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Temperature stress response of heat shock protein 90 (Hsp90) in the clam *Paphia undulata*☆

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ABSTRACT

The Heat shock proteins (HSPs) are a group of molecular chaperones that play a crucial role in cell response to various stresses. A full-length cDNA of the heat shock protein 90 (*PuHsp90*) was cloned and sequenced from the clam *Paphia undulata*. Phylogenetic analysis revealed that *PuHsp90* grouped with the *Hsp90* from other metazoan species. Expression of *PuHsp90* was highly detected in the gonad, followed by digest gland, gills and heart but was found poorly expressed in mantle, adductor muscle and hemocytes. After a heat shock stress at 32 °C up-regulation of *PuHsp90* was detected in the mantle, adductor muscle, gills and hemocytes. Maximal expression levels occurred at 4 h after the heat shock and up-regulation is indicative of protein denaturation and of an increase in energy consumption. In contrast after the heat shock, *PuHsp90* was continuously down-regulated in the digestive gland and in the gonad suggesting modifications of the biochemical pathways and energy budgets involved in the synthesis of other protein, such as catalase and of other Hsp proteins. These results reveal that *PuHsp90* may play an important role in the clam response to a temperature stress.

1. Introduction

Many bivalves are sedentary filter feeders, living in estuarine or intertidal regions. Their health is severely affected by temperature or salinity fluctuations, oxygen levels, toxic metals, or bacterial pathogens (Fabbri, Valbonesi, & Franzellitti, 2008; Li et al., 2009). In the process of adaptation, bivalves acquired defense systems to provide protection from harmful environments. The Heat shock proteins (Hsps) are part of the defense system and their expression can rapidly increase in response to environmental stresses (Craig, 1986; Gao et al., 2008). Generally, they are regarded as molecular chaperones and thus protect proteins and cells from damage when under stressful conditions. Hsps are highly conserved proteins from bacteria to mammals (Craig, 1986; Csermely et al., 1998; Gao et al., 2008). According to their molecular size and sequence homology they are grouped in several families: Hsp110, Hsp90, Hsp70, Hsp60, Hsp47, and low molecular mass Hsps (Heikkila, 2010; Lindquist & Craig, 1988). To date, several studies have focused on Hsp90s and additionally, according to their subcellular locations, they can be classified into four types: cytosolic, endoplasmic reticulum (ER), mitochondria and chloroplast in plants (Picard, 2002).

It was reported that Hsp90 are highly conserved and accounts for approximately 1%–2% of the total soluble proteins in some cells (Buchner, 1999). When organisms are exposed to a stress, such as an environmental insult or pathogenic infection, expression of Hsp90 can significantly increase in a short period of time to prevent irreversible aggregation of damaged proteins. Therefore, in most of eukaryotes Hsp90 plays an important role in cellular protection against stress (Buchner, 1999), tumor repression (Gress et al., 1994; Whitesell et al., 1994), cell cycle control (Aligue, Akhavan-Niak, & Russell, 1994), and antigen recognition (Srivastava et al., 1994). Hsp90 and expression patterns in response to various stressors have been reported in several molluscs, including *Laternula elliptica* (Kim et al., 2009), *Crassostrea gigas* (Choi, Jo, & Choi, 2008), *Haliotis tuberculata* (Farcy et al., 2007), *Haliotis asinine* (Gunter & Degnan, 2007), *Chlamys farreri* (Gao et al., 2007), and *Argopecten irradians* (Gao et al., 2008).

☆ The GenBank accession number of the sequence reported in this paper is AFZ93093.

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Table 1
Primer sequences and annealing temperatures.

Primers	Sequences (5'→3')	Annealing temperature (°C)	Location (bp)
Hsp90-F1	CACCCTGTACATGAARGARGAYCA	53	705
Hsp90-R1	ATGCCCAGGCCCARYTTDATCAT		2033
Hsp90-F2	GAGGTGTCCGACGAYGARGARGA	55	779
Hsp90-R2	AGGGCCTGGGCYTTCATDAT		1922
Hsp90-3′F1	CTACATGACTGACCCTATTGATG	53	1642
Hsp90-3′F2	GAAGAGAGAAGAACAAACAGCTG	56	1762
Hsp90-5′R1	ATTCCTCCTGTGTGATGTCATC	51	989
Hsp90-5′R2	CCTGGTACTTTTCCTTGATCTTC	53	919
Hsp90-rF	TCGATGAACCAACTACTCATGCC	60	2118
Hsp90-rR	CACCAGCCTCCGGGACAACTAC	60	2192
β-actin rF	CGAGAGATTCCGTTGCCCAGAAT	60	
β-actin rR	GATGTCGACGTCGCACTTCATG	60	
AOLP	GGCCACGCGTCGACTAGTACT$_{16}$(A/G/C)		
AAP	GGCCACGCGTCGACTAGTACG$_{10}$		
AP	GGCCACGCGTCGACTAGTAC		

The clam *P. undulata* is one of the important farmed bivalve species in China and South East Asia and mass mortality causes great losses in *P. undulata* aquaculture (Leethochavalit et al., 2004). The reasons of clam mortality are currently unknown but it has been associated with harmful environmental conditions, such as acute changes in temperature. Therefore, in order to understand the physiological responses and protective mechanism in harsh environment, we cloned and characterized *PuHsp90* gene and analyzed its expression in the gonad, heart, adductor muscle, mantle, gills, digestive gland and hemocytes under high temperature stress. Our study provides preliminary information to monitor the physiological status of *P. undulata* under different environmental conditions.

2. Materials and methods

2.1. Animal collection

More than three hundred individuals of *P. undulata*, with average shell length of 44 mm, were collected from the main distribution areas of *P. undulata* in Xiamen, Fujian, China in November 2013 (temperature 19 ± 0.5 °C). Clams were reared in tanks (7 m^3) containing aerated sand-filtered seawater (salinity 20, pH 8.1) at 19 ± 0.5 °C for 7 days, prior to the experiments. Animals were fed with 0.8% *Tetraselmis chui* once every 24 h.

Table 2
Accession numbers and percentage of amino acid homology of the Hsp90s used for the phylogenetic analysis.

Species	Protein	GenBank accession number	Amino acid homology (%)
Paphia undulata	PuHsp90	AFZ93093	100
Laternula elliptica (Antarctic bivalve)	LeHsp90	ACF35426	85
Cristaria plicata (Freshwater mussel)	CpHsp90	ADN87332	82
Argopecten irradians (Bay scallop)	AiHsp90	ABS50431	82
Crassostrea hongkongensis (Hongkong oyster)	ChHsp90	ADL59936	83
Crassostrea gigas (Pacific oyster)	CgHsp90	EKC25687	83
Haliotis discus hannai (Japanese disc abalone)	HdHsp90	ACX94847	82
Mytilus galloprovincialis (Mediterranean mussel)	MgHsp90	CAJ85741	82
Chlamys farreri (Zhikong scallop)	CfHsp90	AAR11781	83
Danio rerio (Zebrafish)	Hsp90 α	NP_001038538	79
Danio rerio (Zebrafish)	Hsp90 β	NP_571385	80
Paralichthys olivaceus (Flounder)	Hsp90 α	ABG56393	78
Paralichthys olivaceus (Flounder)	Hsp90 β	ABG56394	76
Xenopus laevis (African clawed frog)	Hsp90	AAV41061	79
Xenopus tropicalis (Western clawed frog)	Hsp90	AAH90610	79
Gallus gallus (Chicken)	Hsp90 α	NP_001103255	79
Gallus gallus (Chicken)	Hsp90 β	CAA49704	78
Rattus norvegicus (Norway rat)	Hsp90 β	XP_217339	78
Rattus norvegicus (Norway rat)	Hsp90 α	NP_786937	79
Bos taurus (Cattle)	Hsp90 β	NP_001073105	79
Bos taurus (Cattle)	Hsp90 α	NP_001012688	78
Homo sapiens (Human)	Hsp90 α	NP_005339	78
Homo sapiens (Human)	Hsp90 β	NP_031381	78
Bactrocera dorsalis (Oriental fruit fly)	Hsp90	AEJ88466	75
Eriocheir sinensis (Chinese mitten crab)	Hsp90	ADE60732	79
Fenneropenaeus chinensis (Chinese shrimp)	Hsp90	ABM92446.1	78
Penaeus monodon (Black tiger shrimp)	Hsp90	ACO83357	78
Oryza sativa (Rice)	Hsp90	BAD04054	68
Zea mays (Maize)	Hsp90	ACO35045	69