Identification of Genes Differentially Expressed During Heat Shock Treatment in *Aedes aegypti*

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ABSTRACT Temperature is important for mosquito development and physiological response. Several genes of heat shock protein (HSP) families are known to be expressed in mosquitoes and may be crucial in responding to stress induced by elevated temperature. Suppression subtractive hybridization (SSH) was used to identify target transcripts to heat shock treatment in female *Aedes aegypti*. Subtraction was performed in both directions enriching for cDNAs differentially expressed between a non–heat shock control and heat shock treatment. Heat shock treatment of female *Ae. aegypti* was carried out for 1 h at 42°C. Clones from differentially expressed genes were evaluated by sequencing. Target transcripts up-regulated by heat shock included five different HSP gene families and 27 other genes, such as cytochrome c oxidase, serine-type endopeptidase, and glutamyl aminopeptidase. Additionally, some novel genes, cytoskeleton and ribosomal genes, were found to be differentially expressed, and three novel up-regulated sequences belonging to a low-abundance class of transcripts were obtained. Up-regulated/down-regulated transcripts from heat shock treatment were further confirmed and quantified by quantitative real-time polymerase chain reaction (PCR). High temperatures can alter the gene expression of a vector mosquito population, and further characterization of these differentially expressed genes will provide information useful in understanding the genetic response to heat shock treatment, which can be used to develop novel approaches to genetic control.

KEY WORDS heat shock, *Aedes aegypti*, gene expression, suppression subtraction hybridization

Higher temperatures can drastically alter the genetic structure and gene expressions of a vector mosquito population. Several families of heat shock proteins (HSPs) are known to be expressed in insects and may have a cumulative role in determining stress in response to elevated temperature (Mahroof et al. 2005; Yadav et al. 2005; Rinehart et al. 2006a, b; Robich et al. 2007), which itself is governed by several genes. In addition to heat shock, the expression of HSPs has also been reported to be induced under various stress conditions (such as pathogen infection, heavy metal ions, hypoxia, and osmotic stress) in many other animals and insects including mosquitoes (Mosser et al. 1988; Yamuna et al. 2000; Boone and Vijayan 2002a, b; Spees et al. 2002; Cheng et al. 2003; Sim et al. 2005; Chuang et al. 2007; Sim et al. 2007). HSPs are also developmentally regulated. For example, the synthesis of HSP in *Anopheles stephensi* was correlated with the various morphological and physiological events occurring during development (Gakhar and Shandilya 1999). Therefore, HSPs are important for us to better understand the mechanism of heat shock and other types of stress in mosquitoes.

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In this study, SSH was used to identify genes that are differentially expressed during heat shock treatment of female *Aedes aegypti* L. Expression patterns were confirmed by quantitative real-time-polymerase chain reaction (RT-PCR), and the regulated genes identified are discussed in the context of their possible function at high temperature (heat shock treatment). As part of our effort to develop new insecticides for mosquito control, the finding of HSP genes and genes from other families specifically expressed in heat shock–treated female *Ae. aegypti* may provide information needed to identify proteins critical to mosquito survival. Using RNAi technology to knock down these critical proteins may provide additional targets that can be developed as molecular pesticides (Pridgeon et al. 2008).

Materials and Methods

RNA Extraction. *Aedes aegypti* (Orlando, FL, strain, maintained since 1952) were reared in the insectary of the Mosquito and Fly Research Unit at the Center for Medical, Agricultural, and Veterinary Entomology, USDA–ARS, Gainesville, FL. To test how temperatures affect gene expression in mosquitoes, extreme heat shock treatment (42°C) of female *Ae. aegypti* was

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14. ABSTRACT

Temperature is important for mosquito development and physiological response. Several genes of heat shock protein (HSP) families are known to be expressed in mosquitoes and may be crucial in responding to stress induced by elevated temperature. Suppression subtractive hybridization (SSH) was used to identify target transcripts to heat shock treatment in female Aedes aegypti. Subtraction was performed in both directions enriching for cDNAs differentially expressed between a non?heat shock control and heat shock treatment. Heat shock treatment of female Ae. aegypti was carried out for 1 h at 42 C. Clones from differentially expressed genes were evaluated by sequencing. Target transcripts up-regulated by heat shock included ?ve different HSP gene families and 27 other genes, such as cytochrome c oxidase, serine-type endopeptidase, and glutamyl aminopeptidase. Additionally, some novel genes, cytoskeleton and ribosomal genes, were found to be differentially expressed, and three novel up-regulated sequences belonging to a low-abundance class of transcripts were obtained. Up-regulated/down-regulated transcripts from heat shock treatment were further con?rmed and quanti?ed by quantitative real-time polymerase chain reaction (PCR). High temperatures can alter the gene expression of a vector mosquito population, and further characterization of these differentially expressed genes will provide information useful in understanding the genetic response to heat shock treatment, which can be used to develop novel approaches to genetic control.

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conducted to optimize the response and facilitate detection of the highly expressed genes. Specifically, 10-d-old females were exposed for 1 h at 42°C and 56 ± 1.5% RH in an environmental chamber (L-C Incubator; Lab-Line Instruments, Melrose Park, IL) for this study. Untreated females were held at a constant room temperature (23°C) and 56 ± 1.5% RH in an environmental chamber. The total RNAs were extracted using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Poly $(A)^+$ RNA was isolated applying Oligotex-dT suspension (QIAGEN, Valencia, CA). RNA samples were quantified by SmartSpec Plus Spectrophotometry (BIO-RAD, Hercules, CA).

Subtractive Hybridization. Differentially expressed mRNA populations, both from heat shock treatment and from untreated controls, were converted into cDNA using PCR-Select cDNA Subtraction Kit (Clontech; Roche Molecular Systems, Alameda, CA). Subtractive cloning is a powerful technique that allows isolation and cloning of mRNAs differentially expressed in two different populations. In the generalized subtraction scheme, the mosquitoes to be compared are the [+] or tracer (HS treatment) and the [−] or driver (non-HS, control), where mRNAs expressed in the tracer and not the driver are isolated. Hybrids that form include sequences common to both mosquito populations. The unhybridized fraction is enriched for sequences that are preferentially expressed in the tracer mosquito population. Differentially expressed cDNA sequences are used to construct a subtracted cDNA library (Patel and Sive 2001).

PCR-select cDNA Subtraction Library. Forward and reverse subtracted libraries were cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen). Transformed plasmids were inserted into One Shot TOP10 Competent Cells (Invitrogen) and grown overnight on Luria-Bertani (LB) plates containing ampicillin and X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside). For each library, >100 white colonies were isolated and grown overnight in LB-ampicillin broth at 37°C and 235RPM in the Innova 4000 Incubator Shaker (New Brunswick Scientific, Edison, NJ).

Gene Sequencing of PCR-select cDNA Subtraction Library. Clones of the subtracted library were purified with QIAprep Miniprep (QIAGEN). The plasmid DNAs were digested by using *Eco*RI enzyme for 1.5 h and were run on a 1% agarose gel to confirm the DNA insert. Selected clones were sent to the DNA Sequencing Core at the Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida, to be sequenced, and the sequences were analyzed using the National Center for Biotechnology Information (NCBI) BLASTN program to identify sequence homologies. The homologous DNA fragments in the gene or cDNA or mRNA are also recorded in Table 1.

cDNA Synthesis for Real-time PCR. A 5-μg aliquot of purified RNA was reverse transcribed in 20-μl reaction volume using Superscript II first-strand cDNA Synthesis system for RT-PCR according to the manufacturer's instruction (Invitrogen). The reaction was terminated by heat inactivation at 95°C for 5 min. The cDNA samples for heat shock treatment and control were diluted by adding 80 μl ddH_2O (500 ng/μl) and stored at −20°C.

To design gene-specific primers, detailed analyses of the nucleotide sequence of all up-/down-regulated genes were performed using PRIMER3-Design Primer Pairs and Probes program from Biology Workbench (http://workbench.sdsc.edu). The primers for *ACTIN* gene were also designed for an internal control and comparison. The primers are listed in Supplemental Table 1.

Real-time PCR Amplification. The real-time PCR assay for *AeaGene* was performed using Platinum SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen) in a volume of 15 μl on an Applied Biosystems 7300 Fast Real-Time PCR System (Foster City, CA). The PCR mixture consisted of 1 μl diluted cDNA (500 ng/μl), 0.5 μM primers, and 1× master mix. In every RT-PCR run, *ACTIN* was used as an internal control to normalize for variation in the amount of cDNA template. The PCR primers used were *Aea-GENE-F* and *Aea-GENE-R* (Supplemental Table 2). The PCR primers for *ACTIN* and the PCR thermal cycling parameters were the same as described in the previous publication (Zhao et al. 2008). This experiment was replicated three times. Relative expression levels were calculated as follows. First, *AeaGENE* transcript levels relative to a standard (*ACTIN*) using the formula $\Delta C_T = C_T$ (*AeaGENE*) - C_T (*ACTIN*). Second, an average ΔC_T value for each sample was calculated. Third, relative expression levels were calculated using the equation $100 \times 2^{[-\text{average } \Delta CT]}$. Last, fold increases or decreases were calculated using HS treatment RNA relative expression level/non–heat shock treatment control RNA relative expression level.

Results

Identification of Genes Specifically Expressed During Heat Shock Treatment in *Ae. aegypti*. To show how global genes are specifically expressed during heat shock treatment in *Ae. aegypti*, we used subtractive hybridization to successfully construct a subtraction cDNA library of control female mosquitoes and female after heat shock treatment for 1 h at 42°C. Clones of differentially expressed genes were evaluated by sequencing. Using NCBI blast to analyze the sequences, we found 10 clones encoding *Ae. aegypti* heat shock protein genes, including at least three groups: HSP 26 kDa, other HSPs, and heat shock cognate (HSC) 70 mRNA. A full-length cDNA clone encoding an HSP 26 kDa family was isolated and submitted to National Center for Biotechnology Information (NCBI accession no. gb|EU048558.1|). There were 12 clones encoding 10 non-HSP genes. They were *Ae. aegypti* serine-type endopeptidase, glutamyl aminopeptidase, cytochrome c oxidase (subunit VIA), casein kinase, adp/ATP carrier protein, elongation factor 2, ATP synthase delta chain, thioredoxin reductase, methionine adenosyltransferase, and trans-

Table 1. Heat shock treatment response genes from *Ae. aegypti* isolated by suppressive subtractive hybridization with homologous gene blast from NCBI

Clone	Accession no., organism, putative identity, mRNA/cDNA length	Gene regions
HSPs		
51, 61	gb EU048558.1, *Ae. aegypti*, HSP 26-kDa mRNA, complete cds, length = 738	nt: 738–1
62	ref XM_001655592.1, *Ae. aegypti*, HSP partial mRNA, length = 2,605	nt: 1933–2498
50	ref XM_001649702.1, *Ae. aegypti*, HSP partial mRNA, length = 2,602	nt: 1935–2501
12, 21	ref XM_001649702.1, *Ae. aegypti*, HSP partial mRNA, length = 2,602	nt: 1935–2501, nt: 1551–1215
41	gb AY432606.1, *Ae. aegypti*, putative: HSP mRNA sequence, length = 1,275	nt: 49–139
22	gb DQ440299.1, *Ae. aegypti*, HSC 70 mRNA, complete cds, length = 1,956	nt: 34–613
44, 60	gb DQ440299.1, *Ae. aegypti*, HSC 70 mRNA, complete cds, length = 1,956	nt: 1126–1415, nt: 613–34
Non-HSPs		
9, 26	ref XM_001659911.1, *Ae. aegypti*, serine-type enodpeptidase partial mRNA, length = 1,117	nt: 367–7 nt: 7–367
10, 17	ref XM_001658502.1, *Ae. aegypti*, casein kinase partial mRNA, length = 3,840	nt: 1,074–850
14	ref XM_001658180.1, *Ae. aegypti*, glutamyl aminopeptidase partial mRNA, length = 3,060	nt: 2,391–2,225
16	ref XM_001656578.1, *Ae. aegypti*, cytochrome c oxidase, subunit VIA partial mRNA, length = 522	nt: 44–284
20	ref XM_001654983.1, *Ae. aegypti* adp, ATP carrier protein partial mRNA, length = 835	nt: 571–486
36	gb AF331798.1 AF331798, *Ae. aegypti* elongation factor 2 (Ef-2) mRNA, complete cds, length = 2,681	nt: 1,485–1,955
37	ref XM_001654955.1, *Ae. aegypti*, ATP synthase delta chain partial mRNA, length = 902	nt: 655–861
42	ref XM_001662616.1, *Ae. aegypti*, thioredoxin reductase partial mRNA, length = 2,137	nt: 1,950–2,084
45	gb AY432324.1, *Ae. aegypti*, putative: methionine adenosyltransferase mRNA sequence, length = 2,655	nt: 2,004–2,370
49	gb AY431275.1, *Ae. aegypti*, translational inhibitor mRNA sequence, length = 663	nt: 57–521
Unknown		
18	gb AY432385.1, *Ae. aegypti*, conserved unknown mRNA sequence, length = 1,198	nt: 1,149–1,198
25	ref XM_001660484.1, *Ae. aegypti*, hypothetical protein partial mRNA, length = 795	nt: 263–13
35, 59, 64, 68	gb BQ790640.1 M3–1-5-_8–6-01–8-28_PM.ab1 whole midgut #1 (WMG1), *Ae. aegypti*, cDNA 5′, mRNA sequence, length = 493	nt: 5–315
38	gb AY432167.1, *Ae. aegypti*, conserved unknown mRNA sequence, length = 735	nt: 9–245
39	ref XM_001663796.1, *Ae. aegypti*, hypothetical protein partial mRNA, length = 1,602	nt: 187–444
47, 52, 55, 70, 71	gb DV340302.1, *Ae. aegypti*, infected with *Plasmodium gallinaceum Ae. aegypti* cDNA clone NABU360, mRNA sequence, length = 807	nt: 566–214 nt: 214–566
58	gb EF173371.1, *Ae. aegypti*, clone BAC ND22N19, complete sequence, length = 146,563	nt: 64,254–63,965
3, 32	gb BC046262.1, *X. laevis*, HSC protein 70, complete cds, length = 2,202	nt: 1,258–1,502
4, 29, 66	ref XM_001861402.1 . *Culex pipiens quinquefasciatus*, HSP 70 B2 partial mRNA, length = 2,072	nt: 1,915–1,565 nt: 1,475–647
Cytosolic large ribosomal subunit		
1, 69	gb AY431470.1, *Ae. aegypti*, cytosolic large ribosomal subunit L19 mRNA sequence, length = 832	nt: 1–251 nt: 300–39
27	ref XM_001661916.1, *Ae. aegypti*, ribosomal protein L36 partial mRNA, length = 490	nt: 110–11
43	gb AY432710.1, *Ae. aegypti*, cytosolic large ribosomal subunit L27A mRNA sequence, length = 658	nt: 19–299
40S ribosomal protein		
2	ref XM_001658972.1, *Ae. aegypti*, 40S ribosomal protein S3 partial mRNA, length = 927	nt: 83–354
30	gb AY552053.1, *Ae. aegypti*, 40S ribosomal protein S16 mRNA, complete cds, length = 674	nt: 471–329
67	ref XM_001649929.1, *Ae. aegypti*, 40S ribosomal protein S24 partial mRNA, length = 597	nt: 444–28
60S ribosomal protein		
5	ref XM_001657905.1, *Ae. aegypti*, 60S ribosomal protein L31 partial mRNA, length = 632	nt: 21–385
8	ref XM_001647784.1, *Ae. aegypti*, 60S ribosomal protein L12 partial mRNA, length = 498	nt: 1–387
11	ref XM_001657661.1, *Ae. aegypti*, 60S ribosomal protein L8 partial mRNA, length = 1,087	nt: 956–707
24	ref XM_001651406.1, *Ae. aegypti*, 60S ribosomal protein L26 partial mRNA, length = 704	nt: 297–22
33	ref XM_001659089.1, *Ae. aegypti*, 60S ribosomal protein L24 partial mRNA, length = 682	nt: 72–242
40	ref XM_001655416.1, *Ae. aegypti*, 60S ribosomal protein L10 partial mRNA, length = 959	nt: 322–641
Actin		
31, 34	ref XM_001651695.1, *Ae. aegypti*, actin partial mRNA, length = 1,748	nt: 18–286 nt: 296–73

lational inhibitor (Table 1). We also found 19 clones encoding approximately nine unknown genes. Comparison of blastn nucletide sequences of clones 3 and 32 with *Xenopus laevis* Daudin showed a similar putative HSC protein 70 with 76% identity at the nucleotide level; however, comparison of deduced amino acid sequences of clones 3 and 32 with *Anopheles gambiae* Giles and *Culex pipiens* Linnaeus HSP 70 indicated 95% identity at the protein level. Genes of clones 4, 29, and 66 had 80% DNA sequence similarity to the protein-coding regions of the *Cx. pipiens quinquefasciatus* Say HSP 70 B2 partial mRNA. Therefore,

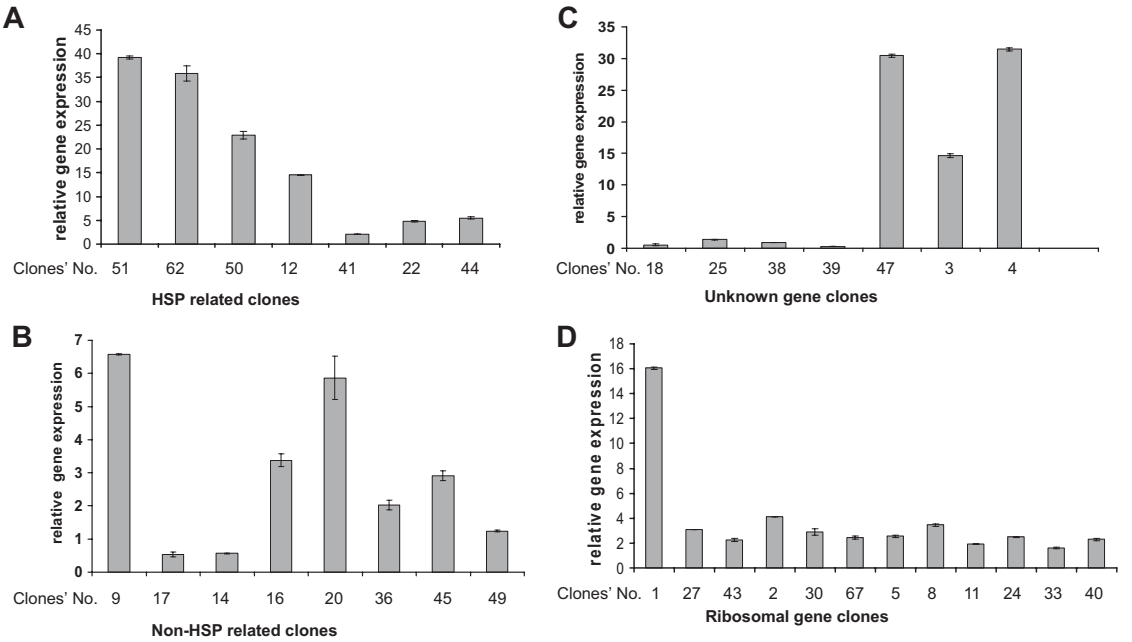

Fig. 1. Quantitative RT-PCR results showing the relative ratio of different gene expressed (up-regulated times) after 42°C heat shock treatment for 1 h compared with the 23°C control. (A) Different HSP genes differentially expressed for after 42°C heat shock treatment 1 h compared with the 23°C control. (B) Different non-HSP genes differentially expressed after 42°C heat shock treatment for 1 h compared with the 23°C control. (C) Different unknown genes differentially expressed after 42°C heat shock treatment for 1 h compared with the 23°C control. (D) Different ribosomal genes differentially expressed after 40°C heat shock treatment for 1 h compared with the 23°C control.

clones 3 and 32, combined with clones 4, 29, and 66, might be new HSC70 cognates or new HSP70 that have not yet been identified in *Ae. aegypti.* In addition, we also found 15 clones that encoded for *Ae. aegypti*, cytosolic large ribosomal subunit, 40S ribosomal protein, and 60S ribosomal protein, as well as cytoskeleton protein actin (Table 1).

High Temperature Effects on Relative RNA Expression Levels of Different Genes in Adult *Ae. aegypti*. To understand whether HSP and other genes in *Ae. aegypti* can be triggered by exposure to high environmental temperature conditions, quantitative RT-PCR analyses were carried out to further confirm differential gene expression of these genes in response to heat shock in *Ae. aegypti.* Seven HSP and HSC genes were identified in the subtractive library (Table 1). According to our quantitative RT-PCR data, all HSP genes and HSC protein genes found in the subtracted cDNA library were up-regulated (Fig. 1B). Compared with the control, the RNA relative gene expression level of HSP family increases dramatically after 42°C treatment of *Ae. aegypti* for 1 h (Fig. 1A). For example, expression of small *HSP genes* (26-kDa mRNA, clone 51) were the most up-regulated genes after 1 h of 42°C treatment of female *Ae. aegypti*, more than a 39-fold relative increase over that found in the untreated control (1 h at 23°C) *Ae. aegypti* female (Fig. 1A). A five- to six-fold increased expression of HSC 70 mRNA (clones 22 and 44) was found after heat shock at 1 h of 42°C treatment of *Ae. aegypti* compared with the untreated control (Fig. 1A; Table 1).

In the non–heat shock group, quantitative RT-PCR was used to further confirm those genes were either up-regulated or down-regulated (Fig. 1B). Six known genes were validated as up-regulated and two known genes as down-regulated in the subtractive hybridization library (Fig. 1B).

For the unknown genes, the quantitative RT-PCR data also confirmed some up-regulated or down-regulated genes (Fig. 1C). Clone 47, whose mRNA sequence was similar to *Ae. aegypti* infected with *Plasmodium gallinaceum* Emile Brumpt clone NABU360, showed more than a 30-fold relative increase when *Ae. aegypti* were exposed 1 h at 42°C than that found after being held continuously at 23°C (Fig. 1C). It is not known if clone NABU 360 represents a gene from *Ae. aegypti* or *P. gallinaceum.* Clones 3 and 32, with ≈76% similarity to the sequence from the *X. laevis* Daudin HSC protein 70, showed greater than a 15-fold relative increase at 42°C than that found in the control (Fig. 1C).

In response to heat shock, expression levels of several ribosomal genes were also significantly modulated, being more than two-fold up-regulated. Quantitative RT-PCR analysis further substantiated the differential expression of three of these genes in response to heat shock treatment (Fig. 1D). Expression levels of *Ae. aegypti* cytosolic large ribosomal subunit L19 mRNA were up-regulated ≈16-fold compared with the non–heat shock treatment control. Most different gene fragments from 60S ribosomal protein and

40S ribosomal protein were up-regulated between two- and four-fold compared with the control.

Discussion

High Temperature Effects on Different Gene Expression in Adult *Ae. aegypti*. In nature, mosquitoes can be subjected to temperature extremes and have developed mechanisms to survive these conditions. We used suppression subtraction hybridization to identify potential critical gene pathways that could be targeted as part of a control strategy. Quantitative RT-PCR analysis further confirmed the differential expression of ≈15 of these genes in response to heat shock treatment at 42°C for 1 h (Fig. 1). Twelve known genes were validated as being up-regulated and five known genes as down-regulated by the subtractive hybridization library.

In general, mosquitoes have a core group of genes that are activated in response to different types of stress. Overexpression of HSPs can also be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, exposure of the cells to toxins, starvation, hypoxia, or water deprivation (Mosser et al. 1988; Yamuna et al. 2000; Boone and Vijayan 2002a, b; Spees et al. 2002; Cheng et al. 2003; Sim et al. 2005; Chuang et al. 2007; Sim et al. 2007). Consequently, HSPs are also referred to as stress proteins, and their up-regulation is sometimes described more generally as part of the stress response. In a previous study, gene expression of *An. gambiae* was significantly modulated in response to O'nyong-nyong virus infection, including a putative HSP 70, HSC, elongation factor 1α, and ribosomal protein L35 (Sim et al. 2005, Sim et al. 2007). Other data have shown an essential role for an RNA polymerase II elongation factor in the regulation of heat shock gene expression in an animal model (Gerber et al. 2005). According to this study, HSP 70, HSC 70B, elongation factor 2, and several ribosomal subunits (L24, L36, and more) were also confirmed to be differentially expressed in response to heat shock treatment.

Increased expression of small HSPs (sHSPs) is known to be a key regulatory mechanism in extending tolerance to a variety of environmental stresses. For example, sHSP are expressed in (1) the wasp *Venturia canescens* after exposure to different temperatures (Reineke 2005); (2) in *Anopheles* vectors because of interaction with *Plasmodium* parasites (Lefevre et al. 2007), and (3) *Drosophila* in response to injuries and aging (Morrow et al. 2004). Our quantitative RT-PCR data also showed significant differences in the expression of sHSP 26 kDa (clone 51, ≈40 times up-regulated compared with the control) in response to heat shock treatment of *Ae. aegypti* female. For the other HSPs, the relative gene expression of clones 62 and 50 (Fig. 1A) were also up-regulated >20 times compared with the control. In summary, elevated gene expressions of these sHSP and HSPs indicate that they are important genes under stressful high temperature conditions and are potential targets for RNAi knock-down studies.

The cDNAs differentially expressed by heat shock conditions were identified using subtractive hybridization library analysis. The quantitative RT-PCR analyses confirmed that genes homologous to serine-type endopeptidase (clones 9 and 26), cytochrome c oxidase (subunit VIA) (clone 16), adp/ATP carrier protein (clone 20), elongation factor 2, ATP synthase delta chain, thioredoxin reductase, methionine adenosyl transferase and translational inhibitor, and some unknown genes were abundant in *Ae. aegypti* female after heat shock treatment. Conversely, glutamyl aminopeptidase and casein kinase were down-regulated in *Ae. aegypti* female after heat shock treatment. Some of the up-/down-regulated genes identified (HSP gene, serine-type enodpeptidase) have previously been shown to play critical roles in response to other types of stress in both invertebrates and vertebrates. For example, it was reported that mitochondrial dysfunction mutations in a gene (designated *levy*) that codes for subunit VIa of cytochrome c oxidase (COX), and the data from *levy* mutants showed a COX-mediated pathway in *Drosophila*. Disruption of this pathway leads to mitochondrial encephalomyopathic effects including neurodegeneration, motor dysfunction, and premature death (Liu et al. 2007). Therefore, relative RNA expressions of serine-type endopeptidase, cytochrome c oxidase (subunit VIA), and adp/ATP carrier protein were up-regulated more than three-fold in response to heat shock treatment and are potential pathways to target for knock-down with RNAi.

This study suggests that genes expressed in response to heat shock treatment and/or temperature play an important functional role in *Ae. aegypti*, perhaps enhancing survival under high temperature conditions. Identification of these groups of gene families may provide critical information needed for designing novel control strategies for medically important disease vectors and identifying new pathways to target for the development of genetic molecular pesticides (Pridgeon et al. 2008).

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