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Tissue related expression of Hsp70 genes in *Drosophila melanogaster*: An *in silico* analysis.

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Summary

To analyze the tissue and developmental-stage specific expression of Hsp70 genes in *Drosophila melanogaster*, an *in silico* analysis was carried out using known EST's, promoter sequences and probable transcription factor binding sequences. A correlation could be observed between the presence of a particular transcription factor binding site and abundance of a particular Hsp70 EST's in a developmental/ tissue specific manner. The analysis suggests that specific transcription factors might be involved in differential regulation of Hsp70 genes at the 87A and 87C loci in a developmental-stage specific manner.

The Hsp70 genes of *Drosophila* are some of the most well studied genes (Ish-Horowicz *et al.*, 1979; Ish-Horowicz and Pinchin, 1980; Torok and Karch, 1980; Bettencourt *et al.*, 2002). The haploid genome of *Drosophila melanogaster* normally carries five, almost identical copies of heat-inducible Hsp70 genes, two copies at 87A2, 87A7 and three copies at the 87C1 chromosome locations. The present copy number of five genes is thought to have evolved from an initial number of two. Recently, Bettencourt and Feder (2001) analyzed the Hsp70 duplication in the *D. melanogaster* subgroup and found that the initial 2 to 4 Hsp70 duplication occurred 10-15 million years ago. In *D. melanogaster*, the fifth gene was a result of tandem duplication and gene conversion at the derived cluster. Analysis of genomic sequences covering these genes reveals that they are almost identical in the coding region and variations are only seen in the flanking regions, which shows a conservation mediated diversification (Bettencourt and Feder, 2002). The evolution of the multiple copy numbers of the Hsp70 genes have raised many questions, including whether all the copies are similarly induced by heat shock and how are these genes regulated in a tissue and developmental stage specific manner; conditions that are not related to heat shock. Recently, using experiments such as *in situ* hybridization of Hsp70 cDNA, 3' untranslated region (specific for 87A7 and 87C1-type Hsp70 transcripts) and their stability in different cell types, Lakhotia and Prashant (2002) have concluded that these genes are differentially regulated in a developmental-stage-specific manner. To understand the tissue specific expression of Hsp70 genes in *D. melanogaster* and find a relation to the regulatory regions of these genes, we carried out an *in silico* analysis of tissue specific Hsp70 Expressed Sequence Tags (EST's), presence of Transcription factor binding sites upstream of the five Hsp70 gene promoters and then tried to correlate the expression patterns with the sequence information.

Promoter analysis of the Hsp70 genes Aa, Ab, Ba, Bb and Bc

The five Hsp70 gene and upstream sequences were obtained from GenBank. Their accession numbers are as follows: Hsp70Aa (AF295933); Hsp70Ab (J01103); Hsp70Ba (AF295946); Hsp70Bb (J01104); Hsp70Bc (AF295958). The Promoter sequence alignments were carried out using the CLUSTALW program (Thompson *et al.*, 1994). Approximately 1000 bases of upstream sequence from the translation start site (ATG) was taken for all the above genes and multiple sequence alignment was carried out using the ClustalW program. All the sequences were almost identical till around 410 bases upstream, after which significant differences were observed among the five sequences (data not shown). Since most of the important elements, such as the TATA box, Transcription and Translation start sites and the first three Heat Shock Elements (HSE's) were in the region of high homology, the differential expression of these genes should be related to the sequences upstream of this region.

Tissue specific EST's of the five Hsp70 genes

Identification and analysis of EST's was carried out at the BDGP server (www.fruitfly.org), using the BLASTN program (Altschul *et al.*, 1990) and the *D. melanogaster* EST databases from BDGP and dbEST cDNAs. When *D. melanogaster* EST databases (BDGP and dbEST cDNAs) were searched using upstream and coding sequences of the five Hsp70 genes, a list of EST's for different tissues of *D. melanogaster* was obtained for each type and are summarized in Table 1A. Table 1B gives an overview of the relative abun-dance of Hsp70 transcripts in differ-ent tissues. Based on EST analysis, maxi-mum expression of Hsp70Aa, Hsp70Bb and Hsp70Bc was observed in Larvae-Early pupae (LP), followed by the ovary-newly eclosed female (GM), a *Drosophila* cell line (Schneider L2 cell line; SD) and Adult Testis (AT), thus showing a similar pattern of expression. The expression of Hsp70Ab was relatively more in Schneider L2 cell lines (SD), followed by the ovary-newly eclosed female (GM), Larvae-Early pupae stages (LP) and the Adult Testis (AT); while interestingly, the Hsp70Ba expression was seen

Table 1A. Tissue related Expressed Sequence Tags for the five *D. melanogaster* Hsp70 genes.

Tissue (code)*	Relative percentage of Hsp70 gene transcripts (as determined from EST's)				
	Hsp70Aa	Hsp70Ab	Hsp70Ba	Hsp70Bb	Hsp70Bc
Larvae-Early Pupae (LP)	53.8	14.3	22.3	53.4	54.2
Ovary-Newly eclosed female, Germarium stage 6 (GM)	23.0	17.2	33.4	23.0	25.0
Adult Testis (AT)	4.0	8.6	11.2	4.0	4.2
Schneider L2 cell line (SD)	11.6	40.0	33.4	11.6	8.4
Embryo, 0-24 hrs mixed stage (LD/CK)	7.0	14.3	---	7.7	8.4
Embryo (RE)	---	---	---	---	---
Adult head (GH)	---	3.4	---	---	---

* (code): as used in the BDGP EST database (www.fruitfly.org)
Only high scoring matches (>1000 score) for the above data sets were taken for analysis. The relative abundance of each EST type is expressed as percentage (rounded off to minimum decimal value).

Table 1B. Preferential expression of Hsp70 genes in different tissues of *D. melanogaster.*

D. melanogaster Hsp70 genes	Relative expression in tissues (based on Table 1A)
Hsp70Aa	LP>GM>SD>LD>AT
Hsp70Ab	SD>GM>LP>AT/CK>LD>GH
Hsp70Ba	GM>SD>LP>AT
Hsp70Bb	LP>GM>SD>LD>AT
Hsp70Bc	LP>GM>SD>LD>AT

Table 2: Transcription Factor binding sites in the Hsp70 upstream regions.

	Number of Binding sites for Transcription Factor	
D. melanogaster Hsp70 genes	BRC-Z (Broad-Complex)	DFD (Deformed)
Hsp70Aa	1	1
Hsp70Ab	2	2
Hsp70Ba	---	---
Hsp70Bb	2	1
Hsp70Bc	1	1

relatively more in the ovary-newly eclosed females (GM). Tissues like adult testis and embryos show lower expression of all the Hsp70 genes and the Hsp70Ab type is more than the others. The Hsp70Ab type seems to be predominant in the cell line (Schneider L2 cell line; SD) and transcripts of only Hsp70Ab type are preferentially seen in adult heads. The above results, based on EST analysis clearly support the differential expression of the five Hsp70 genes in the tissues of *D. melanogaster*, and point towards the presence of specific elements (other than the HSE's) that regulate them in a tissue and developmental specific manner. Analysis of Transcription factor binding sites was carried out to look at such a possibility.

Transcription factor binding sites in the upstream sequences of Hsp70 genes

Transcription Factor binding site analysis was carried out using the Tfsearch program (Yutaka Akiyama, 1995) and the Tfdatabase (Heinemeyer *et al.*, 1998). Search for possible Transcription factor binding sites in around 1000 bases of DNA sequence (upstream of the five Hsp70 genes) showed the presence of such regions, which differed for the above five genes. A list and description of the possible sites is given in Table 2. An analysis of these sites for the five Hsp70 genes, the normal functions of the transcription factors and relating it to the tissue specific expression data of HSp70 genes (Table 1A and 1B) revealed an interesting correlation. Sites for the transcription factors BRC-Z (Broad-Complex) have been observed in the upstream regions of Hsp70Aa, Ab, Bb and Bc. BRC is an ecdysone-responsive key regulator of metamorphosis during third instar stage and early pre-pupal development and EST's of Hsp70 Aa, Hsp70Bb and Hsp70Bc have been predominantly reported in Larvae-Early pupae (LP) (see Table 1A and 1B). Further, the binding sites for DFD (Deformed), a homeotic gene controlling *Drosophila* head development, are associated with Hsp70Aa, Hap70Ab, Hsp70Bb and Hsp70Bc, but not with Hsp70Ba. The HSp70Ba homologous transcripts are found to be less common in embryos (Table 1B). The above observations further strengthen the association of specific regulatory elements to the developmental-stage specific expression of Hsp70 genes in *Drosophila melanogaster*.

The above data represents an attempt to analyze the differential expression patterns of the five Hsp70 genes of *D. melanogaster* with respect to the upstream sequences, which seems to have binding sites for some development/stage specific transcription factors. Due to the presence of these binding sites, the heat shock genes get expressed (in the absence of a heat stress) and must be playing an important role in these developmental processes.

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