Characterization of an inducible HSP70 gene in *Chilo suppressalis* and expression in response to environmental and biological stress

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Abstract

The highly conserved heat shock protein 70 (HSP70) contributes to survival at a cellular level and greatly enhances stress tolerance in many organisms. In this study, we isolate and characterize *Cshsp702*, which encodes an inducible form of HSP70 in the rice stem borer, *Chilo suppressalis*. *Cshsp702* does not contain introns; the translational product is comprised of 629 amino acids with an isoelectric point of 5.69. Real-time quantitative PCR revealed that *Cshsp702* was expressed at maximal levels in hemocytes and was minimally expressed in the midgut. Expression of *Cshsp702* in response to a range of temperatures (–11 to 43 °C) indicated significant induction by extreme cold and hot temperatures, with maximum expression after 2 h at 42 °C. The induction of *Cshsp702* in response to the endoparasite *Cotesia chilonis* was also studied; interestingly, *Cshsp702* expression in *C. suppressalis* was significantly induced at 24 h and 5 days, which correspond to predicted times of *C. chilonis* feeding and growth, respectively. The potential induction of *Cshsp702* as an inflammatory response due to parasitic stress is discussed. In conclusion, *Cshsp702* is induced in response to both environmental and biotic stress and plays an important role in the physiological adaptation of *C. suppressalis*.

Keywords HSP70 · *Chilo suppressalis* · *Cotesia chilonis* · Tissues · Thermal stress · Biological stress

Introduction

Heat shock proteins (HSPs) are highly conserved in all living organisms and are produced in response to environmental and biological stress (Lindquist 1986; Gusev et al. 2011). Protein size and structure have been used to designate HSP families as HSP100, HSP90, HSP70, HSP60, or small HSPs (sHSPs, 12–43 kDa) (Hoffmann and Parsons 1991; Kim et al. 1998; Hoffmann et al. 2003). The HSP70 family is ubiquitous in living organisms (Feder and Hofmann 1999; Sørensen et al. 2003) and functions as a molecular chaperone and anti-apoptotic agent (Johnston et al. 1998; Feder and Hofmann

1999; Yocum 2001; Hellmann 2001; McMillan et al. 2005; Sørensen et al. 2005; Košťál and Borovanský 2009). Furthermore, HSP70 repressed the immune response in rabbits due to activation of IL-10-producing T cells (Abdel-Latif et al. 2018). Interestingly, the activation of tumor cell death by heat treatment was dependent on *hsp70* expression and production of $\gamma \delta$ T cells (Wei et al. 1996). High levels of HSP70 are associated with inflammation (Wachstein et al. 2012); however, the administration of microbial HSP70 can also lead to anti-inflammatory responses (Borges et al. 2012). The contradictory effects of endogenous HSP70 with respect to inflammation are not fully understood (Jong et al. 2009). Despite a high degree of relatedness among different species, effects on the immune system can be markedly different even with the same HSP (Wang et al. 2005).

Chilo suppressalis (Walker) (Insecta: Lepidoptera: Pyralidae), which is known as the rice stem borer, causes significant damage in rice cultivated in the southern parts of Europe, north Africa, and Asia. This pest is widespread in rice fields of China. In Yangzhou (Jiangsu province), *C. suppressalis* completes two full generations and a partial third generation annually and overwinters in the larval form (Lu et al. 2012; Lu et al. 2013). The *C. suppressalis* causes

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significant yield loss in rice, which has stimulated research on biological control methodologies such as the use of natural enemies (Hong 2015). The parasitic wasp, *Cotesia chilonis* (Munakata), is the predominant species within rice fields in Yangzhou. *C. chilonis* is an important endoparasitoid of *C. suppressalis* and has been used for biological control of the rice stem borer (Kazuo et al. 1974; He et al. 1979).

Two HSP70 genes (*Cshsp70* and *Cshsc70*) were previously identified in *C. suppressalis* and were involved in temperature stress (Sonoda et al. 2006; Cui et al. 2010); however, it remains unclear whether additional HSP70s exist in *C. suppressalis*. In this study, we characterize a new inducible *hsp70* (*Cshsp702*) in *C. suppressalis* and describe its structural properties. The expression of *Cshsp702* in response to thermal stress was studied along with expression in various organs and insect tissues. The impact of *C. chilonis*-mediated endoparasitism on *Cshsp702* expression in *C. suppressalis* was also examined.

Materials and methods

Insect rearing

C. suppressalis and *C. chilonis* were collected from rice fields in Yangzhou (32.39°N, 119.42°E). Larvae of *C. suppressalis* were reared under laboratory conditions at 28 ± 1 °C and $70 \pm 5\%$ RH with a 16:8 h light/dark photoperiod and supplied with an artificial food source as described previously (Shang et al. 1979). Populations of *C. chilonis* were maintained as described for *C. suppressalis* except adults which were fed with a 10% honey/water solution and supplied with 5th instar larvae of *C. suppressalis* as a food source.

Extraction of total RNA from *C. suppressalis* and synthesis of cDNA

The SV Total RNA isolation system was used to isolate RNA from *C. suppressalis* (Promega, USA), and the quality and concentration of RNA were evaluated as described previously (Pan et al. 2018). Oligo (dT)₁₈ primers were utilized to transcribe cDNA from total RNA as recommended by the manufacturer (Fermentas, Canada). The *C. suppressalis* database (<http://ento.njau.edu.cn/ChiloDB/>) (Yin et al. 2014) was searched for partial sequences of HSP70 genes; using this approach, an uncharacterized HSP70 gene was obtained and compared with the previously described *C. suppressalis* *Cshsp70* (Cui et al. 2010). Primers (Table 1) were designed and synthesized based on the partial sequence of the new *hsp70*, which was designated *Cshsp702*. 5'-RACE and 3'-RACE were utilized to obtain the full-length cDNA sequence of *Cshsp702* (SMART RACE, Clontech), and the resulting sequence was reconfirmed by 5'-RACE.

Genomic DNA characterization

Genomic DNA was extracted from *C. suppressalis* as described previously (Pan et al. 2018), and primer pairs were designed to amplify *Cshsp* genomic fragments. PCR products were purified, cloned into pGEM-T (Promega, USA), and introduced into cells of *Escherichia coli* DH5 α as described (Pan et al. 2018). Clones containing *Cshsp* inserts were confirmed by PCR and then sequenced (ThermoFisher, Shanghai).

Sample preparation for quantitative real-time PCR

The sample design for the initial experiment has been described previously (Lu et al. 2014). Briefly, each experimental group contained 10 randomly selected 5th instar larvae and eight different insect tissues; experiments were repeated three times. Larvae were anesthetized on ice, and the following tissues were obtained and rinsed with 0.9% NaCl: epidermis (EP), heads (HE), midgut (MG), fat body (FB), Malpighian tubules (MT), foregut (FG), hemocytes (HC), and hindgut (HG). Tissues were frozen in liquid nitrogen and stored at -70 °C until needed.

In another series of experiments, 5th instar larvae of *C. suppressalis* were placed in separate glass tubes, and groups of ten were subjected to a 2-h temperature treatment (-11 , -9 , -8 , -6 , -3 , 0 , 3 , 6 , 9 , 12 , 15 , 18 , 21 , 24 , 27 , 30 , 33 , 36 , 39 , 42 , or 43 °C) in a DC-3010 controlled-temperature incubator (Jiangnan Equipment). Surviving larvae were frozen and stored at -70 °C as described above. Each treatment included three or more surviving larvae.

Fifth instar larvae of *C. suppressalis* in separate glass tubes were also subjected to parasitism by *C. chilonis*. Groups of larvae ($n = 5$) were incubated with *C. chilonis* for the following durations: 0 h (control), 1 h, 10 h, 24 h, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, and 9 days. Surviving larvae were frozen and stored at -70 °C as described above, and each treatment included three or more surviving larvae.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated, prepared, and analyzed by qRT-PCR as described previously (Pan et al. 2018). Each reaction was conducted in a 20 μ L volume that consisted of the following: iTaq™ Universal SYBR® Green Supermix, 10 μ L (Bio-Rad); gene-specific primers, 1 μ L; cDNA template, 2 μ L; and ddH₂O, 6 μ L. The amount of *Cshsp70* mRNA was determined using the $2^{-\Delta\Delta C_t}$ method and normalized using genes encoding elongation factor 1 (*EF1*), histone 3 (*H3*), and tubulin (*TUB*) for temperature exposure, tissues or organs, and parasitism, respectively (Nolan et al. 2006; Bustin et al. 2009; Xu et al. 2017; Pan et al. 2018). Controls (heads and larvae) were maintained at 27 °C (Pan et al. 2018). Following qRT-PCR, melting curve analysis was used to confirm the

Table 1 Primers used in this study

Purpose or target	Primer name	Primer sequences (5'-3')
RACE PCR	<i>hsp702</i> -5'	CGTGGATCTGGCTCTTGTCCATCTT
	<i>hsp702</i> -3'	AAGGACGCCAAGATGGACAAGAGC
Genome amplification	<i>hsp702</i> -F	AGTTCGGTACGAACCGAAG
	<i>hsp702</i> -R	TCAGGATTCACTTTGGTGTCGC
qPCR	<i>hsp702</i> -F	CATCGACTTCTACACGCGCC
	<i>hsp702</i> -R	CGACAGATTCAGTTTCTTCCCG
<i>TUB</i>	<i>TUB</i> -F	GAGGGCATGGACGAGATGGA
	<i>TUB</i> -R	ACGACGGTACGAGTATGACGGG
<i>EF1</i>	<i>EF1</i> -F	AAAATGGACTCGACTGAACCCC
	<i>EF1</i> -R	TCTCCGTGCCAACCAGAAATA
<i>H3</i>	<i>H3</i> -F	CTGCACCAAGCACTGGTGGA
	<i>H3</i> -R	TAGCGGCGGACTGGAAACG

qPCR primers used in this study were validated

homogeneity of PCR products as described previously (Schmittgen and Tamura 2008; Pan et al. 2018). Each PCR reaction was replicated three times.

Bioinformatic analysis

ORF finder (<https://www.ncbi.nlm.nih.gov/orffinder/>) was used to discover ORFs, and CLUSTALX analysis was conducted to obtain aligned amino acid sequences. The predicted CsHSP70 protein was analyzed using Compute pI/MW, Translate, and Blast tools available on the ExPASy Molecular Biology Server (Swiss Institute of Bioinformatics). Deduced protein sequences were used to obtain phylogenetic relationships using maximum likelihood, minimum evolution, neighbor-joining (NJ), and maximum parsimony. Trees were generated from 1000 bootstrap replicates with MEGA X (Felsenstein 1985; Zuckerkandl and Pauling 1965; Saitou and Nei 1987; Kumar et al. 2018).

Statistical analysis

Levene's test was used to measure homogeneity of variances among groups, and Tukey's test was used to assess significance. SPSS v. 16.0 (Pallant and Bailey 2005) was used to analyze data and obtain means $\pm$ SE (standard error).

Results

Features of the *Cshsp702* sequence

Cshsp702 from *C. suppressalis* was deposited in GenBank as accession no. MH373546. The full-length *Cshsp702* cDNA sequence is 2222 bp and is comprised of a 153-bp 5' UTR, a 1890-bp coding sequence, and a 180-bp 3' UTR. The *Cshsp702* protein product contains 629 amino acids with a predicted pI of 5.69 and mass of 69.2 kDa. Analysis using

Motif Scan (<https://prosite.expasy.org/>) revealed that CsHSP702 contains a conserved sequence typical of the HSP70 family (VVLVGGSTRIPKVQA, residues 332–346) and the cytoplasmic structural element EEVD (residues 628–631, Fig. 1). CsHSP702 and CsHSP70 shared 72.43% sequence identity (Fig. 2).

Phylogenetic analysis

The relatedness of *CsHSP702* with HSP70s in other insects was investigated, and phylogenetic trees were assembled using the approaches outlined in Methods. The four trees showed a similar trend when evaluated using MEGA X and CLUSTALX. Interestingly, *CsHSP70* and *CsHSP702* were assigned to separate clusters; the latter protein grouped with *DpHsp70* and *GmHsp70* from *Danaus plexippus* and *Grapholita molesta*, respectively, which belong to large group of Lepidopteran HSP70s (Fig. 3).

Genomic form of *Cshsp702* and expression in diverse tissues and organs

Alignment of genomic and cDNA sequences revealed that *Cshsp702* didn't contain introns, which was also true for *Fohsp703* in *Frankliniella occidentalis* (Fig. 4). The levels of *Cshsp702* expression showed significant variation in tissues and organs ($F_{7,19} = 8.555$, $P < 0.001$). Expression was highest in hemocytes and significantly lower in other tissues and organs; for example, expression was 26.67-fold higher in hemocytes than the midgut (Fig. 5).

Expression of *Cshsp702* during temperature stress

Cshsp702 was upregulated by exposure to extreme cold and hot temperature stress ($F_{20,51} = 33.382$, $P < 0.001$).

1 ATGGGGAGTATTTTAAACAGAGCGATTAAGACGAAATAAGAGATTGGTACGA
 61 ACCGAGTGAATCTACGAGTTATATCAAGCATTACTAGTACAGAGATTAAATTTGGA
 121 TATTTTGAATATAAGAAAGATAAAAACACATGCGAGCTGTAGGAATGATCTCGCA
 1 MAAVGLDLG
 181 CCACGTATCCCTTGGCTGGAGTTTGGCAACAAGGCGTGAATCATTCGCAACGAC
 10 TTYPCVGVVQHGNVEIIAND
 241 AGGGCAACAGGACACCATCATATCGTCCGCTTACGGAACAGAGGGCTAATCGGCG
 30 QGNRTTTPSYVAFDTTERLIG
 301 ATGCGGCGAAGAAACGAGTCTTCAACCCCAATACGGTGTTCGACCGCAAGGAC
 50 DAAKNQVAILNPNNTVFD AKR
 361 TGATCGGTGCAATGTCGAGTCCCAAGATCCAGCAAGACATGAAACATCGGCCCTTCA
 70 LIGRKFFDDPKIQQDMKHWPFF
 421 AGGTGGTCAAAGCTGCGCAACCCGAGATCAAGTGAATTCAGAGCGAAGACGAAAG
 90 KVVVNDCKGKPKIQVEFKGETK
 481 AGTTCCGCCGCAAGGTCGAGCATGGTCTTCTGACGAGATGAAAGGACGGCGGAAG
 110 KFAPEEVS S M V L T K M K E T A E
 541 CGTACTCGGAGCTCGTGGCGCAAGCGGTGATCAGAGTCCGCCCTACTTCAAAGCAT
 130 AYLGSSVRDAVITVPAAYFN
 601 CCCAGCGCAAGCAGCAAGCGGGGCGCATCGGCCCTGAACTGCTTGGCATCA
 150 SQRQAATKAGDAIAAGLVLR
 661 TCAACGAGCCGACGGCGCGCTCGGTATCGGCTCGCAAGAACTCAAAGGCGGA
 170 INEPTAAALAYGLDNKLNKGE
 721 GGAAGTCTCATCTTGGCTGGCGGCGCACCTTCAAGTGTCTCTGACCATCG
 190 RNVLIIFGLGGTFFDVSLTI
 781 ACAGAGGCTCTGTTGAGGTGAAGTCACGCGCGGCAACCACTGGAGGCGGA
 210 DEGSLEFEVKSTAGDTHLGG
 841 ACTTCGAACAGGCTGGTCACTCTGGCGGCAAGTTCACAGCAAGTAAAGAAAG
 230 DFDNRRLVNHLLADEFRKRYKK
 901 ATCTGGTATGAAACCGCGCTGCGCGCTCGCGCGCGCGCGCGCGCAAGC
 250 DLRMNPRLRLRLRTAAERAK
 961 GCACTCTCTGAGTCAAGGCGCATCGAGTCAAGCGACTGACAGGGCATCG
 270 R T L S S S T E A T I E I D A L Y E G I
 1021 ACTTCTACCGGCTCTCGCGCGCTTCAAGAGCTGAAAGCGAGTCTGTTCCGCG
 290 D F Y T R L S R A R F E E L N A D L F R
 1081 GCACTCGAAGCGTGAAGAGGCTTCAAGGAGCGCAAGTGAAGCAAGCAAGTCC
 310 G T L D P V E A A L K D A K M D K S Q I
 1141 ACAGCTGTCTGCTCGCGCGCTCAGAGGATCCGAGGCTCGAGGCTGCTGCA
 330 H D V V L V G G S T R I P K V Q A L L Q
 1201 ACTTCTCTCGCGGAGAACTGAACTGTCGATCAACTCGAAGGCGCTGGCTCA
 350 N F F C G K K L N L S I N L D E A V A Y
 1261 GGGCGCGGTGAGGCGCATCTGAGCGATCGAGCGCTGCAAGTCAAGAGCTG
 370 G A A V Q A A I L S G S T D S K I Q D V
 1321 TGTGTGTCGCTGGCTCCCTGCTGTCGATCGAGGCGGCGGCGGTGATGAG
 390 L L V D V A P L S L G I E T A G G V M T
 1381 AGATCATGAGGCAATCAAGATCCGTGCAAGGTCGAGGCTGCAAGCTTCAAGCT
 410 K I I E R N S K I P C K Q S Q T F T T Y
 1441 CGGACAAACGCGGCTGCAAGTGTGTAAGGCGGCAAGCGGCTGATCAAGG
 430 S D N Q P A V T I Q V Y E G E R A M T K
 1501 ACAACAACTGACGGGCTCGCGCGCGGCGAGGAGTGCAGATCGAGCTCACCT
 450 D N N L T G I P P A P R G V P K I D V T
 1561 TCGAATGAGCGCAAGCATCTGAGCTGTCGCAAGGAGCAAGCAGCGCGCA
 470 F D M D A N G I L N V S A K E N S T G R
 1621 CCAAGAACTCGTCAAGCAAGCAAGCGGCTGTCGCAAGGCGATGCAAGCA
 490 T K N I V I K N D K G R L S Q A E I D R
 1681 TGCTGTCGAGGCGGAGTACAGAGAGGAGCAAGGAGCAAGGCGCTGGCAG
 510 M L S E A E K Y R E E D E K Q R Q V A
 1741 CTCGCAAGCTGAGTGTGATGCTGTCAGCTGAGGAGCAAGCTGGAAGCGCGG
 530 A R N Q L E S Y V F S V R Q A L D V A G
 1801 AGAAGCTGAGCGCAAGGAGCATGCTGTCGCAAGGTCGCAAGGCGCTGGGT
 550 E K L S E Q D K S T A R N E C D E A L R
 1861 GGCTCGAACAACCTTGGCGCAAGGAGTACGCAAGGCTGAAAGGAGCTCG
 570 W L D N N T L A E Q E E Y E H K L D L
 1921 AGCGAGTGTCTGCCCATCATGAGCAAGTCAAGCGCGGAGAGCGCATGCCG
 590 Q R V C S P I M S K M H G A G A G M P R
 1981 GGGAGTGGCGTCAAGCAAGGAGCAAGCGGCGCATCGTGAAGGAGTGAAT
 610 G G V P G A Q Q Q R S N G P T V **E E V D**
 2041 GAAACCCGCAACCAAGTGAATCTGAAAACATAGCATGTATTAATTTAAGAA
 630 *
 2101 CTGATAATTTATTCTTCCCTTAAATGTAATTAATCTGTTTGAAGCAATAATGTC
 2161 TTTGTTTGAACAGAAATAAAGATATTAGTACAAAATAAATAAATAAATAA
 2221 AA

Fig. 1 Nucleic acid and predicted protein sequence of *Cshsp702* from *Chilo suppressalis*. Amino acid residues 332–346 represent a conserved sequence in HSP70, and this area is denoted with a bold, thick horizontal line. The EEVD motif is bordered with a rectangle

With respect to high temperatures, an increase in *Cshsp702* expression was first observed at 33 °C and was still highly expressed at 43 °C (Fig. 6). *Cshsp702* expression increased by 1002.88-fold after 2 h at 42 °C and was 929.59-fold higher than the control at 27 °C (Fig. 6). During cold stress, *Cshsp702* expression was maximal at −11 °C, which was 99.4-fold higher than that of the control (Fig. 6). These results indicate that *Cshsp702* responds to both extreme cold and hot temperature stress and is especially responsive to extreme temperatures.

Cshsp702 expression during parasitism

After *C. suppressalis* was parasitized by *C. chilonis*, *Cshsp702* expression showed an upward trend in expression, and it was significantly higher at 24 h and 5 days ($F_{11, 28} = 4.514$, $P < 0.001$) (Fig. 7). The expression level of *Cshsp702* was highest at the 5-day parasitism time point, where it was 4.35-fold higher than that of the control (*C. suppressalis* larvae not parasitized by *C. chilonis*). The lowest level of *Cshsp702* expression was observed at 8 days, where transcript levels were only 1.38-fold higher than the control. Thus, *Cshsp702* responded to parasitic stress, but expression was not consistently high.

Discussion

In this study, *Cshsp702* was cloned and characterized from *C. suppressalis*; the cDNA encoded a 1890-bp ORF with no introns. The absence of introns was also observed for *hsp703* in *F. occidentalis* (Qin et al. 2017); however, it is important to note that introns have been reported in genes encoding other insect HSP70s (Li and Du 2013). For example, *Fohsp701*, *Fohsp702*, *Nvhs70*, and *Bmhs70* contain four, seven, three, and two introns, respectively (Fig. 4). It has been reported that genes without introns or containing short introns may be expressed at higher levels than genes with multiple or long introns (Comeron 2004). One role of HSP70 is to help maintain the normal functioning of organs and tissues. We observed significant variation in *Cshsp702* expression in different tissues or organs, and mRNA levels were particularly high in hemocytes (Fig. 5). Hemolymph facilitates nutrient transport and thermoregulation; thus, it remains possible that *Cshsp702* helps protect hemocytes from toxins and environmental stressors (Gupta and Singh 1994). The hemocytes are key cells for invertebrates' innate defense reactions (Vazquez et al. 2009; Iwanaga and Lee 2005), and they play an important role in the host immune functions when the organism is attacked by bacteria or viruses (Lee and Söderhäll 2002; Lavine and Strand 2002; Wu et al. 2013). EcFAMeT ascended at 6 h in hemocytes of *Exopalaemon carinicauda* when shrimps were injected with white spot syndrome virus (Duan et al. 2014). We speculated that *Cshsp702* had immune functions for it was upregulated when *C. suppressalis* was parasitized, that was why hemocytes showed the biggest response. The deduced protein, CsHSP702, contained 629 amino acids with a probable mass of 69.2 kDa and isoelectric point of 5.69. CsHSP702 contains the conserved VVLVGGSTRIPKVQA sequence that is typical of HSP70 family members. Furthermore, the C-terminal end of CsHSP702 contains the highly conserved cytoplasm-specific EEVD motif, indicating that CsHSP702 functions in the cytoplasm (Gupta 1995; Zhang and Denlinger 2010). The predicted CsHSP702 shows over 70% identity to CsHSP70 (Cui et al. 2010);

Fig. 2 Amino acid alignment of Cshsp70 and Cshsp702. Sequences are deposited with GenBank as accession numbers GU726137 (Cshsp70) and MH373546 (Cshsp702)

however, it is even more closely related to HSP70 from *D. plexippus* and *Bombyx mori*.

Proteins in the HSP70 family are generally encoded by two genes, *hsp70* and *hsc70*, which are induced and constitutively expressed forms of the gene, respectively (Pardue et al. 1980;

Boutet et al. 2003; Franzellitti and Fabbri 2005). Under most conditions, *hsc70* is stably expressed in all cells and does not respond to environmental stress factors. In contrast, *hsp70* is either not expressed or expressed at a minimal level until induced by environmental or biotic factors (Boutet et al. 2003; Deane and Woo 2005). Both *Cshsp702* (this study) and *Cshsp70* (Cui et al. 2010) encode inducible forms of HSP70, and qRT-PCR experiments revealed that *Cshsp702* was expressed in response to extreme thermal stress (Fig. 6). Cui et al. reported that *hsp70* isoform might be important for routine folding, and this gene in *C. suppressalis* was closer constitutive expression. However, *Cshsp702* was about stress responsiveness, which was induced by an acute cold and high stress in response to increased relative expression of mRNA. Temperature tolerance is largely attributed to the expression levels of HSP-encoding genes (Castillo-Davis et al. 2002; Sørensen et al. 2003; Queitsch et al. 2002). Sun-irradiated *Drosophila* larvae and pupae produced high levels of HSP70 and survived exposure to 40 °C (Feder and Krebs 1997). During thermal stress, *Fohsp703* expression and survival rates were higher in *F. occidentalis* females than males (Qin et al. 2017). Our expression studies indicated that *Cshsp702* was highly expressed in larvae exposed to extreme cold (−8 to −11 °C) and hot temperatures; maximal expression was observed at 42 °C (Fig. 4), which may be the optimal temperature for induction. Similarly, Huang and Kang reported that the highest temperature threshold for *Liriomyza sativae* *hsp70* expression was 42.5 °C (Huang et al. 2007). In addition, *hsp70* was found to be upregulated in unsuitable low-temperature conditions in most organisms. For example, human cells subjected to a 4 °C cold shock upregulated *hsp70*

Fig. 3 Phylogenetic tree showing relatedness of Cshsp702 to other insect HSP70s. The tree was constructed using the neighbor-joining method; numbers represent bootstrap values acquired from 1000 replicates (only values greater than 50 are shown). *C. suppressalis* Cshsp70 and Cshsp702 are bordered by rectangle. Abbreviations, species, and accession numbers are as follows: *Bombyx mori* (Bmshp68, XP_004923548); *Cotesia chilonis* (CcHsc70, KM077499; CcHsp70, KM077498); *Chilo suppressalis* (Cshsp70, GU726137; Cshsp702, MH373546); *Cotesia vestalis* (Cvshp70, AGF34718); *Danaus plexippus* (Dphsp70, OWR47855); *Grapholita molesta* (Gmshp70, ANJ86404); *Liriomyza huidobrensis* (Lhshp70, AAW32098); *Melitaea cinxia* (Mchsp704, AGR84227); *Musca domestica* (Mdshp70, XP_005189456); *Papilio xuthus* (Pxhsp68, KPJ02391); *Pteromalus puparum* (Pphsp70, ACO57618); *Spodoptera litura* (Slhsp68, XP_022825877); and *Stratiomys singularior* (Sshsp70, ADX42270)

Fig. 4 Diagrammatic representation of the genomic forms of *hsp70* in several insect species. Black solid rectangles and horizontal lines represent introns and exons, respectively. Numbers indicate intron position. HSP70 genes are shown for *Chilo suppressalis* (*Cshsp702*), *Frankliniella occidentalis* (*Fohsp701*, *Fohsp702*, *Fohsp703*), *Nasonia vitripennis* (*Nvhs704*), and *Bombyx mori* (*Bmhs70*)

transcript after returning to 37 °C for recovery (Liu et al. 1994). In addition, *hsp70* was expressed strongly in response to low temperature and expression and then persists for the duration of diapause (Yocum et al. 1991, Denlinger et al. 1992). Furthermore, Ali et al. (2003) reported that in carp acclimated to 12 °C, a temperature drop of 7 °C for 1 h resulted in the upregulation of the inducible *hsp70* transcript in both the liver and white muscle. In addition, *Hsp70* in *Culex pipiens* was turned on briefly in response to a cold shock, but the expression did not persist (Robich et al. 2006). The relative expression of *Cshsp702* was significantly upregulated under low-temperature conditions, which was beneficial to the survival of *C. suppressalis* in winter. Otherwise, unlike *Cshsp702*, the Antarctic fish isolated over evolutionary history in the sub-zero waters of the southern ocean. Although there was an inducible heat shock protein, it was not induced by acute thermal

stress and exhibited a constitutive expression pattern (Buckley et al. 2004; Place and Hofmann 2005).

Parasitism of *C. suppressalis* by *C. chilonis* caused a general increase in *Cshsp702* expression, and mRNA levels at the 24 h and 5-day time points were significantly different from the control (Fig. 7). In medicine, HSPs have been used as inducers to help protect and regulate the immune system and to promote inflammatory responses (Odashima et al. 2002; Mayer and Bukau 2005; Oyake et al. 2006). It is tempting to speculate that *Cshsp702* may act as a molecular chaperone to stimulate protective enzymes in *C. suppressalis*, which may help it resist or eliminate the parasitic wasp *C. chilonis*. Although *Cshsp702* was upregulated during parasitic stress, expression levels were relatively low. However, *Cshsp702* expression was significantly induced at 24 h and 5 days, which correspond to predicted times of feeding and growth of the endoparasitoid *C. chilonis*. The prolonged production of

Fig. 5 *Cshsp702* expression in *C. suppressalis* foregut (FG), midgut (MG), hindgut (HG), fat body (FB), Malpighian tubules (MT), heads (HE), epidermis (EP), and hemocytes (HC)

Fig. 6 *Cshsp702* expression in *C. suppressalis* exposed to -11, -9, -8, -6, -3, 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, and 43 °C

Fig. 7 *Cshsp702* expression in *C. suppressalis* parasitized by *C. chilonis* for the following time intervals: 0 h, 1 h, 10 h, 24 h, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, and 9 days

HSPs can negatively impact growth and development of an organism; thus, *C. suppressalis* may selectively downregulate *Cshsp702* expression at selective time intervals during endoparasitism. It also remains possible that the induction of *Cshsp702* during endoparasitism arises due to inflammation, which is worthy of further study.

HSP70 has been widely used as a molecular marker to study transcriptional changes during the stress response, which has furthered our understanding of the molecular mechanisms underlying ecological adaptation (Yocum 2001; Hellmann 2002; McMillan et al. 2005; Sørensen et al. 2005). Insects encounter extreme temperatures and parasites in nature, and the modulation of HSPs in response to environmental and biotic stress is paramount. The current study furthers our understanding of the HSP70 gene family in responding to extreme temperatures and parasitism and provides groundwork for future studies aimed at understanding the molecular mechanisms that are driving the evolutionary adaptation of *C. suppressalis*.

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