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Characterization of heat shock cognate protein 70 gene and its differential expression in response to thermal stress between two wing morphs of *Nilaparvata lugens* (Stål)

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ABSTRACT

Previous studies have demonstrated differences in thermotolerance between two wing morphs of *Nilaparvata lugens*, the most serious pest of rice across the Asia. To reveal the molecular regulatory mechanisms underlying the differential thermal resistance abilities between two wing morphs, a full-length of transcript encoding heat shock cognate protein 70 (Hsc70) was cloned, and its expression patterns across temperature gradients were analyzed. The results showed that the expression levels of *NlHsc70* in macropters increased dramatically after heat shock from 32 to 38 °C, while *NlHsc70* transcripts in brachypters remained constant under different temperature stress conditions. In addition, *NlHsc70* expression in the macropters was significantly higher than that in brachypters at 1 and 2 h recovery from 40 °C heat shock. There was no significant difference in *NlHsc70* mRNA expression between brachypters and macropters under cold shock conditions. Therefore, *NlHsc70* was indeed a constitutively expressed member of the *Hsp70* family in brachypters of *N. lugens*, while it was heat-inducible in macropters. Furthermore, the survival rates of both morphs injected with *NlHsc70* dsRNA were significantly decreased following heat shock at 40 °C or cold shock at 0 °C for 1 h. These results suggested that the up-regulation of *NlHsc70* is possibly related to the thermal resistance, and the more effective inducement expression of *NlHsc70* in macropters promotes a greater thermal tolerance under temperature stress conditions.

1. Introduction

Climate change exerts heavy impacts on the development of insects, which may lead to frequent fluctuations in their populations and changes in geographic distribution (Bale et al., 2002; Stoeckli et al., 2012). Insects are small-bodied organisms with limited ability to maintain their body temperature in response to climate change, and thus are easily influenced by thermal stress (Chen et al., 2014; Piyaphongkul et al., 2012b). Therefore, environmental temperature plays an important role in regulating their behavioral and physiological processes. Evolutionally, insects have developed many molecular and cellular strategies to cope with thermal stress and gain thermotolerance. Generally, molecular mechanisms of tolerance for extreme temperature are complex and many biochemical molecules are suggested to be involved (Clark and Worland, 2008; Neven, 2000). Among the mechanisms reported, heat shock proteins (Hsps), a set of conserved cell-protecting proteins, play a crucial role in the acquisition of thermotolerance in adaptation to temperature fluctuations (Feder and Hofmann, 1999; Rinehart et al., 2007; Xu et al., 2011).

Hsps are a group of molecular chaperones which are highly evolutionary conserved in eukaryotic organisms, and function as chaperones in protecting cellular proteins during the process of protein biosynthesis and refolding (King and MacRae, 2015; Martín-Folgar et al., 2015). These proteins represent a predominant self-protecting mechanism adapting to extreme changes in temperature, and can also be induced by various stress factors, such as starvation, chemical exposure and heavy metal pollution (Jiang et al., 2012; Shu et al., 2011; Wang et al., 2012). Usually, Hsps are grouped in superfamilies according to their molecular weight: Hsp90, Hsp70, Hsp60, Hsp40 and small Hsps (sHsps) (Feder and Hofmann, 1999; Sun et al., 2014). Among them, the Hsp70 family is one of the most highly conserved proteins, which was rapidly synthesized in response to several kinds of environmental stresses (Mayer and Bukau, 2005). Hsp70s also participate in various cellular processes, including protein translocation, cell cycle, apoptosis, as well as degradation of denatured proteins (Daugaard et al., 2007; Thanaphum and Haymer, 1998). Hsp70s have an N-terminal ATP binding domain (45 kDa) and a C-terminal substrate binding domain (25 kDa) (Karouna-Renier et al., 2003). Despite these similarities in the regulatory domains and features, the Hsp70 expression patterns are quite different. According to the expression profiles, Hsp70s can be further divided into two different groups that share many similar structural characteristics. The first group, heat shock protein 70 (Hsp70), is

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inducible and expressed at very low levels under unstressed conditions, but its transcription and translation are induced quickly in response to various stressors, while returning to a normal expression level when transferred to normal conditions (Bahar et al., 2013; Choi et al., 2014; Mahroof et al., 2005a). In contrast, another group, heat shock cognate 70 (Hsc70), is constitutively expressed in high levels in normal conditions and does not change upon exposure to stresses such as heat or cold shock (Kiang and Tsokos, 1998; Zhang and Denlinger, 2010). However, recent reports indicate that the gene expression patterns of *Hsc70* are also variable in different developmental stages and in response to different kinds of stress conditions, such as thermal stress, pesticide exposure, heavy metal treatments, starvation and food shortage (Shim et al., 2006; Sun et al., 2016; Wang et al., 2012; Yoshimi et al., 2009).

The brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae), is one of the most serious rice pests in Asia, and their feeding causes tremendous rice yield losses (Hu et al., 2014). This insect possesses two wing morphs in adult stage, and long-winged macropters has a high migratory ability (Xu et al., 2015). Due to the high thermotolerance of both morphs, *N. lugens* has spread over most rice growing areas in Asia (Piyaphongkul et al., 2012a). The ability to tolerate a wide range of temperatures is associated with *N. lugens* outbreaks, and temperature is often used to forecast population fluctuations (Ge et al., 2013; Piyaphongkul et al., 2012b). It has been reported that adults had greater thermotolerance than nymphs when exposed to high temperature, and adult macropters were more heat tolerant than brachypters (Piyaphongkul et al., 2012a). To reveal the differential thermal resistance between two wing morphs, we studied the differential expression profiles of *NlHsp90* in brachypterous and macropterous females exposed to various temperatures, indicating that *NlHsp90* may be related to the differential thermotolerance of two morphs of *N. lugens* (Lu et al., 2016b). However, except for *NlHsp90*, whether other Hsps participated in the resistance against temperature stresses is largely unclear. Therefore, in the present study, we first cloned the full-length transcript encoding Hsc70 from *N. lugens*, and then investigated the different transcriptional expression patterns of *NlHsc70* between brachypters and macropters in response to different thermal stresses. In addition, we used RNAi to reveal the function of *NlHsc70* in *N. lugens* thermotolerance.

2. Materials and methods

2.1. Insects and temperature exposure

The brown planthopper, *N. lugens*, was collected from a field population of Guangzhou City, Guangdong Province, China. The colony was reared on rice seedlings in a light incubator in our laboratory (Lu et al., 2015). The brachypterous and macropterous females were generated under the same environmental conditions: 26 ± 1 °C, 65% relative humidity and a photoperiod of 16:8 h (light:dark).

Temperature treatments were performed following previously described protocols (Lu et al., 2016b). Briefly, the treated temperatures were set up at 0, 6, 12, 18, 26, 28, 30, 32, 34, 36, 38 and 40 °C, with 26 °C as the control. Newly emerged brachypterous and macropterous female adults were thermal shocked at different temperatures for 1 h in a bath incubator (Polyscience, Niles, IL, USA). The insects exposed to extreme temperatures (40 °C or 0 °C) were allowed to recover at 26 °C for 1, 2, 6, 12 and 24 h, respectively. Three independent experiments were performed with thirty females at each temperature point, and each treatment was replicated three times.

2.2. Cloning the full length of NlHsc70

Total RNA was extracted from the whole body of *N. lugens* with an Eastep™ Universal RNA Extraction Kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. The quality and quantity of RNAs were determined by agarose electrophoresis and spectrophotometer Nanodrop2000C (Thermo Fisher Scientific, West Palm Beach, FL, USA). After DNase I treatment, 1 μg total RNA was used to synthesize first-strand complementary DNA (cDNA) with a PrimeScript RT Reagent Kit (TaKaRa, Tokyo, Japan). The synthesized cDNA was used as template for PCR amplification with degenerate primers with 35 cycles under conditions of 30 s at 95 °C for denaturation, 30 s at 50 °C for annealing and 1 min at 72 °C for extension (Table 1). Then, two gene-specific primers for rapid amplification of cDNA ends (RACE) were used to amplify the 5′ and 3′ ends of *NlHsc70* by the SMART™ RACE cDNA amplification kit (Clontech, Mountain View, CA, USA). Two rounds of nested PCR were performed with an initial denaturation step at 95 °C for 5 min, and 35 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 1.5 min, and a final extension step of 72 °C for 10 min. The final PCR products were subcloned into pGEM-T easy vector (Promega Corporation, Madison, WI, USA) and transformed into *Escherichia coli* DH5α-competent cells (Tiangen, Beijing, China). Positive plasmids were purified using a plasmid extraction kit and sequenced by Life Technologies Company (Guangzhou, China).

2.3. Quantitative real-time reverse transcriptase PCR (qRT-PCR)

Quantitative real-time reverse transcriptase PCR (qRT-PCR) was used to detect the mRNA expression levels of *NlHsc70* in control and thermal shock treatments. The primers used for qRT-PCR were designed based on the full-length of *NlHsc70* (GenBank accession no. GU723301) (nucleotides 1902–2059, 158 bp) and the housekeeping gene *β-actin* was used as an internal reference for data normalization (GenBank accession no. EU179850) (nucleotides 959–1108, 150 bp) (Bao et al., 2010). Reactions of qPCR were carried out using Light Cycler 480 detection system (Roche Diagnostics, Basel, Switzerland) with SYBR Premix^{EX} Taq II Kit (TaKaRa, Tokyo, Japan). Each 10 μl qPCR reaction volume consisted of 5 μl 2× SYBR Premix^{EX} Taq II master mix (TaKaRa, Tokyo, Japan), 0.4 μl each primer (10 μM), 1 μl cDNA equivalent to 50 ng RNA and 3.2 μl deionized water. The qPCR conditions were set as 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 15 s and then 72 °C for 20 s, and a final dissociation curve from 60 °C to 95 °C with an increment of 0.5 °C per 5 s. A calibrator control was set in order to make plate to plate comparisons as described previously (Lu et al., 2016b). To determine the qPCR amplification efficiency (*E*) of primers, a standard curve of five 10-fold serial dilutions was generated from a

Table 1
Primers used in this study.

Primers	Primer sequence
Degenerate primers	
Hsc70-F	5′-AACGARCCBACYGCYGCYGC-3′
Hsc70-R	5′-GCGTCSATSTCRAADGTGAC-3′
For RACE	
UPM	(UPM-long) 5′-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAAC GCAGAGT-3′ (UPM-short) 5′-AAGCAGTGGTATCAACGCAGAGT-3′
NUP	5′-AAGCAGTGGTATCAACGCAGAGT-3′
3′Hsc70-F1	5′-GAAACTGCTGGAGGCGTGATGACTGT-3′
3′Hsc70-F2	5′-TTCACCACCTACTCGGACAATCAGCCC-3′
5′Hsc70-R1	5′-CTTATCCATCTTGGCGTCCCTCAGCGA-3′
5′Hsc70-R2	5′-TTGAGGTCAGGTCCTTCTTGTGCTTCC-3′
For real-time PCR	
QHsc70-F	5′-CCCTCTGCAACCCCATCATC-3′
QHsc70-R	5′-GTCGACTTCTTCGATGGTTGGG-3′
QActin-F	5′-CCCTCGCTCCCTCAACAATG-3′
QActin-R	5′-TGGATGGACCAGACTCGTCGT-3′
For dsRNA synthesis	
Hsc70-Fi	5′-GACGACATCGAGCGCATGGT-3′
T7Hsc70-Ri	5′-ggatcctaatacgactcactataggg ATGGTTGGGCCAGCTCCTGA-3′
T7Hsc70-Fi	5′-ggatcctaatacgactcactataggg GACGACATCGAGCGCATGGT-3′
Hsc70-Ri	5′-ATGGTTGGGCCAGCTCCTGA-3′

Lowercase letters labeled sequences represent for T7 promoter.