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ORIGINAL PAPER

Identification and characterization of novel ER-based *hsp90* gene in the red flour beetle, *Tribolium castaneum*

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Abstract Heat-shock protein 90 (HSP90) is a highly conserved molecular chaperone found in all species except for Archaea, which is required not only for stress tolerance but also for normal development. Recently, it was reported that HSP83, one member of the cytosolic HSP90 family, contributes to oogenesis and responds to heat resistance in *Tribolium castaneum*. Here, a novel ER-based HSP90 gene, *Tchsp90*, has been identified in *T. castaneum*. Phylogenetic analysis showed that *hsp90s* and *hsp83s* evolved separately from a common ancestor but that *hsp90s* originated earlier. Quantitative real-time polymerase chain reaction illustrated that *Tchsp90* is expressed in all developmental stages and is highly expressed at early pupa and late adult stages. *Tchsp90* was upregulated in response to heat stress but not to cold stress. Laval RNAi revealed that *Tchsp90* is important for larval/pupal development. Meanwhile, parental RNAi indicated that it completely inhibited female fecundity and partially inhibited male fertility once *Tchsp90* was knocked down and that it will further shorten the lifespan of *T. castaneum*. These results suggest that *Tchsp90* is essential for development, lifespan, and reproduction in *T. castaneum* in addition to its response to heat stress.

Keywords HSP90 · Heat stress · Development · Reproduction · Lifespan · *Tribolium castaneum*

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Introduction

Heat-shock proteins (HSPs) are highly conserved chaperones typically expressed in response to environmental stress factors and were identified from *Drosophila melanogaster* in 1964 (Ritossa and Vonborstel 1964). Usually, HSPs can be classified into several families, such as HSP110, HSP90, HSP70, and small HSPs (sHSPs) on the basis of their molecular weight and the homology of their amino acid sequences (Csermely et al. 1998; Lindquist and Craig 1988). Heat-shock protein 90 (HSP90) was discovered in all species except in Archaea, and the molecular weight of this family ranges from 82 to 94 kDa in different organisms (Csermely et al. 1998; Kim et al. 1998; Large et al. 2009). They consist of the following conserved domains: N-terminal ATP-binding domain, the charged linker domain, middle-domain, and the C-terminal dimerization domain (Marzec et al. 2012; Meyer et al. 2003; Street et al. 2011).

However, there are variant isoforms of HSP90 that exist among eukaryotes. For example, in *D. melanogaster* and *Caenorhabditis elegans*, only one cytosolic HSP90 homolog exists, and two closely related isoforms exist in mammals as well as in yeast cytosol, which are differentially regulated in tissues and by various forms of cellular stress, while there are three isoforms in zebra fish (Haslbeck et al. 2012; Johnson 2012; Katherine et al. 1989; Krone et al. 1997). Beyond these cytosolic HSP90 proteins, there is another major HSP90 group, which is the ER-based HSP90 homolog (also called as 94-kDa glucose-regulated protein, GRP94, gp96, or endoplasmin), which shows high sequence similarity with cytosolic HSP90 homolog, except that it has an additional signal peptide in its N terminus and ER-targeting motif KDEL in its C terminus (Marzec et al. 2012; Pelham 1989). Moreover, mitochondria, chloroplast HSP90 homologs, and even HTPG (high-temperature protein G in bacterial cytosol) have been

discovered lately, although these three homologs show less sequence similarity with the previous two members. Meanwhile, mitochondria and chloroplast HSP90 function in specific cell organs, and HTPG is specific for prokaryotes (Chen et al. 2006).

Current research has mostly focused on the vital role that cytosolic HSP90 plays against environmental stress such as heat, cold, toxins, and invading pathogens (Boher et al. 2012; Colinet et al. 2010; Kaiser et al. 2011; Liu et al. 2004) and in its development as a chaperone of steroid hormone receptors and signaling kinases in assisting other proteins in folding and maturation with its co-chaperone (Richter and Buchner 2001; Theodoraki and Caplan 2012). However, relatively little is known regarding the chaperone biology of ER-based HSP90. While ER-based HSP90 is an endoplasmic reticulum stress protein of the heat-shock protein (HSP) 90 family with an ER retention signal and has high levels in secretory tissues (Johnson 2012). The upregulation of ER-based Hsp90 is used as a hallmark of responses to ER stress (Eletto et al. 2010). Recent studies have demonstrated ER-based HSP90 is involved in maturation of proteins destined for cell surface display or export and plays a vital role in both the adaptive and innate immune systems (Marzec et al. 2012; Tramentozzi et al. 2011; Yang et al. 2007). Moreover, it is also required during the early developmental stage in *C. elegans*, fly, and mouse (Marzec et al. 2012; Maynard et al. 2010; Wanderling et al. 2007). And, in fly, this HSP90 orthologous protein mutant larvae has pronounced defects in its midgut epithelium (Marzec et al. 2012; Maynard et al. 2010).

Very recently, like the other model species, cytosolic HSP90 was renamed as HSP83 based on its molecular weight and was identified in a recently emerged model insect, red flour beetle *Tribolium castaneum.* It has been shown that *hsp83* contributes to oogenesis, compound eye development, and response to heat resistance in the *T. castaneum* (Knorr and Vilcinskas 2011, Xu et al. 2009; 2010a). However, no information as to the role of the *hsp90* (the homologue of ER-based *hsp90* in *T. castaneum*) gene in *T. castaneum,* either for stress response or for insect development, is available to date. Therefore, based on their molecular weight, we refer to cytosolic HSP90 and ER-based HSP90 as HSP83 and HSP90, respectively, for convenience. In this study, we have cloned cDNA of the *Tchsp90* gene and phylogenetic analysis has revealed that *Tchsp90* and *Tchsp83* evolved separately from a common ancestor and *Tchsp90* originated earlier than *Tchsp83.* In order to understand the function of *Tchsp90*, we further investigated whether this molecule responds to heat or cold stresses. Moreover, an RNAi experiment has illustrated that *Tchsp90* plays vital roles in the development, reproduction, and lifespan in *T. castaneum.*

Materials and methods

Experimental animals

The Georgia-1 (GA-1) strain of *T. castaneum* was reared at 30 °C and 40 % relative humidity in 5 % yeasted flour under standard conditions (Haliscak and Beeman 1983; Li et al. 2011).

Identification and cloning of *hsp90* gene in *T. castaneum*

The *Tchsp90* gene was identified by searching Beetlebase (http://www.beetlebase.org/). Based on the predicted sequence, we designed the primers to amplify the full-length of *Tchsp90* cDNA. The primers used for polymerase chain reaction (PCR)-based cloning are listed in Table 1. cDNA from the late larva was used as the template for cloning, and PCR products were cloned into the pEASY-T3 Cloning Vector (TaKaRa) and sequenced in the Majorbio of Shanghai.

Sequences and phylogenetic analyses

To identify candidate HSP83 and HSP90 genes (cytosolic HSP90 and ER-Based HSP90 isoforms) from other organisms, BLASTP and TBLASTN were used to search the following databases: FlyBase (http://flybase.org/), BeeBase (http://hymenopteragenome.org/beebase/), Silkworm Genome Database (http://silkworm.genomics.org.cn/), AphidBase (http://www.aphidbase.com/aphidbase), VectorBase (http://www.vectorbase.org/index.php), BeetleBase (http://www.beetlebase.org/), and NCBI (http://www.nibi.nlm.nih.gov/). The *E* value for evaluating all sequences in homology search is 10^{-6}. These candidate genes were further examined by reciprocal Blast in NCBI to ensure they are real HSP90. A total of 21 *hsp90* homologues and 31 *hsp83* homologues were derived from 21 species which have completed genomic data from invertebrate to vertebrate animals for subsequent analysis (Electronic supplementary material Table S1). The gene structural prediction was analyzed online using PredictProtein (http://www.predictprotein.org/) and InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/). Multiple sequence alignments of the amino acid sequences were performed online using ClustalW2 from the website (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The alignments were viewed and edited with the BOXSHADE software (http://www.ch.embnet.org/software/BOX_form.html). Phylogenetic trees were constructed using MEGA 5.0 software with the neighbor-joining and maximum likelihood (ML) methods (Tamura et al. 2011). One thousand bootstrap tests were performed, and values lower than 50 % are not shown.

Table 1 Oligo-nucleotide primers used in this study

Primer	Direction (3′–5′)	Fragment length (bp)	Sequence	Remarks
hsp90-L1	Forward	2349	ATGAAGCAGTTGATTGTTTTAGCG	Whole ORF of hsp90
hsp90-R1	Reverse		TTACAACTCGTCATGATCCCCA	
hsp90-L2	Forward	303	CACACGAGCAGATGAATCTATTG	hsp90 transcriptional level
hsp90-R2	Reverse		CAAGCGGATCTTATCTAAAGCGT	
hsp90-L3	Forward	511	TAATACGACTCACTATAGGGTTAAAAGAAC TGCGCGACAAAGC	dsRNA of hsp90
hsp90-R3	Reverse		TAATACGACTCACTATAGGGCACAATGGAA AAGCTTGACGAATC	
P1			GCGAGCACAGAATTAATACGACTCACTATA GGTTTTTTTTTTTTVN	3′RACE cDNA synthesis
P2	Reverse		GCGAGCACAGAATTAATACGACT	3′RACE
P3	Reverse		CGCGGATCCGAATTAATACGACTCACTATAGG	
hsp83-L	Forward	324	CTTCCAGACTCGATTCAGGCAAA	hsp83 transcriptional level
hsp83-R	Reverse		GTGAAGCTACCACCAGCTGA	
rps3	Forward	260	TCAAATTGATCGGAGGTTTG	Internal control transcriptional level
rps3	Reverse		GTCCCACGGCAACATAATCT	

Quantitative real-time RT-PCR

Total RNAs from pools of three individuals were extracted at each of the following developmental stages: early eggs (EE, 1 day old), late eggs (LE, 3 days old), early larvae (EL, 1 day old), late larvae (LL, last-instar larvae), early pupae (EP, 1 day old), late pupae (LP, 5 days old), early adults (EA, 1 day old), and late adults (LA, 1 week old) with the RNAiso Plus reagent (TaKaRa). Reverse transcription was performed using 1 μg total RNA. PCR amplification was performed under the following conditions: one cycle of 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s; and one cycle of 72 °C for 7 min. The *T. castaneum* ribosomal protein S3 (*rps3*, GenBank accession numbers CB335975) gene served as an internal control and was run for 28 cycles under the same conditions (Arakane et al. 2008). The primers are listed in Table 1.

RNA interference

Double-stranded RNAs (dsRNA) was designed on nucleotides 199–710 (511 bp) of the CDS region of the *Tchsp90*, and these regions span exons 3–4. The nucleotide sequence identity between *Tchsp90* and *Tchsp83* in the targeted region is only 59.5 %, while the longest stretch of identical matching sequence for the region is only 14 bp. About 200 ng of dsRNA in 200 nl solution was injected into last-instar larvae and early pupae (Arakane et al. 2005; Tomoyasu and Denell 2004). On the sixth day after dsRNA injection, the insects were used to detect whether RNA interference is effective and whether it shows cross interference between *Tchsp90* and *Tchsp83*. Afterwards, these insects were reared in the same conditions mentioned above, and the developmental status was observed.

Behavior analysis

Larval injections were followed by the observation of the noticeable morphological defects and mortality. Three biological replications were carried out for the experiments with 25 beetles/replication/group. Mortality occurring less than 5 days after injection was attributed to injection injury rather than to target transcript knockdown. In general, mortality due to injection damage was <10 % (Begum et al. 2009). Pupal injections, which did not cause any detectable defects in adult eclosion, were regarded as parental RNAi experiments (Bucher et al. 2002) and were followed by the assessment of oviposition rates and progeny survival. Individuals in two replications of RNAi were utilized for single-pair matings (10 to 15 pairs/replication, respectively). Three-day ovipositions (13-day- to 16-day-old females) were collected, counted, and held for hatch-rate measurement, and their development was observed. Numbers of offspring were counted 15 days after the eggs were collected. Negative controls consisted of injections of either vermilion dsRNA or an equal volume of buffer only (Begum et al. 2009). The data analysis was done using STATISTIC version 10.0 by one-way ANOVA.

Stress treatment

To examine whether *Tchsp90* is a response to thermal and cold stress, 24 late larvae for each group were reared at 45 °C and 4 °C condition. Six insects for each time point were collected after the insects were treated with 45 °C and 4 °C for 1, 2, 4, and 12 h, respectively. In total, three biological replications were carried out for these experiments. Thereafter, the expression level of *Tchsp90* was measured.

For a starvation tolerance assay, parental RNAi was carried out. Once the wild-type and *Tchsp90* RNAi insects were treated under starvation conditions, the expression level of *Tchsp90* and survival rates were investigated. Two biological replications were carried out for the experiments, and 25 beetles were used in each group.

Results

Identification of *hsp90* gene from *T. castaneum*

According to the known *D. melanogaster* cytosolic and ER-based *hsp90*s sequences, we mined out two candidates from *T. castaneum* genome and named them as *Tchsp83* and *Tchsp90*, respectively, based on their predicted molecular weights. *Tchsp90* was identified in *T. castaneum* for the first time and is located on chromosome 9 of the *T. castaneum* genome. *Tchsp90* contains a 2,349 bp open reading frame (ORF) interrupted by three introns, which produced a putative protein of 782 amino acids with the predicted molecular mass of 89.5 kDa. The 3′ non-translated region of the *Tchsp90* is 115 bp and contains a typical motif polyadenylation signal (accession number: KF922652). TcHSP90 is highly conserved with the homologs of HSP90 from *Acromyrmex echinatior* (accession number: EGI61481.1) and *D. melanogaster* (accession number: CG1242) with 72.8 % and 73.3 % identity, respectively. HSP90 shares 48.8 %–99.5 % identity across animals; HSP83 shares 47.1 %–99.3 %, but HSP90 and HSP83 share 24.6 %–43.3 % identity across animals (Electronic supplementary material Table S2, Table S3, and Table S4). However, TcHSP90 only showed 41.6 % amino sequence identity with TcHSP83, although both subfamilies of HSP90 and HSP83 contain four conserved domains and an ATP binding domain (Fig. 1). For TcHSP90, beside these domains, there is an extra ~20aa with Gln-rich signal peptide that only exists in the N-terminal of HSP90 not in that of HSP83. Moreover, the C terminus of HSP90 has a conserved HDEL motif which is an endoplasmic reticulum targeting sequence, while HSP83 has an MEEVD motif (Fig. 1).

Phylogenetic analysis

In order to understand the evolutionary history of HSP83 and HSP90, 21 genome sequenced invertebrates and vertebrates were selected. A total of 34 *hsp83* homologs and 21 *hsp90* homologs were derived from the genome database of these organisms. Phylogenic analysis revealed that HSP83s and HSP90s possessed the same origin and diverged before the differentiation of invertebrates and vertebrates (Fig. 2 and electronic supplementary material Fig. S1). Thereafter, another major duplication occurred before the differentiation of vertebrates and which led to the fact that vertebrates have two copies of HSP83, except that *Danio rerio* has three HSP83s which are located on chromosome 20, and these three HSP83 share 79.2 % identity. On the other hand, invertebrates have only one HSP83 except that *Apis mellifera* and *Acyrthosiphon pisum* have two HSP83s derived from species-specific gene duplication. The two HSP83s of *A. mellifera* are located on chromosome 1 and chromosome 7 separately, and share 82.1 % amino acid identity with each other. The two HSP83s of *A. pisum* are located on scaffold GL349748 and GL349707 with 87.1 % amino acid identity. However, each species has only one highly conserved *hsp90* gene in the group based on their genome sequences.

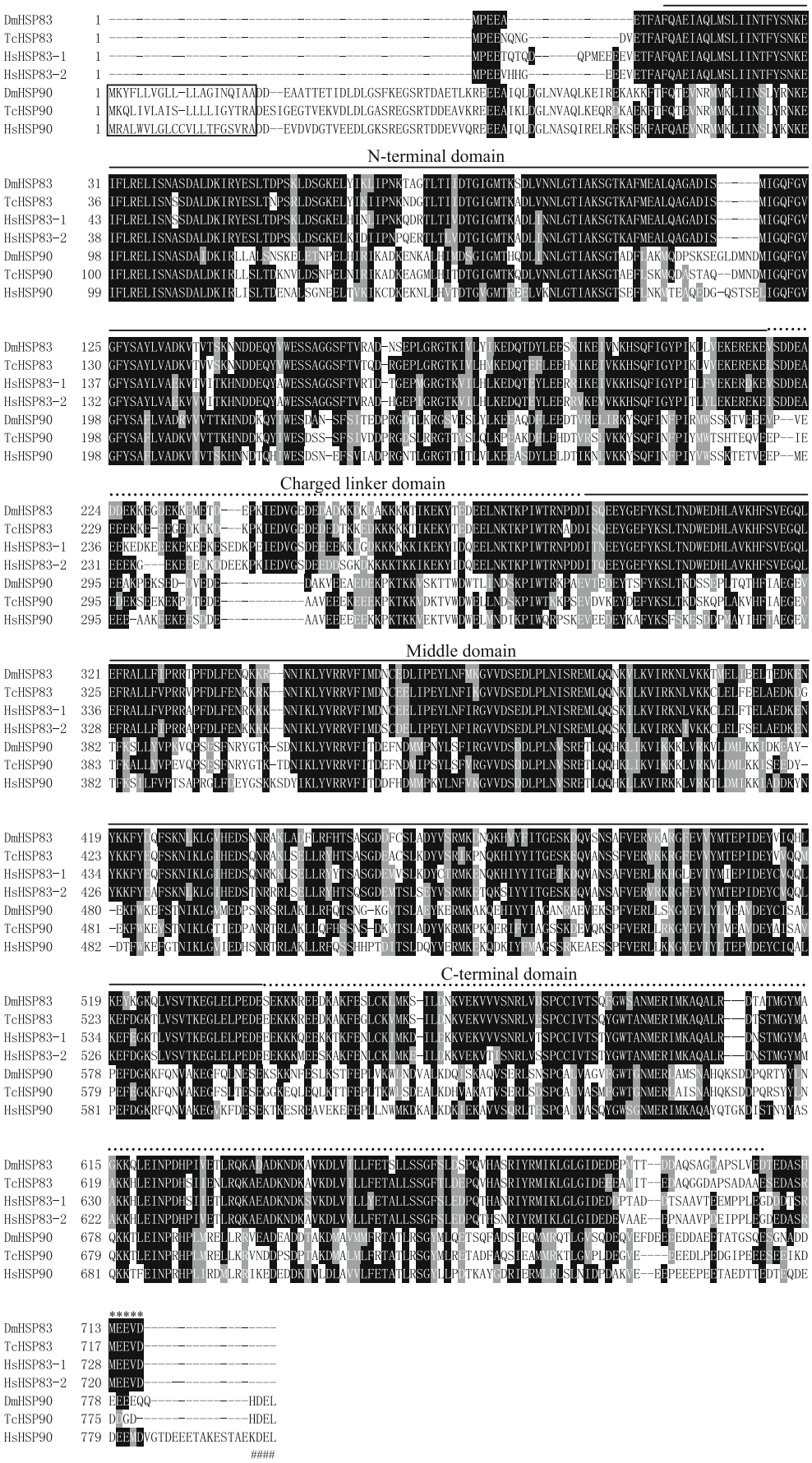
Fig. 1 Alignment of the amino acid sequences of HSP90s and HSP83s. The signal peptide was marked with *black box*. The first 50 amino acids of HSP90 (GRP94) are distinct from the N terminus of HSP83 in both length and sequence. The ATP-bind domain located in N terminus both HSP90 and HSP83. The C-terminal conserved signature sequences of HSP83 were marked with *asterisk* while the HSP90 were marked with *number sign*. The HSP90 sequences derived from *D. melanogaster* (DmHSP90, CG5520); *Homo sapiens* (HsHSP90, CAI64497.1); *T. castaneum* (TcHSP90,TC012185), and the HSP83s derived from *D. melanogaster* (DmHSP83, CG1242); *H. sapiens* (HsHSP83-1,NP-005339.3; HsHSP83-2,NP-031381.2); *T. castaneum* (TcHSP83,TC014606)

Expression pattern of *Tchsp90*

The expression patterns of *Tchsp90* gene were investigated by quantitative real-time polymerase chain reaction (qRT-PCR)using the *rps3* gene for normalization. *Tchsp90* transcript abundance was highly expressed at the early stage for each developmental stage and formed three expression peaks from larvae to adults except for the embryo stage, while it was least expressed at the later larval stage and was expressed most at the early pupal stage (Fig. 3a).

Response to stresses

As shown in Fig. 3b, *Tchsp90* expression level was significantly increased after heat stress and reached a peak after 2 h of heat treatment at 45 °C, the expression level is 3.7~8.3-fold, higher than that without the thermal treatment, and its expression level gradually returned to normal. However, *Tchsp90* did not show any response to cold stress (4 °C). After starvation, the qRT-PCR analysis indicated that *Tchsp90* expression levels declined gradually and reached the lowest expression level on the sixth day, remaining at this level until the beetles died (Fig. 4a). Pupa RNAi resulted in suppression of transcript level for *Tchsp90* (Fig. 4b). However, *Tchsp90* knock-down insects could survive 12.5±0.14 days under starvation conditions and showed comparable starvation tolerance with the wild-type and buffer-injected insects which survived 13.5±0.61 and 12.9±0.64 days, respectively, under starvation conditions (Fig. 4c).


N-terminal domain
Charged linker domain
Middle domain
C-terminal domain
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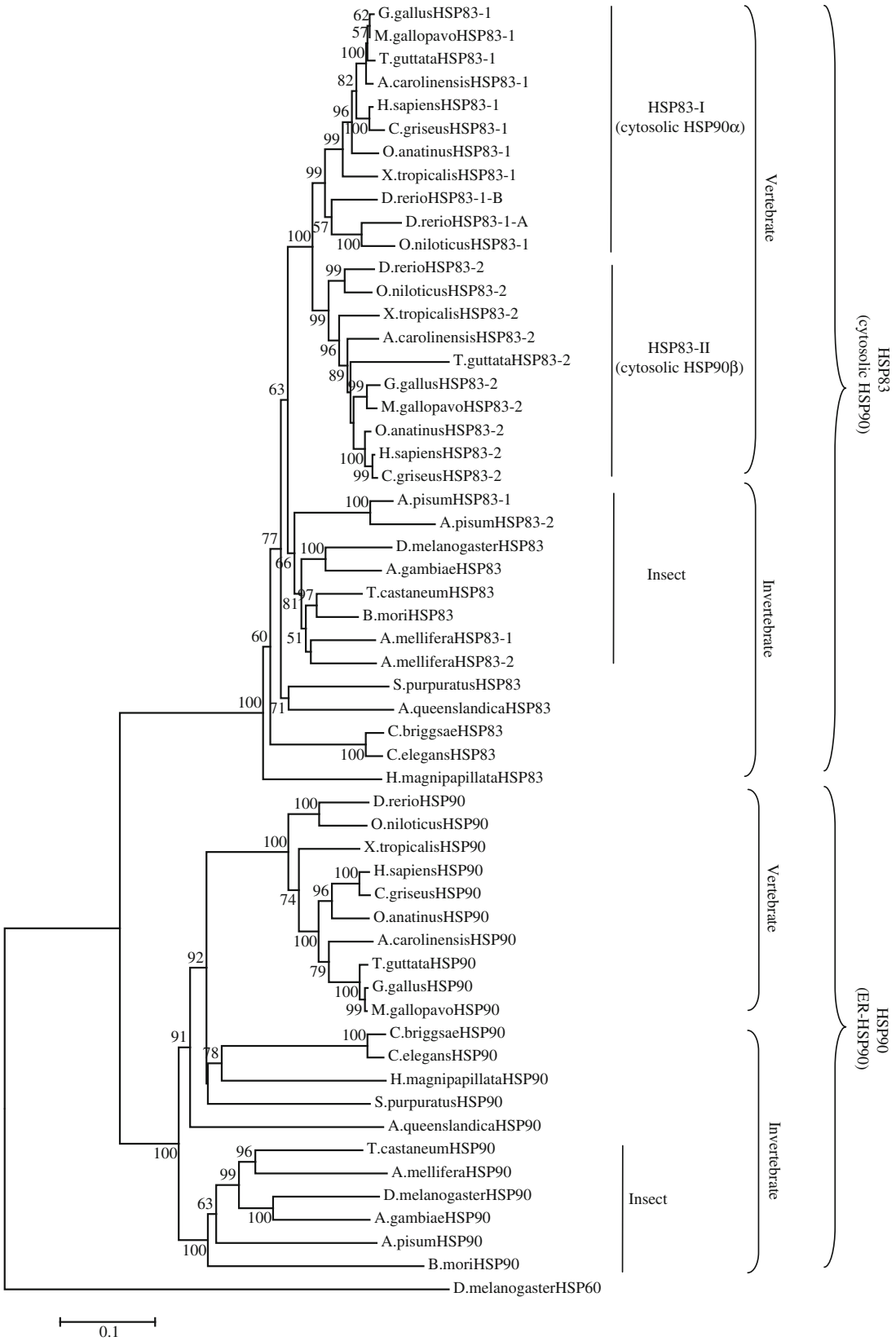

Fig. 2 Phylogenetic analysis of HSP90s and HSP83s. Bootstrap values below 50 were removed from the tree. The ML tree is rooted by the *D. melanogaster* HSP60. The details of the gene information which used in the analysis were descript in Electronic supplementary material Table S1

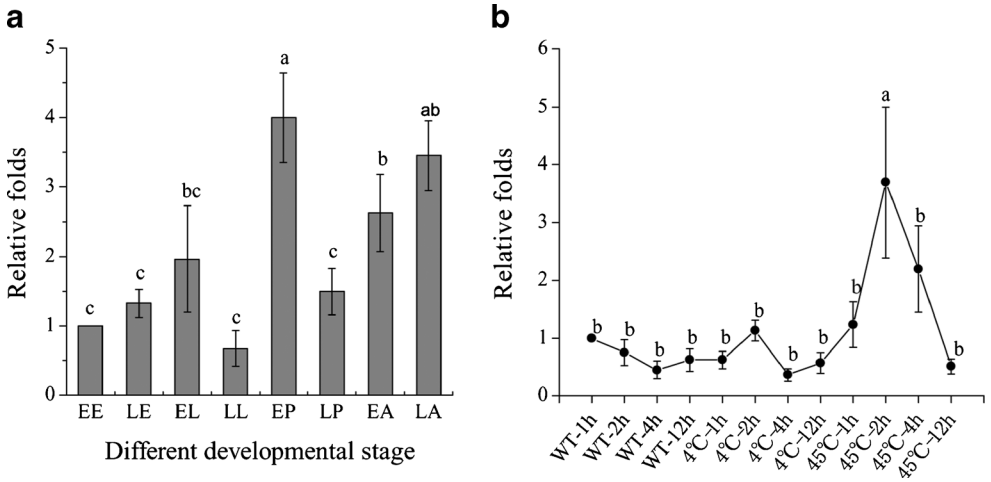

Fig. 3 The expression pattern of *Tchsp90* in different developmental stages and under cold and heat treatment. **a** Quantitative real-time PCR analysis of *Tchsp90* mRNA relative transcript levels were normalized to ribosomal protein s3 (*rps3*) in the same cDNA samples. Different development stages are *EE* early egg (1-day-old); *LE* late egg (3-day-old); *EL* early larval (1-day-old); *LL* late larval (7-day-old); *EP* early pupae (1-day-old); *LP* late pupae (6-day-old); *EA* early adult (1-day-old); *LA* late adult (7-day-old). **b** The expression of *hsp90* in cold (4 °C) and heat (45 °C) treatment. The *lowercase letters* were analyzed by HSD multiple compartment. *Error bars* represent standard deviations among three biological replications

RNAi phenotypes of *Tchsp90*

Injection of dsRNA of *Tchsp90* during the late larval stage resulted in defects in larval development and suppression of transcript levels for this gene (Fig. 5). Interestingly, larval RNAi of *Tchsp90* caused approximately 74 % insects to fail to molt subsequently and were unable to completely shed larval cuticles, and they finally died at larval stage. The remaining 26 % insects could molt to the pupal stage with morphological deficiency and eventually died at pupa stage (Fig. 5).

To examine later stages of RNAi phenotypes, we injected 2-day-old pupae and assessed oviposition rate and the survival rates of the offspring. The injection of RNAi for *Tchsp90* at the pupal stage caused a complete inhibition in egg production, whereas the wild-type and IB beetles laid an average of 5.4 and 5.6 eggs/day/female. Meanwhile, the inhibited oviposition rates were 40.5±0.6 and 39.1±2.3 were recovered by back-crossing with wild-type and IB females and could not be recovered by back-crossing with wild type males (Fig. 6a). But the egg hatch rate had no apparent differences among ds-*hsp90* ♂× WT♀group, ds-*hsp90* ♂× IB♀group, IB group, and WT group (Fig. 6b).

In order to examine if Tchsp90 is involved in longevity of *T. castaneum*, we injected 2-day-old pupae and assessed their survival days. The pupae with injected ds-*hsp90* all died during the 80 days after eclosion. However, the survival rate of wild-type and buffer-injected adults (WT and IB) remained

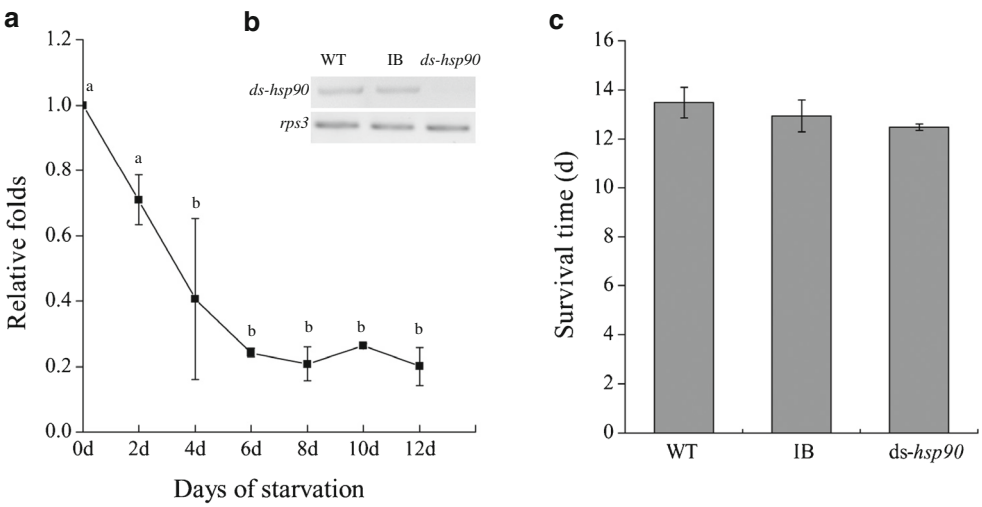

Fig. 4 The expression pattern of *Tchsp90* under starvation and the survivorship after the pupa RNAi. **a** Time course analysis of *Tchsp90* expression under starvation stress. **b** The *Tchsp90* expression level after RNAi. **c** The survivorship of n WT, IB, and ds-*hsp90* beetles under starvation

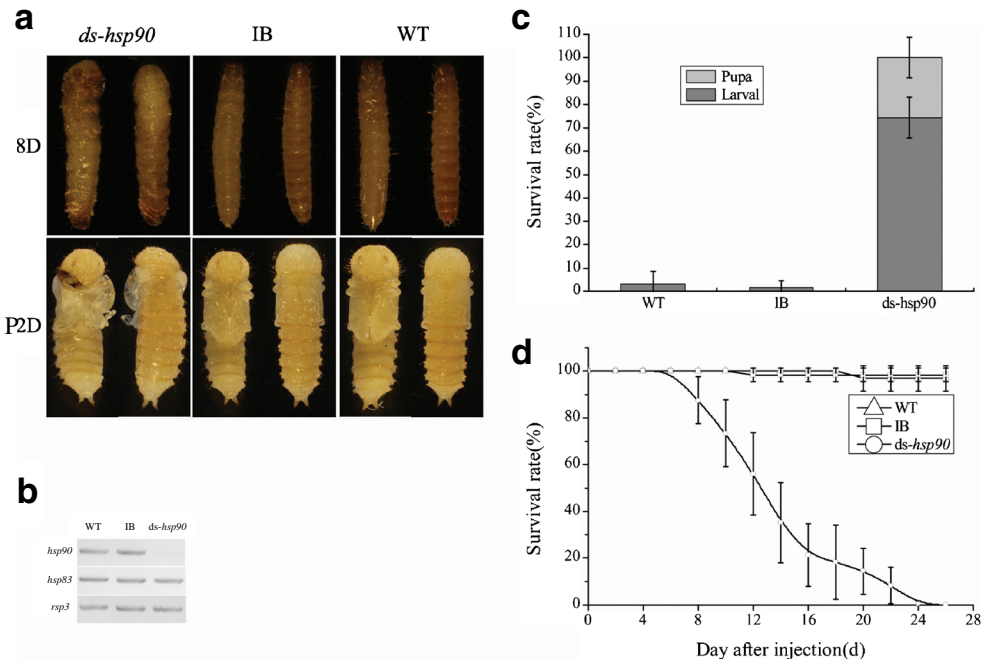

Fig. 5 RNAi phenotype of *Tchsp90*. **a** RNA interference resulted in lethal phenotypes in both of larval/pupal stages. **b** Knocking down levels of *Tchsp90* using larval RNAi. **c** Progressively increasing mortalities after late larval RNAi of ds-*Tchsp90*. **d** Cumulative adult mortality after late larval RNAi. Each point represented the mean days of three biological replications

at 97.6±1.7 and 96.7±3.0 percentage, respectively. Results showed that the suppression of *hsp90* significantly shorten the lifespan of *T. castaneum* (Fig. 6c).

Discussion

In this study, we identified *Tchsp90*, a novel ER-based *hsp90* member, based on the genome sequence of *T. castaneum*. Sequence analysis showed that there are 48.8 %–99.5 % amino acid identities among HSP90 groups, while there are 47.1 %–99.3 % amino acid identities among HSP83 groups. However, there are only 24.6 %–43.3 % amino acid identities between HSP83 and HSP90 groups. Moreover, phylogenetic analysis revealed that *hsp90* and *hsp83* were clearly divided into two groups, and they evolved separately from a common ancestor but *hsp90* originated earlier than *hsp83*, indicating that theses two molecular chaperones may be under differential selections during their evolution.

Basically, both these two groups and other homologs of HSP90s, such as mitochondrial TRAP1 (tumor necrosis factor receptor-associated protein 1) and chloroplast HSP90, exhibit similar ATPase rates and undergo similar conformational changes. One of the key differences is that cytosolic HSP83 interacts with a large number of co-chaperones that regulate the ATPase activity of HSP83 or have other functions such as targeting clients to HSP83 (Johnson 2012). All vertebrates contain at least two isoforms of cytosolic HSP83s. Previous studies have proposed that two HSP83s evolved by duplication of a common ancestral gene more than 500 million years ago, close to the time of emergence of the vertebrates (Krone and Sass 1994; Moore et al. 1989). However, *D. rerio* had three *hsp83* genes which are located on the same chromosome (CL20), and it seems that HSP83-1-A and HSP83-1-B were

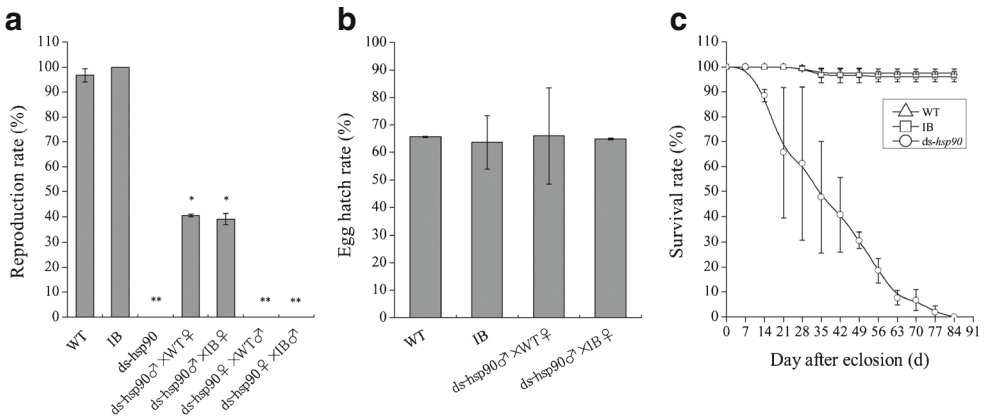

Fig. 6 Reduced egg laying (**a**) and the egg hatch rate (**b**) as well as cumulative adult mortality (**c**) after injection of ds-*Tchsp90* in 2-day-old pupae. Confidence intervals calculated using Student's *t* test for 21 single pair mating and show standard deviations and significant differences compared with the control using *single asterisk* indicates $p<0.05$; *double asterisks* indicates $p<0.001$

duplicated by additional recent gene duplication. It was further confirmed that these two genes possessed different functions for muscle development between HSP83-1-A (HSP90α1) and HSP83-1-B (HSP90α2) in *D. rerio* (Du et al. 2008). Interestingly, most invertebrates have only one HSP83 except that *A. mellifera* and *A. pisum* have a species-specific gene duplication that has resulted in two HSP83s. As the *A. mellifera*-specific HSP83 is caste- and age-specifically expressed in adult bees (Aamodt 2008), it was proposed that the duplicated gene might be associated with special morphological and behavioral differentiation of castes in *A. mellifera* (Xu et al. 2010b). However, both *A. mellifera* and *A. pisum* lived on the frequently changed humidity condition as their feed behavior to ingested plant fluids and secrete honey dew, thus the duplication might be associated with this environmental stress selection as was shown in HSP83 which has been reported to be involved in the dehydration, rehydration, and overhydration processes in *Antarctic midge* (Lopez-Martinez et al. 2009). However, further investigation incorporating environmental stress is required in understanding the function of these transcripts.

Induction of expression of the *hsp83* gene under heat, cold, and other environmental stress conditions has been found in many species, such as *D. melanogaster* (Boher et al. 2012), *Lucilia cuprina* (Concha et al. 2012), and *Spodoptera exigua* (Jiang et al. 2012), and the induced expression of *hsp83* under heat-shock conditions was observed in the whole body and ovary extracts of both newly hatched and mature adults of *T. castaneum* (Xu et al. 2010a). Currently, there are very limited studies on the expression of the HSP90 protein under environmental stress conditions, since it has been recognized that HSP90, unlike HSP83-I and II, was not induced by high temperature or other stresses that are unique to the cytosol HSP83 (Marzec et al. 2012; Subjeck and Shyy 1986). However, its upregulation is often used as a hallmark of responses to ER stress. Interestingly, in this study, a significant increase of HSP90 expression was observed under heat stress at 42 °C, and it indicated that HSP90 could also play a pivotal role in the response to heat stress as it was shown in grass carp that *Cihsp90* (ER-based *hsp90*) was upregulated after heat shock at 34 °C or cold stress at 4 °C (Wu et al. 2012). However, *Tchsp90* was only upregulated in response to heat stress but had no change under cold stress at 4 °C in *T. castaneum*. These results indicated that HSP90 might be involved in differential protection processes from variant stress conditions in different organisms.

Moreover, we found that *Tchsp90* was significantly downregulated under starvation conditions, which is consistent with Dd-GRP94 (*Dictyostelium discoideum* glucose-regulated protein 94) expression that rapidly declined within 1 h in response to starvation (Morita et al. 2000). Moreover GRP94 had low expression level in energy restriction mice liver in which secretory tissues showed high expression level at normal conditions. It is possible that long-term food deprivation reduced the level of GRP94 mRNA by reducing the level of malfolded proteins in the endoplasmic reticulum of hepatic cells (Eletto et al. 2010; Stephen et al. 1990). However, one conflicting result has shown that food-deprivation (7 days) enhanced ER-based HSP90 expression in larvae of gilthead sea bream and rainbow trout. This increase of HSP90 may be associated with enhanced protein catabolism in feed-deprived fish (Cara et al. 2005). Thus, ER-based HSP90 might be involved in differential mechanisms under starvation/food-deprivation conditions for various organisms.

In addition to the response to ER-stress and environmental stress, *hsp90* is also required at normal development for many organisms. Previous studies have shown that GRP94 is critical in mouse embryos as its absence provoked an embryo-lethal condition (Mao et al. 2010; Wanderling et al. 2007). Moreover, ER-based HSP90 is also required during the early developmental stage in *C. elegans* and fly (Marzec et al. 2012; Maynard et al. 2010). Interestingly, once knocked-down, the expression of *Tchsp90* is not only arrested the larvae/pupae development, but also significantly reduced the beetle's lifespan and their reproduction in *T. castaneum* besides its response to heat stress. The loss of Gp93 expression is larval-lethal in *Drosophila*; it seems because GRP94 expression was essential for the integrated secretory and absorptive functions of the midgut that nutrient assimilation-coupled growth control is enabled (Maynard et al. 2010).

Moreover, ds-*Tchsp90* female had no offspring, and the male had low reproduction rates. This implied that *Tchsp90* was a maternal effect gene similar to GRP94 (Li et al. 2010). These data suggested that *Tchsp90* might be involved in the oogenesis and spermatogenesis as it was discovered that this gene is required during early embryo development of mouse, which failed to develop mesoderm, primitive streak, or proamniotic cavity once this gene was deleted (Mao et al. 2010). Meanwhile, grp94−/− ES cells grow in culture and are capable of differentiation into cells representing all the three germ layers. However, these cells do not differentiate into cardiac, smooth, or skeletal muscle (Wanderling et al. 2007). Although eggs produced by ds-*Tchsp90* male have comparable hatch capability with wild-type, it is suggested that this gene seems not to be involved in the later embryo development in *T. castaneum*. Furthermore, we first discovered that *Tchsp90* is important for beetle's lifespan, and once this gene was knocked down, all adult beetles died within 80 days, which is significantly shorter lifespan than that of the wild-type. One possible explanation is that the decreased expression of *Tchsp90* would reduce the ER stress resistance and when the misfolded proteins constant increased beyond the ER capacity would induce ER-stress-induced apoptosis (Tabas and Ron 2011). Finally, it caused a greater reduction in lifespan in *T. castaneum*. However, further investigation into the details of how this gene is involved in the beetle's lifespan and its mechanism is needed to further understand the function of this gene.

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