Heat shock loci 93D of *Drosophila melanogaster* and 48B of *Drosophila hydei* exhibit a common structural and transcriptional pattern

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## ABSTRACT

A comparison of gene structure, sequence, and transcription pattern of heat shock loci 93D of <u>Drosophila</u> <u>melanogaster</u> and 48B of <u>Drosophila hydei</u> has been performed. Both heat shock loci consist of an unique region that is flanked by an internally repetitive element. Different members of these elements are highly conserved, repeat unit length, however, and primary sequence diverged totally. Whereas the overall gene structure in both species is substantially related, sequence conservation is only observed at very few sites in the unique region. These represent primarily sequences that are identified as regulatory elements for faithful transcription and processing. The number and size of transcripts obtained from heat shock locus 48B in third instar larvae closely resembles the pattern of heat shock locus 93D. Thus their quite alike structure and transcription pattern suggest strongly a conserved hitherto unknown function.

## INTRODUCTION

The cellular response to elevated growth temperature or stress in general results in a characteristic alteration of RNA and protein synthesis (1-3). In consequence, the production of a small number of specific polypeptides, the heat shock proteins (hsps), is induced or enhanced. In Drosophila, cytological examination of heat shocked third instar salivary gland nuclei revealed transcriptional activity at several hspuff sites (4,5). Most of these puffs are known to contain genes that code for heat shock proteins. Since the majority of the heat shock genes are sequenced in Drosophila and distant species like man and bacteria, it is evident now that they represent surprisingly well conserved gene structures.

In <u>D. melanogaster</u>, however, in situ hybridizations revealed an additional heat shock locus at the cytological map

position 93D. Attempts to identify a protein product which might be encoded by the 93D derived heat shock RNA have not been successful. Several other Drosophila species contain one heat shock puff that is clearly related to 93D (6). Loci 48B of D. hydei and 93D of D. melanogaster are the only ones that have been cloned and structurally mapped and are thus available for closer examination (6,7,8). Several features shared by the two loci suggest that they represent equivalent heat shock puffs. Both gene loci consist of a unique structure, that is flanked by an internally repetitive element (9,10). In third instar larvae they have been shown to accumulate unique giant ribonucleoprotein particles (11,12). In search for a functional relevance of this specific type of heat shock response it has been proposed that they might represent a storage site for certain RNP antigens.

Even though cytological examination of both puffs lead to the conclusion that they are equivalent heat shock loci, no crosshybridization of <u>in vivo</u> labeled heat shock RNA from <u>D</u>. <u>hydei</u> to polytene chromosomes of <u>D</u>. <u>melanogaster</u> was observed (13). This finding suggests that the characteristic features of both loci are somehow preserved despite of very dramatic nucleotide sequence changes.

In this report, the sequence of heat shock gene 48B and of a series of repeat units of heat shock locus 93D is determined. By cross hybridization experiments and direct sequence comparison we identify small areas of strong sequence conservation. We examine the transcriptional activity of heat shock locus 48B of <u>D. hydei</u> and compare it with the activity of heat shock puff 93D of <u>D. melanogaster</u>.

## MATERIALS AND METHODS

## Crosshybridization experiments

The DNA cloning and mapping experiments as well as Southern blot analyses were performed according to published procedures (14). For reduced stringency hybridizations, Southern blots (15) were prehybridized in 5 x SSC, 5 x Denhardt's solution (16), 250  $\mu$ g ml<sup>-1</sup> sonicated, boiled herring sperm DNA, 50 mM phosphate buffer, pH 6.8, 0.1 % SDS, 43 % deionized formamide at 37 °C for 2-3 h. Hybridization was carried out with nick-translated probes under the same conditions for 24 to 48 h, then the blots were washed twice in 2 x SSC, 0.1 % SDS for 5 min at room temperature, followed by two washes for 15 min each at 37 °C (17).

# Northern blot analysis

RNA of heat shock third instar larvae and control larvae was isolated as described previously (18). For Northern blot analysis, prehybridization and hybridization was performed in 50 % formamide, 5 x SSC, 20 mM phosphate buffer, pH 6.8, 5 x Denhardt's solution, 100  $\mu$ g/ml sonicated, boiled herring sperm DNA, and 250  $\mu$ g yeast RNA at 42 °C. After hybridization for 24 h to 48 h, the filters were washed twice in 2 x SSC, 0.1 % SDS for 5 minutes at room temperature and two times for 15 minutes at 50 °C in 0.1 x SSC, 0.1 % SDS.

# S1 mapping and sequencing

S1 mapping was performed as previously described (18). For sequencing we followed the methods of Maxam and Gilbert (19) and of Sanger (20) using pEMBL8+ and pEMBL8- vectors (21).

#### RESULTS

# A. Crosshybridization between D. melanogaster 93D and D. hydei 48B sequences

Crosshybridization experiments of <u>in vivo</u> labeled <u>D. hydei</u> heat shock RNA to polytene chromosomes of <u>D. melanogaster</u> described by Peters et al. (13) had revealed no positive result at the 48B counterpart heat shock locus 93D. However, by using fragments of cloned DNA of the same loci, we were able to define small areas of homology in Southern hybridization experiments.

The cloned DNA of both heat shock gene regions is depicted in Figure 1, part I and II. Cosmid cos 7 contains the complete transcription unit of heat shock locus 93D of <u>D. melanogaster</u> (8). It consists of a unique gene sequence that gives rise to a polyadenylated transcript and is in third instar larvae in addition partially read through into the neighbouring internally repetitive sequence (18). This is indicated in Figure 1 as dou-



Figure 1

Cross hybridization between the heat shock gene regions 93D (D. melanogaster) and 48B (D. hydei).

I. Restriction map of a cosmid and lambda clones of a genomic walk in heat shock locus 93D (8): A represents a HindIII ( $\uparrow$ ) XhoI ( $\chi$ ) fragment of the in the transformation vector pC20 subcloned HindIII fragment (pC20/7) of cosmid 7 (cos7); B, C, D, E, F, J, and K are EcoRI ( $\downarrow$ ) fragments cloned in phage lambda. p5A is a subclone of the heat inducible region (10). The transcription unit is indicated as double arrow. The left arrow corresponds to the unique part of the heat shock gene, while "TaqI repeat" shows the extend of the repetitive part.

II. Restriction map of the cosmid clone cDh171 (9). EcoRI fragments used for crosshybridization are designated as a, b, c, and d. Again, the left arrow corresponds to the unique part of the heat inducible region while the extend of the repetitive part is delimited by hybridization with clone N09-15 containing only the <u>D. hydei</u> 48B repeat (7).

III. Cross hybridization between DNA of the two species. The arrows below the isolated subfragments of cosmid cDh171 that have been used in the crosshybridization experiments point to the cloned <u>D. melanogaster</u> DNA tested in each case. All clones are digested with EcoRI as indicated in part I of the Figure. pC20/7, however, is digested by XhoI and HindIII. All fragments containing <u>D. melanogaster</u> DNA are indicated by capital letters as in part I. In lane pC20/7 trace amounts of pUC sequences in the isolated hybridization fragment give rise to a strong signal for the homologous cosmid vector sequences. The extend of crosshybridization is summarized below: the solid bar indicates the region of strong crosshybridization, the cross hached bar represents a weak sequence relation whereas the open bar corresponds to areas of no crosshybridization. The cDh 171 HindIII-Xba (Xb) fragments e and the HindIII-EcoRI fragment f are used for crosshybridization to the 93D clone p5A as shown in Figure 2.

ble arrow. Subcloned p5A and  $\lambda$  590 comprise only parts of the heat shock locus.

In order to obtain a measure for the degree of fluctuation of gene arrangement and sequence information between D. hydei and D. melanogaster outside the heat shock locus, we included a stretch of 10 - 15 kb DNA that is flanking the 3' borders of each heat shock gene in the crosshybridization experiments. For <u>D. melanogaster</u>, this area is contained in clones  $\lambda_{13}$  and  $\lambda_{12}$ (Fig. 1, part I). Cosmid cDh171 includes heat shock gene region 48B of D. hydei as indicated in Figure 1, part II. Single EcoRI fragments of cDh171 were subcloned and hybridized under reduced stringency (17) to the lambda- and cosmid clones containing previously identified overlapping DNA fragments from heat shock puff 93D (8). Lambda clones 12, 13 and 590 are cut by EcoRI and give rise to the fragments indicated by capital letters in part I. The HindIII fragment of cos 7, however, that spans the region between  $\lambda$  13 and  $\lambda$  590 is first subcloned in the transformation vector pC20 and then cut by XhoI and HindIII. DNA fragments containing D. melanogaster DNA in the Southern blots are marked with capital letters as in part I. Figure 1, part III, shows that a strong sequence homology is observed between each pair of the EcoRI fragments that flank the 3' ends of heat shock gene 48B and 93D. Fragment "d" of cosmid cDh171 hybridizes specifically to fragment "D" of  $\lambda$ 12, fragment "c" recognizes homologous sequences in fragment "C" that is contained in  $\lambda_{12}$  and  $\lambda_{13}$ . In addition, it hybridizes to part of fragment "B". Thus, crosshybridizing fragments located are ar-



Figure 2

Evidence for homologous sequences in heat shock gene 93D and 48B.

I. Restriction map of clone  $p5\lambda$  that consists of the unique part of heat shock gene 93D (Fig. 1) and part of the TaqI repeat subcloned in pUC8. Only relevant restriction sites are shown.

II. Restriction map of EcoRI fragment "a" of cosmid cDh171 of <u>D. hydei</u> (Fig. 1). The single XbaI site separates the unique portion of the transcription unit (defined by fragment e and f in Fig. 1) from the N09-15 repeat structure.

III. Crosshybridization: 1. EcoRI-HindIII fragment f of <u>D</u>. hydei cDh171 hybridized to EcoRI-ClaI and EcoRI-PstI digests of clone p5A. 2. HindIII-XbaI fragment e of cDh171 hybridized to an EcoRI-SacI digest of p5A. 3. 2.2 kb AvaII fragment of <u>D</u>. <u>melanogaster</u> p5A hybridized to HindIII-EcoRI fragment f of <u>D</u>. <u>hydei</u> digested with SalI. 4. The 0.6 kb SacI fragment of <u>D</u>. <u>melanogaster</u> p5A hybridized to the HindIII-XbaI fragment e of <u>D</u>. <u>hydei</u> digested with Sau3a. The leftmost HindIII-Sau3a fragment is strongly and the neighbouring Sau3a fragment is slightly positiv.

Abbreviations used are:  $\lambda = \lambda vaII$ , C = ClaI, D = DraI, E = EcoRI, H = HindIII, P = PstI, S = Sau3a, Sc = SacI, Sl = SalI, Xb = XbaI. \* denote vector sequences; o indicate positive hybridization to traces of undigested DNA due to incomplete digestion.

ranged in the same order downstream of the heat shock loci in D. hydei and D. melanogaster. The sequence conservation diminishes towards the 3'-end of the heat shock transcription units, since <u>D. hydei</u> fragment "b" hybridizes to fragment "B" of cosmid cos7 only to a smaller extend compared to the hybridization strength of the neighbouring fragments as mentioned above. Hybridization of D. hydei repeat elements as contained in cDNA clone NO9-15 (7) revealed no crosshybridization signal in the Southern blot analysis (data not shown). If there is any sequence homology between the repetitive portion of both heat shock loci, it is not sufficient to form stable hybrids under our experimental conditions (see also sequencing data below). However, when the complete D. hydei 48B heat shock DNA (fragment "a") is used, a limited crosshybridization to fragments "A", "H", and "J" resumes in the unique part of the heat shock gene 93D.

investigate this site of sequence homology in more TO detail we isolated subfragments of the unique heat shock gene regions of <u>D. melanogaster</u> and <u>D. hydei</u>. Figure 2, part I, shows a restriction map of the <u>D. melanogaster</u> heat shock clone p5A. We have previously identified the structure of the unique 93D heat shock gene (18). A single intron is contained in the middle of the gene. In all RNA preparations analyzed so far we observed a substantial amount of unspliced 93D RNA. In part II of Figure 2, a restriction map of EcoRI fragment "a" of cosmid cDh171 of D. hydei is depicted. Subfragments "e", "f", and "h" used as probes are indicated. Probing the D. melanogaster clone p5A with the 1.6 kb EcoRI-HindIII subfragment "f" of D. hydei, a crosshybridization signal is obtained in the exon1-intron region of heat shock gene 93D as shown in part III of Figure 2. The 1.0 kb ClaI fragment and the 1.5 kb EcoRI-PstI fragment (Fig. 2, part III, 1) hybridize specifically to the D. hydei probe. Crosshybridization to fragment "f" is therefore mostly restricted to the exon 1-intron transition of the 93D gene between the ClaI and PstI sites.

Furthermore, the neighbouring <u>D. hydei</u> 1.5 kb HindIII-XbaI subfragment "e" (Fig. 2, part II) hybridizes to the 550 bp SacI fragment of an EcoRI-SacI double digest of clone p5A (part III,



### Figure 3

Transcription analysis of the 48B gene region.

RNA isolated from third instar larvae kept as mass culture was used. Lane 1: 10  $\mu$ g of poly(A)<sup>-</sup> RNA and lane 3: 2  $\mu$ g of poly(A)<sup>+</sup> RNA of larvae kept at 25 °C. Lane 2: 10  $\mu$ g of poly(A)<sup>-</sup> RNA and lane 4: 2  $\mu$ g of poly(A)<sup>+</sup> RNA of heat-shocked animals (37 °C/45 min). Hybridization was performed with <sup>32</sup>Pnicktranslated probes. Hybridization probes: panel I; XbaI-EcoRI fragment h containing <u>D. hydei</u> 48B repeat sequences (Fig. 2); panel II; 1.5 kb HindIII-XbaI fragment e containing <u>D. hydei</u> 48B unique sequences (Fig. 2); panel III; a longer exposure of lane 3 and 4 of panel II. Capital letters denote major RNA species of 10, 2.5, 2.0, and 1.3 kb.

2 of Fig. 2). The reverse experiment using the 2.2 kb AvaII and 550 bp SacI subfragments of the <u>D. melanogaster</u> heat shock clone p5A as hybridization probe for isolated fragments of the corresponding DNA region of <u>D. hydei</u> (Fig. 2, part III, 3 and 4) confirm the sequence homology between these heat shock loci. It is restricted to very small areas of the unique part of both heat inducible transcription units.

B. Transcription of the D. hydei 48B heat shock gene

Evidence of Peters et al. (7, 9) existed that demonstrated the transcription of the repetitive <u>and</u> the unique



<u>Figure 4</u> <u>S1-mapping to determine the 5' start position of the 48B</u> transcripts.

The EcoRI-SalI fragment g (Fig. 2, II), 5'end labelled at the SalI site, was used. Lane 1:  $\lambda$  + G sequencing track; lane 2: S1 mapping using 20000 U S1/ml and 46 °C as hybridization temperature, lane 3: S1 mapping using 5000 U S1/ml and 49 °C as hybridization temperature; lane 4: C + T sequencing track. The arrow indicates the deduced transcription start site.

part of the 48B heat shock puff. A library prepared from oligo dT primed cDNA of <u>D. hydei</u> heat shock RNA contained a clone that includes several units of the heat shock repeat element. In addition, <sup>32</sup>P-labeled cDNA of <u>D. hydei</u> heat shock RNA hybridized strongly to cloned DNA fragments of the neighbouring unique part (9). Therefore, we expected both regions to be transcriptionally active.

	10	)	20	30	40	50	60	70	80	90	100
			•	•	•••••••••		AC-CGT AGG	ACCACCTATA	CGATGTATGT		TACTCA
1	T AT AAAAGA	AATATATG	TGTTTGTCAA	GAGTATACGA	TGAAGTGAAT	* ***R#* * CACGTAG3CA	ACACTCAGC	AGTGTATTCA	CTCACTCCTT	***R R TGATAAAA	* * * TTGTAG
53	CTCCAACCC	CAATGCCG	CAGACCCGAG	AGACAGGCAG	ATTTTTCCCG	AACCCGC-GG	CTTCCCT A	A.	cecct-cetc	COTCAC	TCACTO
101	*** ¥ -GCCATAC	CAAGGGAG	* * ** -ACA-AATAG	• Y Y •	* *Y ** * AATCGTCGAG	** *** * AAGACGCTGG	SYRRANS CGCTAGGTA	*******R** TAAATAAAGG	Y* ** * * TGACTCCCTG	ACTGC AC	***Y** TCATTC
	<u> </u>		•	<b>.</b> .	•	•	•	•	•	6	
151	TCAAATGAA	AAGAGFTC.	AAGTGCA-TT ***** * **		GCTGAAAAAA R## ####	Y * *	****** R	FACAAAGAAA * y <b>R</b> **   *	* ** *Y*	TGCAAT **** R*	* *R**
196	GAAAAAAA	AAGTGETG	AAGTGAAGTT	<b>G</b> TCTAGIGAA	ACTTAAAACT	GTTTGAAGAA	TAAAATATA	TTTCTGGATA	AGTGCTGTTT	TGCATTGT	TCTACA
244	AGCAGTAGC	Tacaasca	AAAATGGAAA	AGTGTAAAAA	TCOTOTCCCA	GCAGACGAGG	AGCAGCAGT	ACGAGTATTG	CAAAATGCAG	CGGCAAGG	GCCCAC
296	TAAATAATA	FACAA-CT	GCAATGGAGA	AGTGTACAGT	ATTTGTTGC-	GAAGGCGAG-	-ATTGC	-GGAGCT-	- AT AATA-	TGTCAC	GTCGAC
344	GTAGTATTT	TTCCACGT	CGGGCATTTA	ATECTCICEA	GTTGGAAACA	AAGAAACCAT		CCCTGGAAAA	GATGTGATTA	GTCAT	-CGATT
379	*RR ** * -TGAAATAA	** * * TTGCTAG-	CGCACCGTTC	• ••• •¥ AAGCTGTTTT	GCTGTGAACA	****** ** AAGAAAA1	* ** *Y 'AAAATCT	*YY* * GCTCGTCATC	GTCGTGGGCA	**¥ * GTTTTCGG	GCGTTT
440	TTGCTGGTC	AGCGTC	GGGGAATTTC	GCAATGCAG	AGGCAGTTTT		GUCT AG-AA	AGTGACCCAC	TAGGCAGTCT	GAGGCAGT	·
473	TTGCATTTG	** R*Y Aggaaatt	**** * ¥ * GGGGCAACAC	G-AAAGCAGC	GGCCAAGTTI	¥**** **** TTCAACATTI	GGCTAGAAA	*R* R* * AATTGCACTG	******* ** TAGGCAGGCT	****** Gaggcaga	* * TGCAGG
		•	•	•	•	•	•	•	•		<u>C</u>
532	TCCAGGA	TGTAAGGA *R * **	TGTCTG ** * *	CAGTACTC	TCFGFGFC-G Y R* Y* *	AATGGCGATG •R•• K•	*Y******	TGCGGATTTT ** *R **	GAAAGATTGA *R*R * **	AAT AGGAA R	GCCAGG
572	AGTTTGCGA	TAAATTGA	TGAGGCAGGA	AAGACCTAGO	CAGATCCCGG	AGTETATETT	GTCCTGACC	TG-GACTTAA	GGAGTTTGGA	CHTAGGAA	GCCAGG
623	TATACATAC	• AC AGC AAC	GAATGCAGCG	TOTTCTATA	TCTTAATTCG	TAAGCCAA	ACCTATOTT	TAAGACGTAG	TTTATATAAC	CGTACATA	TGTATC
671	TATA-AACC	GAAACGGC	GAAACCAGC-	-TTCGTACCI	TATT-ATACG	TAAGCGGAAA	ATCTAAGCA	TAAGGCCTAG	TTT-TATTAC	T-CAAC	TATATG
721	TAGCGAAAC	ACGTTTTC		TCGAAGCCAT		GCCATTGCAA	TTTCATCGT	CTTTGCGTAA	TEGEAAATTG	T-AAATGT	GTCCST
763	Y*RY ** CAATTTAAA	ACGTTTTC	AAGCCTGACG	TCAGCGTAAT	** ** * * TT-TTGTTTT	* * *R * TCGAGTAAAC	GTTIATACT	Y* R R***	GCGAATATTG	• ••• Y TGTAATTC	R+Y+ + ATTCGT
820	ACTATTICA	CATTTGTG	TGT ATCCA	AGTEGAAAGT	TCTCGAACTA		A-GCAACCC	CCGGATCG	AAAGGCCA		ectro
861	* ****YR AATTTTG	ү <b>* ***</b> ү* Т <i>А</i> -ТГССС	•R • Y • TAAAATAATT	*****R * TTTCGAAGC1	*** * **** TCTAGCACTA	** * * Y TTTCCACCCC	* **** * ATGCAAAGC	* * ** CATGTCTTCC	* R *Y CATCTATCTT	Y*RR* * TCGGCCAT	TTCGAG
909	tteretete	CCCAG-AA	ACAGCTTA	CTCATACGTI	TACAATTGAC	TCTCTAC-CI	ATCGGGTAT	ACACATTTT	ATATATGTAT	ACACTCAG	AGACAC
958	* ¥**** TAAATTCTC	* * GACTGTTC	ү <b>*ү***</b> ТТТСТТГАЛГ	*¥* ** R CCCCTAAAAG	* *RY * Y Gagageager	GCCTGCTCI	* * RK** 'AAC-AATAA	*Y* ** * ATAGCATTGT	** *RYR*R* Atttgcatgt	•••¥ ¥* Acatatat	* * *Y Ataaat
1005	CCCAATCCC	CCCCCATA		G-ATGTGTGA	ATGTATCOCA	GTTGGACGAG	AACGGCGCT	GCGC-AGAAA	- ACGCTGCGG	CGT-AC	ATACAA
1057	*Y*R Y * CTCGTATAC	ACGTATIC	¥ * * * ¥ TCTCTCGCTT	* *Y **Y* GCACTTGCGG	*RY**Y** TTACATTGCC	**** RY*R GTTGCGTGG	* <b>******</b> -ATGGCGCT	GCGCGATAAA	***Y ** CTCGCCTCGT	GCTCAGTT	GTTCGA
1099	ACGAGCTCT		 			ATACGTATA		ATCAGCGGTA	ATCT-COCCA	GCCTGACT	
1156	* * **Y* CCCACCTTT	* ** GTCTTACA	*** *** * ATGTATTIGT	у• • • GTATTTCCA1	** ******* CTCTTTCTCT	****R*** * ATACATAI-/	* R*R *	** ** Ataagactad	ATCTACTTGA	* * * Y* GACAGTTT	* * TCTCAA
1189				-							
1255	********** ACT GTC GCTT	* **** AT AAT TGG	**** *** CACATCTCTT	* *****R* * *****R*	TCATAGGTTC	****R* **	TAAACCCTAG	TGTTAATCT	TATTTATAAAG	-TGTCAA	* **
	•		•		<u> </u>	•			<b>-</b> :	•	•
1354	AACCTGCCATA * Y** ** TAATTGTTA	ACACTTA ACACTTA	ACC AAGC AAT ********** ACC AAGC AAT	AT GT ATT TC 1	TTCTCTAAAC	********** ***********	GGGCGTTGAA ********* - <u>GGCGTTGA</u>	AGTTGATAT	CGATCGAA	CGTG-AA R®® ® GATGTTA	AAGTEG • •YR AC.TTA
1378	ATACCCTGCGG	CAAGCATG	GGGCGGCATA	TGCGTGCTG	AAACGCACTC	GCCCGATC	CCGATTGCAG	COTTATTCO		TOCOACC	TGACT
1450	*YR* *R**1 ACGCAATACG1	Г <b>СЛ</b> САЛЛА	GGCCTTACAA	* ***R*	***Y** Y AAATGCTGAT	*YY**R**	** ***** CCATGTAG	* YR* *	AGAG-TATATO	* <b>R**</b>	GCGACT
1478 (	GAGATCATATO	GCGTACAT	AT ATCTAATG		TGGGCCAGCC	AGGGTGCTC	GATTCTGTCA	GATTGATTG	GCGGATTOTO	TTATAGO	
1540	*R** R*R*	TGTGTGTCG	RY*** CGGCCT AC	* ***** AC AGGGGT AG	*RR * *Y*	GTTTTT ATC	* Y*** -AGATTGT	GATTGCGTG	RY ** *R* Gattgaatat-	++++++ ATATAGO	• •• AAC-
1578	GTGT ATCOA		CTCCACTA		AGGAT 4		•	• GA- AAAGOCI	•	* *	
1627 -	********* CTGT ATCGA	*******	***¥*** CTCT ACT AAG	ACTTGTTCA	AAGAAAGGCG	*** *** CCCAGT FAC.	AT AT GT ACT /	* *** *	GTCTCTTAT	TACCTAC	CATCGA

#### Figure 5

Comparison between the sequences of heat shock loci 93D and 48B.

Upper part shows the sequence of heat shock gene 93D as determined by Hovemann et al. (18), lower part the sequence of heat shock gene 48B. Computer alignment for maximal homology is shown between both sequences. ('\*': homologous base, 'Y': pyrimidine exchange, and 'R': purin exchange). Areas of strong sequence homology are boxed. A: TATA box; B: start site of transcription with  $\bullet$ 5' end of 93D RNA and  $\circ$ 5' end of 48B RNA as determined by S1 analysis (Fig. 4); C: exonI-intron boundary of heat shock gene 93D; D: intron-exonII boundary of heat shock gene 93D. The intron-exon boundaries of the 93D gene are indicated by a filled rhombus; E: polyadenylation site. The AATAAA motifs in this region are underlined.

Following this line of evidence, we subcloned DNA of the repetitive and the unique part of heat shock locus 48B and performed hybridizations with RNA of third instar larvae. The results are shown in Figure 3. A single RNA hybridization signal in the 10 kb size region is obtained using the XbaI-EcoRI subfragment "h" that consists primarily of repeat sequences. This transcript is present in the  $poly(A)^+$  and in the  $poly(A)^$ population (Fig. 3, panel I). Its transcription might be slightly enhanced after heat shock. The XbaI-HindIII subfragment "e" of the neighbouring unique DNA (Fig. 2, part II)

# **Nucleic Acids Research**

hybridizes to a series of RNA bands (Fig. 3, panel II). Size and relative intensity of the signals observed for D. hydei very closely resemble the result that we previously described for the corresponding heat shock gene locus 93D of D. melanogaster (18). As in D. melanogaster, the heat induction of the RNAs in the 2 kb size range is five- to tenfold. The high molecular weight transcript contains sequences of the unique and the repetitive region. In D. melanogaster, the heat inducible transcription unit 93D gives rise to a series of RNAs due to low splicing and 3' end processing efficiency (18). Number, size, and relative intensity of bands observed in hybridization with the equivalent heat shock sequences of D. hydei imply a very similar transcription and processing pattern.

transcriptional activity Since the is comparable, determination of the transcriptional start point in heat shock gene 48B should allow to line up the homologous regions in D. hydei with respect to gene 93D. This is of particular importance because of the failure in identifying a translational reading frame used within the 93D transcript. We determined the 5' transcription start site using the 1.0 kb EcoRI-SalI subfragment "g" of cDh171 (Fig. 2, part II) for S1 protection analysis. As depicted in Figure 4, a 1173 bp DNA fragment, 5' end-labeled at the SalI site, is protected against nuclease **S1** digestion. The start point for the transcription unit is determined to 185 bp upstream of the SalI site. It serves as a landmark for the sequence comparison of both heat shock genes. C. Sequence of the unique part of the 48B heat shock

## gene of D. hydei

Crosshybridization experiments, Northern, and S1 analysis delimit the unique part of the heat shock gene in locus 48B. Using the chain termination (20) and the chemical method (19) we sequenced the DNA of the crosshybridizing fragments "e" and "f" of cDh171. A comparison of this sequence with the corresponding <u>D. melanogaster</u> 93D sequence as depicted in Figure 5 reveales several areas of homology. However, the number of consecutive identical nucleotides is, although clearly above a fortuitous rate, generally low. Primarily sequences surrounding

		10	20	30	40	50	80	70			100
1	1 1 1	ACATCTACA - CAA ACATCTACA - CAA ACATCTACA - CAA ACATCTACA - ACATCTACA - CAA	CAAATATCC ACAAATATCC ACAAATATCC ACAAACATCC ACAAATATCC	TCCGAAAGAA CCCGAAAGAA TCCGAAAGAA CCCGAAAGAA YCCGAAAGAA	AGCGTTATAC AGCGTTATAC AGCGTTATAC TGCGTTATAC AGCGTTATAC	AAAACTGAGT AAATATGACT AAAACTHAGT AAAACTGAGT ****.*	GAAATTGCAC AAAATTGGAA GAAATTGGA GAAATTGGA GAAATTGGA GAAATTGGA	GAGTGTGTGA GAGTGTGTGA GAGTGTGTGA GAGTGTGTGT	AACCATAGAT AACCATAAAT AACCATAAAT AACCATAAAT AACCATAAAT	ATTICCCTCA	ATAGGTA ATAGGTA ATAGGTA TTAGGTA ATAGGTA
	99 99 99 95	GGTGATATACAAAT/ GGTGATATACAAAT/ GGTGATATACAACT/ GGTGTTATACAAAA/ SSES_SSESS GGTGATATACAAAT/	ACT ACT ACT ACT ACT ACT								
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	193 194 196 197 191 193 192 193	ТТААСАА-СТССАА ТСААСАА-СТСССАА ТТААСААССТСССАА ТТААСАА-СТССАА ТТААСАА-СТССАА ТТААСАА-СТССАА ТТААСАА-СТССАА ТТААСАА-СТСССАА	CATGACCCAG CATGACCCAG CATGACCCAG CATGACCCAG CATAACCCAG CATAATTCAG CATGACCCAG CATGACCCAG	AATTTTATG AATTTTATG AATTTTATG AATTTTATG AATTTTATG ACTTTTATG AATTTTACG AATTTTACG	ACTCTTTACC MC-TTAAC ACTCTCTAAT ACTCTTTACC ACTCTTTAAT MC-TTAAT ACTCTTTAC	CAATAGGTTT CAATAGGTTT CAATAGGTTAT CAATAGGTTTT TAATGGTTTT CAATAGGTTTT CAATAGGTTT CAATAGGTTT	TATTTATCCCC TATTTAT-CCC GATAAAACCCC GATAAAACCCC GATAAAACCCC TATTTACCCCC TATTTACCCCC TATTTACCCCC	CATTACCCA CATTACCCA CATACCCA CATACCCA CATACCCA CATACCCA CATACCCA CATACCCG CATACCCG	ATCACTATCC ATCACTATCC ATC-TTATCC ATCACTATCC ATCACTATCC ATCACTATCC ATCACTATCC ATCACTATCC ATCACTATCC	r r T T T T	

#### Figure 6

Commparison of the repeat sequences of both heat shock genes.

A consensus sequence deduced from nine 280 bp <u>D.</u> <u>melanogaster</u> TaqI repeat units is depicted (part II) together with a consensus sequence deduced from four 115 bp <u>D.</u> <u>hydei</u> NO9-15 repeats (part I) as determined by Peters et al.(9). A short stretch of nucleotides conserved between species is underlined.

the transcriptional start, the intron borders, and the poly(A) addition-site of heat shock gene 93D are retained in the 48B gene. These homologous regions of functional relevance are boxed in Figure 5. Upstream of the TATA-motif we observed no significant homology over a length of 800 bp (data not shown). The comparison between this sequence and the one obtained for 93D (18) indeed exhibits the strongest homology at the regula-

tion and processing sites. These are the only sequences that are retained to a high extend.

This result together with the RNA pattern obtained in the Northern hybridization and the positioning of the transcription start site favour strongly our conclusion that locus 48B gives rise to a heat enhancible transcription product very similar in size and processing behaviour to locus 93D.

# D. Sequence of TagI repeats of heat shock locus 93D

of D. melanogaster

A series of repeated sequence units is linked to the 3' end of each of the heat shock genes in locus 48B and 93D. We had already sequenced five 115 bp units of the 48B locus (9). In <u>D. melanogaster</u> the repeat length is 280 bp. In order to allow a direct comparison of the repeat elements of both heat shock loci we sequenced randomly chosen and subcloned single TaqI repeat fragments of the 10-12 kb repeat structure.

A comparison of nine TaqI repeat sequences reveals a highly conserved consensus sequence (Fig. 6). None of the repeat sequences contains a longer open reading frame. Apart from a small stretch of nucleotides (ATTT-C-T-ATAGGTAGG) retained in both species there is no substantial sequence conservation between the <u>D. hydei</u> and <u>D. melanogaster</u> repeat units, a result that explains the failure of getting cross hybridization in the repetitive part of both heat shock loci.

### DISCUSSION

Heat shock genes usually retain highly conserved sequences in different species. This is especially apparent for the gene coding for the 70 kd heat shock protein (hsp 70). Heat shock puff 93D of <u>D. melanogaster</u> clearly differs from the common type of heat shock genes: cytological analyses demonstrated that the major portion of the transcription products are retained in the nucleus and unique giant ribonucleoprotein particles accumulate. RNA derived from this locus does not even crosshybridize to polytene chromosomes of <u>D. hydei</u>. Still, in every Drosophila species studied a 93D like puff has been observed.

Cloning the 93D gene we revealed the unusual structure of

this heat shock puff (8, 10). It contains a transcription unit of unique and neighbouring repetitive sequence. Transcription is only observed from one strand (10). We previously showed that the puzzling pattern of different 93D RNA transcripts derived from this puff is due to inefficient splicing and 3' end processing of a single primary transcript (18). Since not even sequence analysis of the unique portion of the 93D heat shock locus gave us conclusive evidence as to the usage of a translational reading frame we now compare the gene 93D with the analogous heat shock locus 48B of the distantly related species <u>D. hydei</u>.

Heat shock loci 48B of <u>D. hydei</u> and 93D of <u>D. melanogaster</u> both contain heat inducible gene structures that by the sequence data presented here and previously do not seem to code for a protein (sequence of Fig. 5 and 18, 23, 24, 25), but are transcribed into a series of different RNAs. Since there is no obvious coding frame that can be deduced from the gene sequences the question for the functional relevance of these transcription units becomes a difficult task. However, the highly related structure and transcription pattern of these two genes from quite distantly related species might indicate prerequisites of function. The possibility that both 48B and 93D transcription units happened to become fused to a heat inducible promoter accidently and independently in evolution is very unlikely. Since the structure of both genes is clearly "homologous" such an event must have happened before the species got separated in evolution. Interestingly, neighbouring sequences are more strongly retained during evolution than the heat shock puff itself (Fig. 1). We interprete this result as an additional argument against a protein coding function. However, there clearly exist a strong selection against nucleotide changes in the areas that are likely to contain transcription and processing signals. This evolutionary constraint acts mainly on sequences that result in a low processing efficiency and heat induced transcriptional enhancement. A prerequisite for function of these gene products seems to be the neighbouring repeat structure. In both species a tandem array of repeat elements spans over 10 kb. The structural arrangement seems to be

# Nucleic Acids Research

important.Except a short conserved sequence element (ATTT-C-T-ATAGGTAGG) the repeat sequence diverged totally. Since both species retained a high internal sequence stability in the repeats, a concerted evolution mechanism can be anticipated (26). Heat shock loci 48B and 93D represent active gene loci, that retained structural more than sequence homology. They cannot be considered as pseudogenes because of their uniqueness in the genome. With this comparison at the nucleotide level it becomes evident that the functional clue of these genes is likely to be contained in the structure retained during evolution.

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