity against viruses (Bieniasz 2004). Effect of DNA repair molecule, Rad18, upon HIV-1 infection has been demonstrated suggesting the cells lacking Rad18 were highly susceptible to viral infection than Rad18 included controls. Moreover, Rad18-negative cells are more susceptible to other viruses such as murine leucemia virus (MLV) and adenovirus (Lloyd *et al.* 2006).

Complete cDNA sequence of MjRad23 was identified, which is similar to Rad23 of many other organisms such as *H. sapiens*, *B. mori*, *Pediculus humanus corporis* and *Nasonia vitripennis* in relation with domain structures (ubiquitin domain, ubiquitin-associated domain and heat-shock chaperonin-binding motif) and TATA boxes in it. Rad23 has been reported to induce resistance to methyl mercury in yeast cells by suppressing the protein degradation through UBA domain-mediated mechanism (Hwang *et al.* 2005). ubiquitin-like domain (UbL) at the amino terminus of Rad23 suggested a potential proteolytic function, especially achieved when UbL23 was replaced by ubiquitin domain (Watkins *et al.* 1993). To determine the level of MLV infectivity in the human T cells with presence and absence of Rad18 was demonstrated (Lloyd *et al.* 2006). It showed that $Rad18^{-/-}$ cells were highly prone to get infected *vice versa* the $Rad18^{+/+}$ cells were less prone to infection. In the case of MjRad23, different expression profile was observed from 0 to 24 h hemolymph samples collected WSSV-infected *M. japonicus*. Our finding on presence of DNA repair protein (MjRad23) in *M. japonicus* is the first report in crustaceans. In conclusion, mechanisms related to DNA repair process are unexplored still now. Further studies are required to understand the integrity of MjRad23 in DNA repair mechanisms, which has significant consequences in viral parasitism.

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