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GENOMIC STRUCTURE AND SEQUENCE ANALYSIS OF *Lucilia cuprina* HSP90 GENE

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Abstract

The HSP90 family is one of the highly conserved chaperone families, varying between eubacteria to higher vertebrates. The HSP90 protein has been assigned different functions including thermal protection, but having major role in development. The present study is a detailed analysis of the structural characteristics of *hsp90* gene (*lchsp90*) of sheep blowfly, *Lucilia cuprina*. The gene isolated by PCR revealed absence of intron. The nucleic acid and amino acid comparison revealed significant level of sequence similarity among species of various taxa. Significantly, the analysis of the amino acid sequence revealed that the HSP90 of *L. cuprina* belongs to *hsp90*β class.

Key words: Heat shock, *hsp90*, Blowfly, *Lucilia cuprina*.

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Abbreviations: HSP 90: Heat shock protein 90 protein; ***hsp*90**: Heat shock protein 90 gene; ***lchsp*90**: *Lucilia cuprina* heat shock protein 90 gene; ***hsp*90β**: Heat shock protein 90 beta gene; **HSC 82**: Heat shock cognate 82; **HtpG**: Bacterial homologue of HSP 90; **Grp94/gp96**: Glucose Regulated Protein 94/gp96; **TRAP1**: TNF Receptor Associated Protein 1

INTRODUCTION

The heat shock proteins or HSPs are the class of proteins, which elicit their presence in the cells during stress, including thermal stress and sustain the cell survival. These heat shock proteins are classified into different families based on their molecular mass in kilo Daltons (36,17). The 90 kD heat shock protein (HSP90) is one of the most abundant proteins (1% - 2% of total cellular proteins) present in the living cells (57). It is an ubiquitous chaperone found in eubacteria, all eukaryotes, but absent in archea. The first ever report of HSP90 being the heat shock protein was evident from the elevated levels of HSP90 in *Drosophila melanogaster* and in avian cell transformation (9, 42,).

The HSP90 chaperone family of the eukaryotes is one of the highly conserved chaperone families that includes the cytosolic HSP90 with dimeric structure (49), termed variously as HSP90α and HSP90β in humans, HSP86 and HSP84 in mice, HSP83 in *Drosophila*, and HSC82 and HSP82 in yeast. Other family members are HtpG in the bacterial cytosol, Grp94/gp96 in the endoplasmic reticulum of eukaryotes, and the recently discovered HSP75/TRAP1 in the mitochondrial matrix (1, 16,52,60). HSP90 consists of three major domains, namely, a highly conserved

amino-terminal ATPase domain, a middle domain and a carboxy terminal dimerization domain (33,7). In mammalian cells, *hsp90* homologues are encoded by two genes, namely, cytosolic *hsp90*α and *hsp90*β (corresponding to a major and minor isoform), with a sequence homology of about 95%. In higher eukaryotes, homodimers of α-α, β-β and heterodiomers of α-β exist (40).

Unlike HSP70 chaperones or chaperonins (HSP60), the cytosolic HSP90 does not participate in nascent protein folding (49). HSP90 is needed for the maintenance of the activity of many signalling molecules and plays a crucial role in signal transduction (60). The substrates for HSP90 include steroid hormone receptors, transcription factors, signalling kinases, tumor suppressor p53, etc. (43,57,58,60). HSP90 binds to substrate proteins, which are in partial renatured form, i.e., with a defined secondary structure but whose tertiary structure has not been completed (23). The ability of HSP90 to prevent the aggregation of denatured proteins *in-vitro* is ATP independent (23,57). The chaperone activity of HSP90 can be attributed to its two domain structure, namely, carboxy and amino terminal domains, with distinct substrate specificities (59, 46,26,35) thus, preventing protein aggregation and hold substrates in folding competent state (57).

HSP90 is studied not only as a stress protein but also as the one which has its role during various developmental stages of organisms (15), where it is required in the expression of many development related protein kinases such as Torso and Sevenless in *Drosophila* (10, 11). It is found accumulated in *Drosophila* oocytes during oogenesis and in the early stages of development (61,14). In the flesh fly, *Sarcophaga crassipalpis* and blowfly *Lucilia sericata*, the level of HSP90 is significantly reduced during diapause, but restored once the diapause is terminated (44). In higher organisms also, the expression pattern of HSP90 appears to be similar during oogenesis and embryogenesis (37,12). The isoform HSP90α is suggested to be important in muscle development, whereas HSP90β participates in placental, neural, and retinal development (28,56, 31,55).

The sheep blowfly, *Lucilia cuprina* (Weidemann) is a wide-spread menace in Asia and Australia, where it is a major sheep pest species. The fly is an inhabitant of high temperature zone and can withstand extreme changes in the ambient temperature throughout its life cycle. Earlier studies carried out in our laboratory on heat shock response in this fly attributed several characteristic features unique to it. The most significant observations were on the occurrence of developmental-stage specific thermotolerance and tissue-specific expression of HSPs, including HSP60 and HSP70 (27,47,48,53, 54). Despite being a major heat inducible protein, having significant cellular functions in mammalian cells, it still remains a least understood molecular chaperone in the ectothermic organism, such as insect. The present study is a prologue for our ongoing studies on functional and regulatory characteristics of HSP90 gene/protein during various developmental processes, including oogenesis, embryogenesis and metamorphosis in the blowfly *L. cuprina*.

MATERIALS AND METHODS

Fly culture

L. cuprina, commonly known as Australian Sheep Blowfly, belongs to the family Calliphoridae, order Diptera, was reared in our insectary at a rearing temperature between 26°C ± 2ºC with about 50-60% relative humidity. The adult flies were fed on raw goat meat and sugar meal and larvae on raw goat meat.

Identification, cloning and characterisation of hsp90 gene

The hsp90 gene of *L. cuprina* was amplified from the genomic DNA isolated from adult flies (3), using primers designed from *D. melanogaster* hsp90 cDNA sequence.

Primer designing and PCR and cloning

The primers for *lchsp90* coding region were designed (using the program Primer) from the cDNA of *hsp83* of *D. melanogaster* (GenBank accession No. NM_079175). Three sets of primers 90-1F (5’ CCA GAA GAA GCA GAG ACC TT 3’) 90-1R (5’ GAC TCA TAG CGG ATC TTG TC 3’) 90-2F (5’ GGA GCT GAA CAA GAC CAA GC 3’), 90-2R (5’ GGG AAT GAA GAG CAG AGC AC 3’) 90-3F (5’ CTG CTG TCC TCT GGA TTC TC 3’), 90-3R (5’ TAA TCG ACC TCC TCC ATG TC 3’) were designed for all the hsp90 related genes in order to get amplification of most of the coding region. The amplification for *lchsp90* was performed in 25 µl volume with 100 ng of genomic DNA, assay buffer with 1.5 mM MgCl2, 200 µM dNTPs, 10 pM of each primer, 1.0 U of *Taq* polymerase (Roche, Germany; B’Genei, India). The cycling conditions for the amplification of *lchsp90* were 94 °C for 30”, 52 °C for 1’, 72 °C for 2’ followed by a final extension at 72 °C for 20’. The resulting *hsp90* amplicons of *L. cuprina* were cloned in pTZ57R/T vector using InsTAclone PCR Product Cloning kit (MBI Fermentas, Lithuania) as per manufacturer’s instructions. The ligation mixture was transformed in the host, i.e., *E. coli* strain DH5α by calcium-chloride mediated transformation (45) and the positive colonies were identified using blue/white selection.

IN SILICO ANALYSIS

Sequencing, alignment, similarity search and ORF mapping

The positive clones of *lchsp90* of size 2150 bp (product of 901F – 903R combination) were got sequenced commercially (M/s Bioserve, India). The identity of the sequences were confirmed by using BLAST Alignment Tool (www.ncbi.nlm.nih.gov/blast) (4) and aligned with *Drosophila*, mice, rat, human and other related sequences using ClustalW Multiple Sequence Alignment Tool (www.ebi.ac.uk/services/clustalw) (22). The sequence search maps of the genes were done using the program SuperFamily (http://supfam.org/SUPERFAMILY) (19, 32). The open reading frames of the gene sequences were deduced from the program GeneTool (ver 1.5).

Primary, secondary and tertiary structure prediction

The primary structural parameters of the gene products were predicted by using the program ProtoParam (http://expasy.org/tools/protparam.html) (18). The secondary structural parameters of the proteins were predicted by using the programme PredictProtein (http://cubic.bioc.columbia.edu/pp) and the tertiary structures were predicted from the programme SwissModel (http://swissmodel.expasy.org/workspace) and visualised by using the programme YasaraView (http://yasara.org).

Phylogenetic Analysis

The homology similarities of the sequences between species of different phyla were done by ClustalW Multiple Sequence Alignment Tool (www.ebi.ac.uk/services/clustalw) and the phylogenetic tree was constructed by using the programs BioEdit (21) and MEGA 3.1 (29).

RESULTS

Isolation and structural characterisation

Structure of hsp90 gene

The primer combinations designed from *Drosophila hsp83* cDNA, amplified the complete coding region of the *lchsp90* gene of *L. cuprina*. The 901F-903R combination of primer spanned a length of 2149 bps. The internal primer combinations of 90-1F-90-2R and 90-2F-90-3R spanned a length of 954 bps and 1335 bps, respectively (Fig. 1). The fragments were sequenced and the sequence (Fig. 2A) from the combination of 90-1F-90-3R was characterised and submitted to GenBank (Accn. No. EF584332). The coding sequence of *lchsp90* revealed a single open reading frame on +1 strand, assuring that the sequence is not a pseudogene.

Structure of HSP90 protein

The putative amino acid sequence of *L. cuprina* HSP90 (81.5 kD) is comprised of 717 amino acids. The amino acid sequence along with its functional domains is represented in Fig. 3. The primary structural properties of the LCHSP90 protein, comprising of N-terminal, hinge, middle and C-terminal domains are tabulated in the Table 1. The domain structure of LCHSP90 is represented in Fig. 4A.

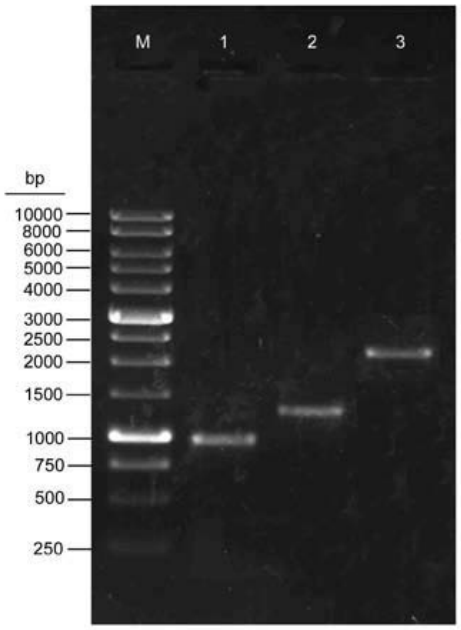

Figure 1. Amplification of *hsp90* gene of *L. cuprina*: Lanes 1- 90-1F-90-2R amplicon, 2- 90-2F-90-3R amplicon, 3- 90-1F-90-3R amplicon; M- 1 Kb ladder.

The secondary structure of the protein consists of 42.3% helix, 18.3% sheet, 6.7% turns and 32.7% coil. The tertiary structure of the monomer of HSP90 with its functional domains is shown in Fig. 4B. The putative LCHSP90 protein deduced from the amplified sequence of *L. cuprina* hosts some of the functional domains in its structure: the two Nuclear Localising Signals (NLSs), the first being 254 – KKDKDAKKKKTIK - 266 and the second 337 - ENQKKRNNIK-347, the three Nuclear Export Signals, 23 – NTFYSNKEIFLR - 34, 422-FYDQFSKNLKLG - 433 and 647 – LVILLFETSLLS - 658, the ATP/Geldanamycin (GA) binding pockets in N-terminal domain between 35 and 174 amino acids, HSP70 binding region at 401-LVKKTMELIEELTED-415 and the putative ATP binding region in the C-terminal domain at 646-DLVILLFETSLLSSGFSLQS-665 amino acids. However, the sequence appeared devoid of the polyglutamine site (EEEEE), a characteristic feature of vertebrate *hsp90α* isoforms (Fig. 3B).

Table 1. Different functional domains in the coding sequence of HSP90 (Figures in brackets indicate the percent similarity with the reference organism)

Domain **Organism**	**Nuclear Export Sequence (11)**	**First ATP / GA binding residues in N-terminal (partial domain) (30)**	**Ca-EF hand (38)**	**NLS (38)**	**Second ATP binding site at C- terminal (putative)**	**HSP70 Binding (putative) (11)**
Lucilia cuprina	NTFYSNKEIFLR (100)	GVGF (100)	EKEREKEVSDDEADDEK-KE (73)	KKDKDAKKKKT-IK (76)	DLVILLFETSLLSSGFSLQS (80)	LVKKTMELIEELTE D (66)
D. melanogaster	NTFYSNKEIFLR (100)	GVGF (100)	EKEREKEVSDDEADDEK-KE (73)	KKDKDAKKKKT-IK (76)	DLVILLFETSLLSSGFSLDS (90)	LVKKTMELIEELTE D (66)
Homo sapiens	NTFYSNKEIFLR (100)	GVGF (100)	EKERDKEVSDDEAEEKED KE (95)	KKDGDKKKKKK-IK (100)	DLVILLYETALLSSGFSLE D (100)	LVKKCLELFTELAE D (100)
Mus musculus	NTFYSNKEIFLR (100)	GVGF (100)	EKERDKEVSDDEAEEKEE KE (100)	KKDGDKKKKKK-IK (100)	DLVILLYETALLSSGFSLE D (100)	LVKKCLELFTELAE D (100)
S. cerevisiae	NTVYSNKEIFL R (92)	GVGF (100)	TKEVEKEVPIPEEEKKDEE K (45)	PKTKK-VK (57)	DLTKLLYETALLTSGFSLD E ((75)	IVKKLIEAFNEIAED (60)
E. coli	HSLYSNKEIFLR (76)	GVGF (100)	REEKDG (33)	---	EWVELLLDQALLAERGTL ED (45)	LTKRVLQMLEKLA KD (40)
Reference organism	**Human**	***P. falciparum***	**Human (placenta)**	**Human (placenta)**	**Mice**	**Human**


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ATGCCAGAAG AAGCAGAGAC CTTTGCATTC CAGGCTGAGT TTGCTCAGCT GATGTCCCTG   60
ATCATCAACA CATTCTACTC GAACAAGGAG ATTTTCCTGC GCGAGTTGAT CTCGAAAGCT  120
TCCGATGCCC TGGACAAGAT CCGCTATGAG TCCCTTACTG AGCCCAGTAA GCAGGACTCT  180
GGCAAGGAGC TGTACATCAA GCTGATCCCT AACAAGAGGC CTGGTACTCT GACCATGATT  240
GATGCCGGTA TCGGTATGAC CAAGTCCGAC CTGGTCAACA ACTTGGGAAC CATTGCCAAG  300
TCCGGAACCA AGGCCTTCAT GGAGGCTCTG CAGGCTGGTG CCGACATTTC CATGATCGGT  360
CAGTTCGGTG TGGGTTTCTA CTCCGCCTAC CTGGTCGCCG ACAAGGTGAC TGTCACCTCC  420
AAGAACAACG ATGACGAGCA GTACGTTGGG GAGTCCTCTG CCGGAGGCTC TTTCACAGTC  480
CGTGCCGACA ACTCTGAGCC CCTGGGCCGT GGCACCAAGA TCGTGCTGTA CATCAAGGAG  540
GACCAGACCG ACTATCTGGA GGAGAGCAAG ATCAAGGAGA TTGTTAACAA GCACTCCCAG  600
TTCATTGGCT ACCCCATCAA GCTGCTCGTG GAAAAAGAAC GTGAAAAAGA AGTGAGCGAT  660
GACGAAGCGG ATGACGAGAA AAAGGAGGGG GACGAAAAAA AGGAAATGGA GACAGATGAA  720
CCAAAGATTG AAGATGTGGA GGGGGATGAG GACGCCGATA AGAAGGACAA GGATGCCAAA  780
AAGAAGAAGA CCATCAAGGA GAAGTACACT GAAGATGAGG AATTAAATAA AACAAAACCG  840
ATCTGGACCC CGAATCCCGA TGATATCTCC CAGGAGGAGG ACGGCGAGCC TACAAAGTCC  900
CTCAACAACG ATTGGGAAGA TCATTTGGCT GTCAAGCACT TCTCCTGTGA AGGTCAACTT  960
GAATTCCGTG CCCTCCTCTT CATTCCTCGT CGCACCCCAT TCGATCTCTT CGAAAACCAA 1020
AAGAAACGCA ACAACATTAA GTTGTACGTC CGTCGTGTCT TCATCATGGA CAACTGCGAA 1080
GATCTCATTC CTGAATACTT GAACTTCAAG AAGGGTGTTG TCGACTCTGA AGATTTGCCC 1140
CTCAACATTT CTCGTGAAAT GCAGCAACAA AACAAAGTCC TAAAGGTGAT CCGAAAGAAC 1200
CTGGTCAAGA AGACCATGGA ATTGATTGAA GAACTTACCG AAGACAAAGA AATGTACAAG 1260
AAGTTCTACG ATCAATTCAG CAAGAACTTG AAATTGGGTG TCCACGAAGA TACCAACAAC 1320
CGTGCCAAAT TGGCCGATTT CTTGCGTTTC CACACCTCTG CTTCTGGTGA CGATGCCTGC 1380
TCCTTGGCCG ACTACGTATC TCGCATGAAG GAGAACCAAA AACACATCTA CTTCATCACT 1440
GGTGAATCCA AGGAACAAGT TGCCAACTCT GCCTTCGTTG AACGTGTCAA GGCTCGTGGG 1500
TTCGAAGTCG TATACATGAC CGAACCCATC GATGATACGT CATCCAACAC TATGAAGGAG 1560
TACAAGGGCA AACAAGTGGT TTCCGTTACC AAGGAAGGTT TGGATTGCCT GGAAACGAGG 1620
AGCGAAAAAA GAAGACGCGA AGAAGATAAG GCCACATTCG AAAACCTCTG CAAGCTAATG 1680
AAATCCATCT TGGACAGCAA AGTTGACAAG GTTGTTGTAT CAAACCGCTT AGTTGAATCA 1740
CCCTGCTGTA TCGTCACCTC CCAATTCGGT TGGTCCGCCA ACATGGCACG TATCATGAAG 1800
GCTCAAGCTT TGCGTGACAC CTCCACCATG GGCTACATGG TCGGCAAGAA ACATTTGGAA 1860
ATCAATCCCG ACCACGCCAT CATTGAGACT CTGCGCCAGA AGGCCGATGC CGACAAGAAC 1920
GACAAGGCTG TCAAGGATTT GGTTATCCTG TTGTTCGAGA CCTCCCTCTT GTCATCTGGC 1980
TTCTCATTGC AAAGCCCCCA GGTGCACGCT AGCCGCATCT ACCGCATGAT AAAGCTCGGC 2040
TTGGGCATCG AGACGAGCGA ACCAATGACC ACCGACGATG CCCAGAGCGC CGGAGATGCC 2100
CCCTCGCTGG TTGAGGACAC AGAGGACGCT TCCCACATGG AGGAGGTCGA TTAA 2154
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Figure 2. Nucleotide sequence of *hsp90* gene of *L. cuprina* with initiation codon ATG at 1 and stop codon TAA at 2154.

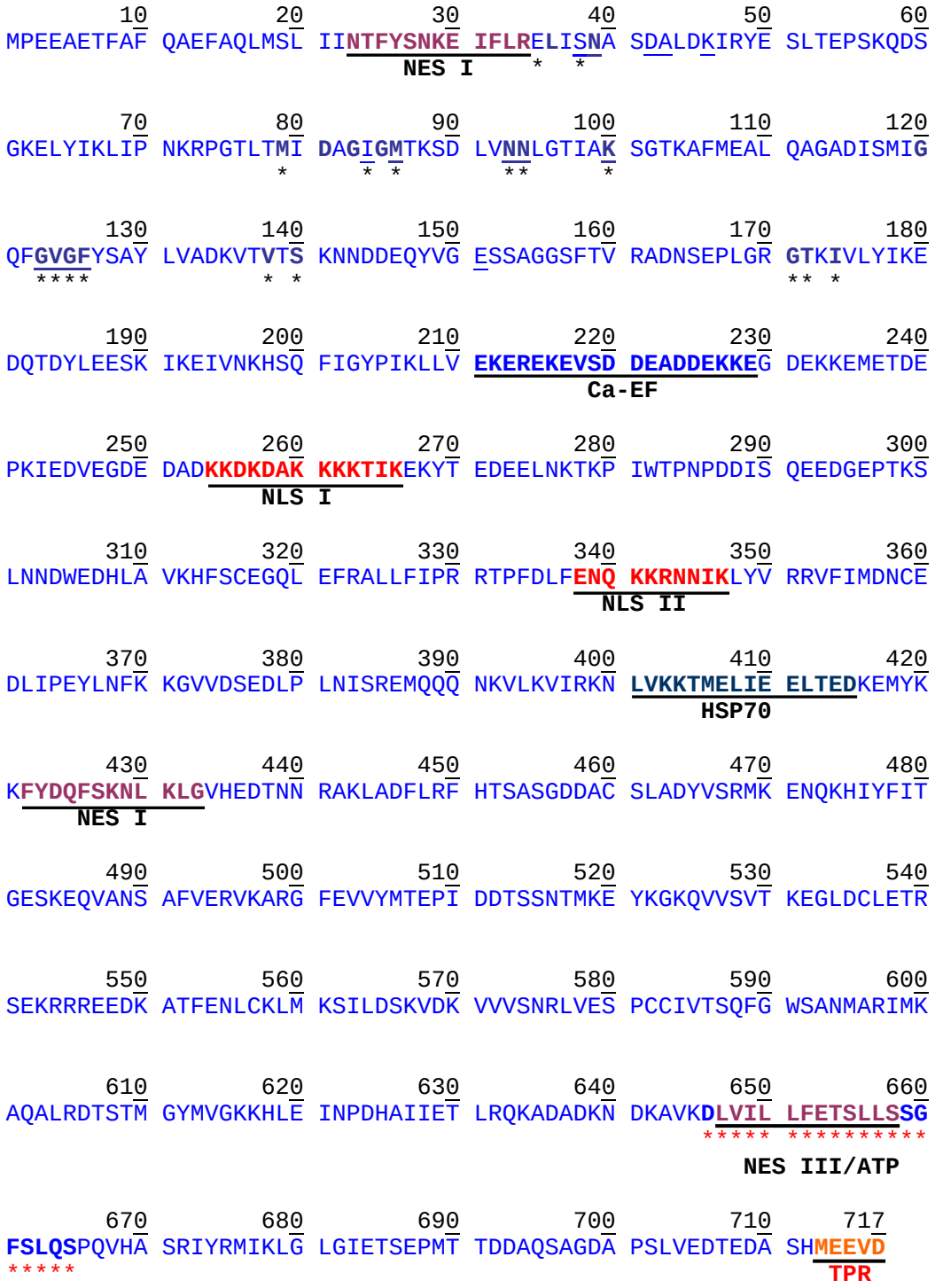

Figure 3. Amino acid sequence of HSP90 of *L. cuprina*. The different binding domains of the HSP90 are shown. NES- Nuclear Export Signal; NLS- Nucleolar Localizing Signal; Ca-EF-Calcium EF arm; HSP70- HSP70 binding region (putative); TPR- Tetratricopeptide binding domain. The exact ATP binding and Geldanamycin binding pockets are shown with (*) and (--), respectively. The putative second ATP binding region in the C-terminal domain is overlapping the NES and represented with (*) in red.

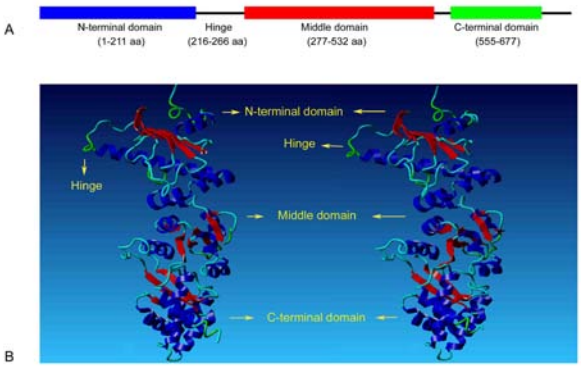

Figure 4. (A) Domain map of HSP 90 and **(B)** Tertiary structure of HSP 90 With its functional domains

Phylogenetic analysis of the hsp90 gene and its product

The BLAST analysis showed that the *lchsp90* sequence is the homologue of *hsp90*β of higher vertebrates. The multiple sequence alignment of *hsp90* sequences revealed that there is a high percentage of homology of *hsp90* gene among individuals of various species of different phyla. The *hsp90* sequence of *L. cuprina* is having an 88% similarity with *Drosophila* and with decreasing similarities of 71%, 69% and 13% between human, mice and *E. coli*, respectively. The sequence alignments with the representative species is shown in the form of phylogenetic tree representing the phylogenetic relationship between and among various species.

Similarly, the amino acid alignments from different species also showed the decreasing homologies from *Drosophila*, human and mice. The LcHSP90 sequence is having a 92% homology with *D. melanogaster* HSP90 homologue (HSP83) with a decreasing homology of 74%, 73% and 37% with human, mice and *E. coli*, respectively. The phylogenetic tree based on the amino acid sequence alignment is shown in Fig. 5.

Though, the overall similarity of the amino acid sequence is varying, the functional domains are highly conserved in the HSP90 protein. The two Nuclear Localising Signals (NLS), the three Nuclear Export Signals (NES), the ATP/Geldanamycin (GA) binding pockets in N-terminal domain, HSP70 binding region and the putative ATP binding region in the C-terminal domain are all highly conserved among the species. The sequence of the domains and the percentage homologies are tabulated in Table 1.

Figure 5. Cladograms showing the phylogenetic relationshionship of *L. cuprina hsp90* gene with other family members based on amino acid sequences.

DISCUSSION

HSP90 proteins, like HSP70, are an important component of the chaperone network, assisting in a variety of vital cellular functions (34). The amplification of *hsp90 g*ene from the genomic DNA of *L. cuprina* confirmed that, like *hsp60* or *hsp70*, it also lacks introns in the gene sequence. The structural analysis of the nucleotide sequence revealed a single ORF of 717 amino acids, with an approximate molecular weight of 81.5 kD, as also reported for *D. melanogaster* (717 aa; 81.9 kDa), human *hsp90*α (732 aa; 84.7 kDa), mice *hsp90*α (733; 84.9 kDa) and *E. coli* (624 aa; 71.4 kDa). The protein is highly stable, is evident from its instability index (39.93).

Like hsp60 or hsp70 the gene sequence of the *L. cuprina hsp90* is devoid of introns as is evident from the amplification of the *Lucilia hsp90* with primers designed from the cDNA of *D. melanogaster hsp90* (*hsp83*) and also from other species (50). But there are exceptions too, e.g., the mouse *hsp86* has eleven exons interrupted by ten introns (13), similar to that of human *hsp90*α with ten introns and eleven exons (25). Further, based on our genomic amplification result and reports in literature (51), the copy number of the *hsp90* gene in *Lucilia cuprina* is expected to be one which, however, requires to be ascertained further. But, there are exceptions also, e.g., *Anopheles albimanus* (6), which has two copies of *hsp82* gene. The vertebrates have two *hsp90* genes with a significant degree of homology, encoding two cytosolic forms, namely, *hsp90*α and *hsp90*β (20).

The *in-silico* analysis of various functional domains in the primary sequence of the protein revealed highly conserved NLS, NES, ATP binding domains of both N- and C-terminal ends,

the HSP70 binding region (38, 11, 30) and also the TPR binding domain at the C-terminal end (41). This suggests that even though some differences do exist in the sequence homology, the functional domains remain conserved across species (8). The absence of polyglutamine sequence domain in the HSP90 protein of *L. cuprina*, which is the characteristic feature of vertebrate alpha-isoforms, makes the sequence closely related to the beta-isoforms of vertebrates (51), a feature common to all the insect proteins.

L. cuprina, being a dipteran, is a holometabolus insect with vermiform larva and co-arctate pupal forms. The development of the fly starts from an egg to adult through larval and pupal stages, which involves various developmental and morphological changes brought up by steroid hormones, like ecdysone or juvenile hormone (5), during metamorphosis. During metamorphosis of an insect, increased synthesis and secretion of steroid hormone 20-hydroxyecdysone (active form of ecdysone) leads the destruction of larval tissues and its replacement by adult tissues (24). HSP90 being a steroid hormone regulating protein, has an important role in the insect development. It binds to the Ecdysone receptor (EcR), which is the primary target for the chaperone complex. Studies in *Drosophila* cell line revealed that HSP90 and its cohort HSP70 function in the binding of the EcR, its stabilisation and translocation to the nucleus (2). Although *L. cuprina* is closely related to *Drosophila*, in most of its developmental processes, the role of LcHSP90 homologue is still to be elucidated.

Evolutionary significance

HSPs are the most conserved of all the proteins, whose homologues occur in all the species of all the taxa (15). Based on the cellular locale of the protein, the HSP90 gene family has been divided into five subfamilies ,namely, HSP90A, HSP90B, HSP90C, TRAP and HtpG, which indicate cytosolic, ER, chloroplast, mitochondrial and prokaryote, respectively (8). In most of the vertebrates, HSP90A exists in two cytosolic isoforms, namely, HSP90AA (HSP90α) and HSP90AB (HSP90β) (20, 8), where as in *S. cerevisiae*, two forms exists, one is heat inducible and the other being constitutive in expression (20). The nucleotide *L. cuprina* has a maximum homology with its dipteran counterparts and is having a close relation in origin to *hsp90* beta-isoforms of vertebrates, which is evident from the phylogenetic analysis also. Similarly, the amino acid sequence analysis of HSP90 of *L. cuprina* also revealed its considerable homology with those of insect species and might be the predecessors for HSP90-beta isoforms of the higher vertebrates. The phylogenetic analysis of HSP90 sequences, of nucleotide and amino acid sequences, point towards a gene duplication, which might have taken place in the early history of the *hsp90* gene family (20).

Even though, many-a-study has been carried out to reveal the structural and functional characterisation of *hsp90* in different models, very little is known in insect species. Further studies are underway in our laboratory to elucidate the functional role of HSP90 protein in *L. cuprina* during development and exposure to thermal or chemical (pesticide and heavy metal) stressors.

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Other articles in this theme issue include references (62-77).

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