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Heat shock factor 1 regulates heat shock proteins and immune-related genes in *Penaeus monodon* under thermal stress

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ABSTRACT

Heat shock factors (HSFs) participate in the response to environmental stressors and regulate heat shock protein (Hsp) expression. This study describes the molecular characterization and expression of *Pm*HSF1 in black tiger shrimp *Penaeus monodon* under heat stress. *Pm*HSF1 expression was detected in several shrimp tissues: the highest in the lymphoid organ and the lowest in the eyestalk. Significant up-regulation of *Pm*HSF1 expression was observed in hemocytes ($p < 0.05$) following thermal stress. The expression of several *Pm*Hsps was rapidly induced following heat stress. Endogenous *Pm*HSF1 protein was expressed in all three types of shrimp hemocyte and strongly induced under heat stress. The suppression of *Pm*HSF1 expression by dsRNA-mediated gene silencing altered the expression of *Pm*Hsps, several antimicrobial genes, genes involved in the melanization process, and an antioxidant gene (*Pm*SOD). *Pm*HSF1 plays an important role in the thermal stress response, regulating the expression of Hsps and immune-related genes in *P. monodon*.

1. Introduction

Aquatic organisms encounter various environmental stressors such as heat, chemicals toxicants, and viral and bacterial pathogens. These stressors induce multiple changes in a cell that ultimately affect protein structure and function. An important mechanism that protects cells from damage caused by these stressors is the activation of the heat shock response. This response is predominantly regulated by various heat shock factors (HSFs), which bind to heat shock elements (HSEs) in the promoters of heat shock genes to induce their expression (Morimoto, 1998).

HSFs were first characterized in *Saccharomyces cerevisiae* (Sorger and Pelham, 1988; Wiederrecht et al., 1988) and *Drosophila melanogaster* (Clos et al., 1990). The mammalian HSF family consists of four members [HSF1, HSF2, HSF3, and HSF4 (Akerfelt et al., 2010a)]. Distinct HSFs possess unique and overlapping functions, exhibit tissue-specific patterns of expression and multiple post-translational modifications, and have several interacting protein partners (Akerfelt et al., 2007, 2010b; Anckar and Sistonen, 2007; Fujimoto et al., 2010). HSF1 and HSF2 are the well-studied factors because of their co-expression in most tissues and cell lines (Ostling et al., 2007; Pirkkala et al., 2001). HSF3 has only been identified in avian species, while HSF4 has been identified in mammals and is expressed predominantly in the lens and brain (Hu and Mivechi, 2006; Inouye et al., 2003; Min et al., 2004; Nakai, 1999; Nakai et al., 1997; Somasundaram and Bhat, 2004). Mammalian HSF1 corresponds to the single HSF in yeast, nematodes, and fruit flies (Clos et al., 1990; Hilgarth et al., 2004; Sorger and Pelham, 1988; Wiederrecht et al., 1988) and is considered the *bona fide* stress-activated transcription factor. HSF members are characterized by their conserved structure of the DNA binding domain (DBD) and hydrophobic heptad repeat (HR-A/B). The DBD, which is the best preserved domain throughout evolution, belongs to the family of winged helix-turn-helix DBDs (Damberger et al., 1994; Harrison et al., 1994; Vuister et al., 1994). The common activation process in all eukaryotic HSFs begins with the conversion of the inactive monomeric HSF1 to high-affinity DNA-binding trimers (Baler et al., 1993; Sarge et al., 1993). The trimerization of HSFs is mediated by arrays of HR-A and HR-B via the formation of a coil; this process is dependent on intermolecular hydrophobic non-covalent interactions (Lu et al., 2009; Sarge et al., 1993).

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Table 1
Summary of primers used in this study.

Primer name	Sequence (5′ → 3′)	Purpose
PmHSF1fl-F	GGACCAAAACGTGTGTAAACAAA	cDNA cloning
PmHSF1fl-R	TCAGGCCAAGCATTCACTTC	cDNA cloning
PmHSF1fm-F	TGGGAATGTCCCGGCGTTTT	RT-PCR, qRT-PCR
PmHSF1fm-R	ACATGTTCAGCTGTCTCACA	RT-PCR, qRT-PCR
PmHsp10-F	TCGACCGTGTGCTGGTCCAA	qRT-PCR
PmHsp10-R	GTGGTGCCAGCATCAGTTCT	qRT-PCR
PmHsp21-F	CACGAGGAGAAGTCTGACAAC	qRT-PCR
PmHsp21-R	GAGAGCGAGGACTTGATGAG	qRT-PCR
PmHsp60-F	CCCCAAGGGTCGAAATGTAA	qRT-PCR
PmHsp60-R	GGCAACATCTTGGACCAACT	qRT-PCR
PmHsp70-F	CTTCGACAACCGCATGGTGA	qRT-PCR
PmHsp70-R	GAAGAGGGAGCCGATCTCCA	qRT-PCR
PmHsc70-F	TGTCGGTATTGATCTGGGAA	qRT-PCR
PmHsc70-R	TTGGCGTCGAATACAGTGTT	qRT-PCR
PmHsp90-F	CGTCGAGAAGGAGAGGGACA	qRT-PCR
PmHsp90-R	TCAGCTCTTCGTCCTCCGTG	qRT-PCR
PmPEN3-F	ATGCGTCTCGTGGTCTGCCT	qRT-PCR
PmPEN3-R	CCTGTGAATACACGAGGA	qRT-PCR
PmPEN5-F	TTGGTCTATGCTTTGCAAGG	qRT-PCR
PmPEN5-R	ACAGATAGTTAAAGTGAAAGAC	qRT-PCR
PmALF3-F	ATACTAGAATTCCAAGGGTGGGAGGCTGTGGCA	qRT-PCR
PmALF3-R	TATTATGGATCCCTATGAGCTGAGCCACTGGTTGGCCT	qRT-PCR
CrustinPm1-F	CTGCTGCGAGTCAAGGTATG	qRT-PCR
CrustinPm1-R	AGGTACTGGCTGCTCTACTG	qRT-PCR
CrustinPm7-F	GGCATGGTGGCGTTGTTCCT	qRT-PCR
CrustinPm7-R	TGTCGGAGCCGAAGCAGTCA	qRT-PCR
PmproPO1-F	GGTCTTCCCCTCCCGCTTCG	qRT-PCR
PmproPO1-R	GCCGCAGGTCCTTTGGCAGC	qRT-PCR
PmproPO2-F	GCCAAGGGGAACGGGTGATG	qRT-PCR
PmproPO2-R	TCCCTCATGGCGGTCGAGGT	qRT-PCR
PmPPAE1-F	CGTCTGCTTCATTGAGGGAGTG	qRT-PCR
PmPPAE1-R	GTAGTAGATGGTGCCCCAGCCT	qRT-PCR
PmPPAE2-F	GCGGCGGTCACGCTCCTTGTTC	qRT-PCR
PmPPAE2-R	ACTCTCGGGGGCACGCTTGTTG	qRT-PCR
PmSOD-F	GCTGCTACAAAGAAGTTGGT	qRT-PCR
PmSOD-R	GGACTGGAATGATCCAAAGC	qRT-PCR
PmPrx-F	GTTCAGGCCTTCCAGTTCAC	qRT-PCR
PmPrx-R	CTTCAAGGCTTCCCTTTGCT	qRT-PCR
EF1-α-F	GGTGCTGGACAAGCTGAAGGC	RT-PCR, qRT-PCR
EF1-α-R	CGTTCCGGTGATCATGTTCTTGATG	RT-PCR, qRT-PCR
siRNA_HSF1F1	GGATCCTAATACGACTCACTATAGGAAGGACCTGCAATGGATGT	RNAi
siRNA_HSF1R1	AATGACTGGGTTACAGCACTT	RNAi
siRNA_HSF1F2	AAGGACCTGCAATGGATGT	RNAi
siRNA_HSF1R2	GGATCCTAATACGACTCACTATAGGAATGACTGGGTTACAGCACTT	RNAi
siRNA_GFPF1	GGATCCTAATACGACTCACTATAGGATGGTGAGCAAGGGCGAGGA	RNAi
siRNA_GFPR1	TTACTTGTACAGCTCGTCCA	RNAi
siRNA_GFPF2	ATGGTGAGCAAGGGCGAGGA	RNAi
siRNA_GFPR2	GGATCCTAATACGACTCACTATAGGTTACTTGTACAGCTCGTCCA	RNAi

HSFs have been reported to play diverse functions in response to a variety of physiological and environmental stimuli (Pirkkala et al., 2001; Shabtay and Arad, 2006). In mammals, HSF1 acts as a classical stress-responsive factor that regulates the expression of heat shock proteins (Hsps). During stress, HSF1 triggers the expression of a broad range of heat-responsive genes in *Drosophila*, including Hsp90 (Westwood et al., 1991). In small abalone, the expression level of HSF1 is up-regulated under thermal and hypoxia stresses; this protects cells from damage (Huang et al., 2014). Moreover, alterations in HSF1 mRNA levels following heavy metal exposure have also been reported in goldfish; cadmium exposure has been shown to induce strong expression of HSF1 mRNA in several tissues (Kim et al., 2015). In the Pacific white shrimp *Litopenaeus vannamei*, *Lv*HSF1 is up-regulated in response to infection by *Vibrio alginolyticus* and *Staphylococcus aureus* (Yan et al., 2014); however, the genes that regulate *Lv*HSF1 have not been identified to date.

In this study, the cDNA encoding *Pm*HSF1 was cloned and characterized from hemocytes of the black tiger shrimp *Penaeus monodon*. The temporal expression of *Pm*HSF1, as well as that of other *Pm*Hsps transcripts, was determined under conditions of heat stress. The effect of *Pm*HSF1 gene knockdown on the expression of several *Pm*Hsps and immune-related genes was also investigated. Our results provide novel insights into the molecular mechanisms underlying the role of HSF1 against heat stress in the black tiger shrimp.

2. Materials and methods

2.1. Shrimp samples

Juvenile *Penaeus monodon* shrimp (wet weight: 2–5 g) were kindly provided by the Shrimp Biotech Center I, Charoen Pokphand Foods Philippines Corporation in Chantaburi province, Thailand. The animals were acclimatized in laboratory tanks at ambient temperature (28 ± 4 °C), and maintained in aerated water with a salinity of 15 ppt for at least one week before use in the experiments. Each container was stocked with maximum density of 40 shrimp in 40 L of water.

2.2. Cloning of PmHSF1 from P. monodon

A 1925-bp cDNA fragment of *Pm*HSF1 was obtained by polymerase