DEVELOPMENTAL GENETICS 17:303–311 (1995)

Spatial Expression of the *hsr-omega (93D)* Gene in Different Tissues of *Drosophila melanogaster* and Identification of Promoter Elements Controlling Its Developmental Expression

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ABSTRACT Developmental expression of the heat shock inducible non-protein coding *hsr-omega* gene in several larval and adult tissues of *Drosophila melanogaster* was examined by *in situ* hybridization to transcripts in intact organs and by X-gal staining in the germline transformants carrying the *lacZ* reporter gene under the control of *hsr-omega* promoter. This gene is expressed in a specific spatial pattern in all the larval and adult tissue types examined; however, its transcripts were specifically absent in certain gonadal cell types like the male as well as female gonial cells and in follicle cells and oocytes in ovary. All polytenised tissues like the prothoracic and salivary glands, certain regions of larval gut and the Malpighian tubules showed a greater abundance of *hsr-omega* transcripts with a strong hybridization in nuclei. Our results with promoter deletion variant germline transformants suggest that a region between −346bp to −844bp upstream contains major regulatory element/s for developmental expression of this gene in most of the larval and adult tissues examined; however, this region is not sufficient for its normal expression in male and female reproductive systems. An analysis of the base sequence of the *hsr-omega* promoter (upto −844bp) reveals putative ecdysone receptor element half-sites and two GAGA factor binding sites which may be involved in its developmental expression and its ready inducibility. The widespread expression in most tissue types and the known lethality associated with its homozygous deletion, suggest that the variety of non-protein coding transcripts of the *hsr-omega* gene have vital "house-keeping" functions. © 1995 Wiley-Liss, Inc.

Key words: Heat shock, non-coding RNA, germline transformation, prothoracic gland, house-keeping gene

INTRODUCTION

The heat shock inducible *93D* or the *hsr-omega* locus of *Drosophila melanogaster* is different from the other major stress genes due to its several unique features: it is singularly induced by several agents like benzamide, colchicine, thiamphenicol etc. [reviewed by Lakhotia, 1987, 1989; Lakhotia and Sharma, 1995b; Pardue *et al.*, 1990], but none of its multiple transcripts [Hovemann *et al.*, 1986; Garbe *et al.*, 1986] have a typical translated product [Lakhotia and Mukherjee, 1982; Fini *et al.*, 1989]; in spite of the rapid DNA sequence divergence at this locus, its genomic organization and inducible properties have been strongly conserved in the genus [Lakhotia and Singh, 1982; Garbe and Pardue, 1986; Garbe *et al.*, 1989; Ryseck *et al.*, 1987]. This locus is developmentally expressed, is one of the early puffs in salivary glands of late third instar larvae after the rise in ecdysone titer [Ashburner, 1972] and is essential for viability [Mohler and Pardue, 1982; Lakhotia, 1987]. Earlier studies on its developmental expression using northern and *in situ* hybridizations [Bendena *et al.*, 1991] showed that the *hsr-omega* transcripts were absent in egg but appeared after the preblastoderm stage and continued to be present in most of the embryonic, larval and adult tissue types examined.

The developmental expression of the *hsr-omega* gene in a wide variety of tissues, its multiple transcripts and its complex pattern of inducibility with the different inducers suggest a complex regulation [Lakhotia, 1987, 1989; Lakhotia and Sharma, 1995b]. Our laboratory has initiated studies to define the various upstream regulatory elements controlling expression of this gene. In the present study we have used promoter deletion variants to express the *lacZ* reporter gene in germ-line transformed flies to examine the cis-acting sequences that regulate its developmental activity in different tissues. The normal developmental expres-

Received for publication May 17, 1995; accepted July 21, 1995.

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sion of this gene in several larval and adult tissue types was also examined by *in situ* hybridization to cellular RNA in intact organs and by X-gal staining for reporter gene activity in the germ-line transformants. We show that the *hsr-omega* locus expresses in almost all the tissue types examined but rather than being expressed constitutively in all cells, it is expressed in a specific spatial pattern in different cells of a given tissue. Our results suggest that a region between −346bp to −844bp upstream of this gene contains major regulatory element/s for developmental expression of this gene in most larval and adult tissues.

MATERIALS AND METHODS

All flies and larvae were reared under uncrowded conditions at 22°±1°C on the standard *Drosophila* food supplemented with additional yeast.

In situ Hybridization to RNA in Intact Organs

Digoxigenin labeled 5kb insert of the *p5A* [Ryseck *et al.*, 1985] clone of the *hsr-omega* gene (see Fig. 1) was used to hybridize *in situ* to *hsr-omega* transcripts in various tissues of wild type Oregon R^+ larvae and flies (see Results). The upstream region present in this probe (see Fig. 1 and below) does not include any other transcribed sequence [Ryseck *et al.*, 1987]; likewise, only one of the strands of the *hsr-omega* gene is known to transcribe [Ryseck *et al.*, 1985]. Therefore, the *p5A* probe can hybridize only to the *hsr-omega* transcripts. To facilitate tissue penetrance and hybridization [Kramer and Zipursky, 1992], the 5kb *hsr-omega* probe was digested with AluI and RsaI to generate smaller fragments which were labeled with dig-dUTP (Boehringer and Mannheim) by random priming. The probe was diluted to 40ng/ml of the hybridization buffer (50% de-ionized formamide, 5×SSC, 0.1% Tween-20, 200μg/ml yeast tRNA and 100μg/ml sonicated salmon sperm DNA) and heat denatured before use. Tissue-fixation, hybridization and detection procedures were essentially as described by Kramer and Zipursky [1992].

DNA Constructs for P-element Mediated Germline Transformation

The 5kb EcoRI genomic insert in the *p5A* clone [Ryseck *et al.*, 1985], which contains 844bp of *hsr-omega* upstream sequence and a part of the transcription unit (see Fig. 1), was digested with XhoI; the resulting 1076bp EcoRI-XhoI fragment includes 844bp upstream sequences and a part of the first exon (Fig. 1) of the *93D* locus (since the EcoRI sites on this clone are not of genomic origin, these are not counted). This fragment was partially digested with AluI and the largest (951bp) and the smallest (498bp) fragments were cloned into *pUC12* at EcoRI and SmaI sites. The inserts from these two subclones were taken out with EcoRI and PstI and recloned into EcoRI and PstI sites of the

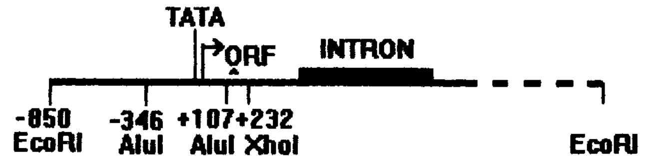

Fig. 1. Map of the 5kb genomic DNA insert in the *p5A* clone (for details see Ryseck *et al.*, 1985), showing locations of the AluI sites used for constructing the two promoter deletion variants for germ line transformation as described in text. Locations of the TATA box, transcription start point (→), the short open-reading frame (ORF, see Fini *et al.*, 1989) and the intron are indicated; the dashed lines indicate some of the 3′ 280bp tandem repeats (not to scale); the EcoRI sites at the two ends are not genomic; therefore, the genomic sequence begins at −844bp.

pBluescript vector. Finally, both the clones were digested with EcoRI and BamHI and the resulting insert fragments (951bp and 498bp genomic sequence) were put into transformation vector *pCaSpeR-AUG-beta-gal* [Thummel *et al.*, 1988] and *pCaSpeR-hs43-beta-gal* [Thummel and Pirotta, 1992], respectively. The two constructs were designated *p951lacZ* and *p498lacZ*. The 498bp insert differs from the 951bp insert in not having the region from −346bp to +107bp (Fig. 1) and thus does not include the TATA-box and transcription start point. Therefore, it was cloned into *pCaSpeR-hs43 beta-gal* vector which provides a basal promoter ahead of the *lacZ* reporter gene [Thummel and Pirotta, 1992].

Germ Line Transformation

For P-mediated transformation, preblastoderm embryos of *w; delta2-3* [Robertson *et al.*, 1988] were microinjected with the above constructs as described by Rubin and Spradling [1982]. Resulting G_0 flies were individually mated to *yw; Sco/CyO* flies (for details of gene markers, see Lindslay and Zimm, 1992] and the G_1 transformant (red eyed) flies were used for establishing transgenic lines. Since in both the cases, the initial transgenic lines were homozygous lethal, the lines were crossed with *w; delta2-3* [Robertson *et al.*, 1988], to mobilize the inserts to other regions which may not be lethal. Integrity of inserts was checked by Southern blot analysis [Lakhotia *et al.*, 1993] of genomic DNA from flies of the different lines (not shown). For *p951lacZ,* two insertion lines and for *p498lacZ,* 3 insertion lines were used. Henceforth the lines carrying the 951bp of *hsr-omega* will be referred to as *951lacZ* and those carrying the 498bp of *hsr-omega* promoter will be referred to as *498lacZ* lines. The viability of these lines and locations of the inserts, mapped by *in situ* hybridization and/or genetic crosses, are shown in Table 1. *In situ* hybridization to DNA in polytene chromosome spreads was carried out using the DIG-labeled *p5A* clone as described [Lakhotia *et al.*, 1993].

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-844 CTTGTTTTTA AATGTCTGTC ATTATCGATA AGCGTGGGTA TTTTATTTTA CAATACGGCG
-784 CATAATTGAC ATCTACAACT GTGTTCTTTA TTGGTACTAC TGGTTGACAT CGGGTTTTTA
-724 CATGTATCTT ACATTGGAAA ACTACGTACG CATTAGATAA ATGTTTGCCT TGTTTTAACT
-664 AAACTTATAT GCATTTTAAT GAAAACGATA TTTCTATAAA ATGTAACCAA ATTTGTGTGT
-604 TTTAATTTTT ATTTATTTCT TCACGACGAA ATTAATATCG ATATTCTTTC GTTAAATTCG
-544 GCATGGAAAA AGCAACCCTG TGAATCAATA AAAAAAAGCA TGCGGTCCCT CTCTCGAAAA
-484 CTGAACATTA TTTGTCTCCT TCTGGATATT TCCATTCTAC ACTAATCAAC AAGAATTGCT
-424 TTATTTTTTT CGAAAGCGAT GTACATTTGT ACATAATATA TATATTTTTT CCGCATTTGT
-364 TTATTGTAAC CACATAGCTT TAGAGCAGTA ATTAAAGCAT AAGAAAGAAT ATCGCTGAAG
-304 CACGAAATTC TTAGACATTA TATGTGTGCA TATTCAGATT TGGTTAGTAT GTGCAGTAGT
-244 TTCCAGAACA CAAGAGAAAA ATCCATATGT ATGTGCTTAA CCGGCTTACC CACCTTTCTC
-184 ACGAAATGAG GGTAGTTTTT GTAGCACAGT GATGTAGACA CTCGTGAGAA ACCGTAGGAG
-124 CACGTATACG ATGTATGTAT ATAGTGTACT CGCTCCAACC CCAATGCCGC AGACCCGAGA
 -64 GACAGGCAGA TTTTTCCCGA ACCCAGCGGT TGGGTATAAA TAGAGCCGCC TCAGTCCGGT
  -4 CACGT
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Fig. 2. Sequence of the 844bp upstream region of *hsr-omega* highlighting different motifs: the putative GAGA binding sites are shown in italics, the putative hormone receptor elements are single underlined, while the dotted underlined region depicts the TATA box. The bold T at the end is the transcription start site, as defined by Ryseck *et al.*, [1987].

TABLE 1. Chromosomal Locations of the *hsr-omega* promoter *lacZ* Fusion Transgenes in Different Transformed Lines Used in This Study

Clone	Chromosome	Position	Viability
951lacZ	Second	30EF	Homozygous lethal
951lacZ	Second	30B	Homozygous viable
498lacZ	Second	44E	Homozygous viable
498lacZ	Second	Not checked	Homozygous lethal
498lacZ	Second	Not checked	Homozygous lethal

Beta-galactosidase Staining

Different tissues from larvae (salivary glands, brain ganglia, gut, various imaginal discs) and from adult flies (gut, brain ganglia, ovaries, testes etc.) of the transgenic lines were dissected in modified Poels' salt solution [Lakhotia and Mukherjee, 1980], and processed for X-gal staining as described [Mukherjee *et al.*, 1995].

DNA Sequencing

Since only a part of the upstream sequence (up to −580bp) of the *93D* locus has been described in literature [Hovemann *et al.*, 1986; Garbe *et al.*, 1986], we sequenced the remaining upstream region upto −844bp. The region to be sequenced was subcloned into *pUC12* and was sequenced in both directions by the di-deoxy chain termination method [Sanger 1977], using the *pUC* sequencing kit (Boehringer & Mannheim) and ^{35}S-dATP as label. The complete sequence of the 844bp upstream region is shown in Fig. 2. This sequence is deposited in the EMBL nucleotide sequence database under accession number X85039.

RESULTS

Localization of *hsr-omega* Transcripts in Different Tissues

A DIG-labeled DNA probe was used to hybridize to *hsr-omega* transcripts *in situ* in intact organs from wild type larvae and adults (see Materials and Methods). Transcripts were present in all the imaginal discs of late third instar larvae examined with certain areas of each disc showing a stronger signal. Thus, in wing imaginal discs, the hybridization signal was stronger in pouch area and also in the presumptive pteropleura, mesopleura and the notum regions (Fig. 3a). The distribution of *hsr-omega* transcripts in late third instar larval eye-antennal imaginal discs as shown in Fig. 3d was also not uniform. Likewise, all the leg discs gave a strong signal in the pouch areas (not shown).

Hsr-omega transcripts were present both in the larval gut and Malpighian tubules (Fig. 3g): strong hybridization signal was observed in the proventriculus, gastric caeca, section II and III of mid gut [Masucci *et al.*, 1993], hind gut and Malpighian tubules (Fig. 3g).

Hsr-omega transcripts were present in larval brain hemispheres (specially the optic lobes) and the ventral ganglion; the prothoracic glands showed a very strong signal (Fig. 3h). In the larval salivary gland cells, a nuclear as well as cytoplasmic signal was seen (Fig. 3i). In all polytene cells (prothoracic glands, salivary glands, midgut and Malpighian tubule cells), the nucleus had a stronger signal (Fig. 3o-q) with a still more pronounced hybridization in a localized region of the nucleus.

In the ovaries of adult flies (Fig. 4a), a strong hybridization was seen in the nurse cells as reported earlier [Bendena *et al.*, 1991]. The germarium region did not show a detectable hybridization. The *hsr-omega* transcripts were also completely absent in follicle cells and the oocyte. In whole mounts, certain nurse cells appeared to give a stronger signal than others (Fig. 4a).

In the testes of adult flies, the germarium region gave a very weak signal, but in the middle part, the hybridization was very strong, followed by weaker signal in the region housing the mature sperms (Fig. 4e). Transcripts were found in all the accessory male reproductive organs (not shown) except for the seminal vesicles.

Expression of the Reporter Gene Under Different Lengths of the *hsr-omega* Promoter

For analyzing the spatial and temporal expression of *hsr-omega,* reporter gene activity was examined in a wide variety of larval and adult tissues from the transgenic lines containing either the full length 951bp (from −844 to +107) or only the distal 498bp upstream region (from −844bp to −347bp) of *hsr-omega* gene (see Materials and Methods). In each case, larval and adult tissues from more than one transgenic lines, with the fusion gene inserted into different chromosomal re-

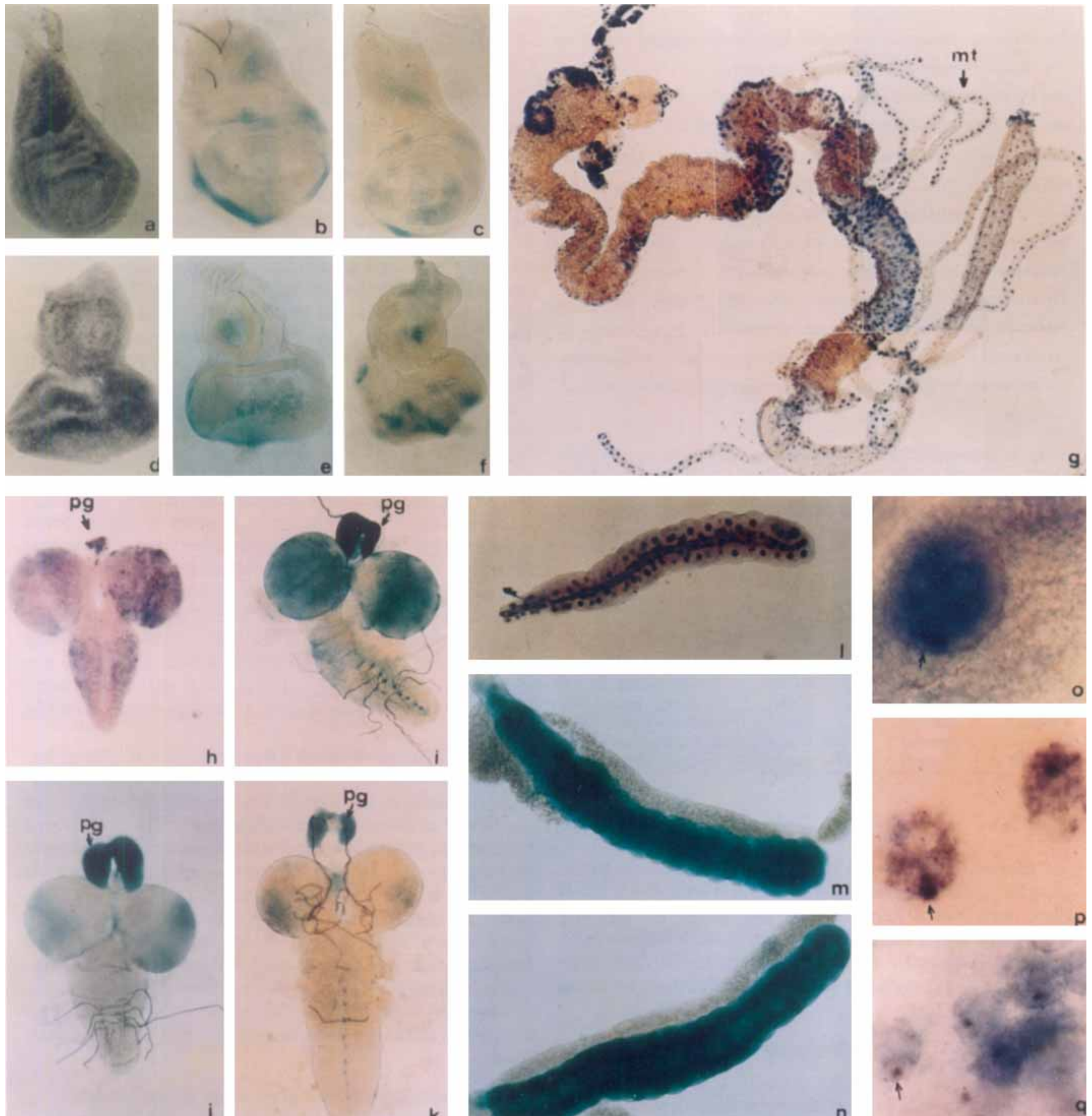

Fig. 3. Expression of the *hsr-omega* in different larval tissues as revealed by: *RISH* (RNA *in situ* hybridization) to *hsr-omega* transcripts (a,d,g,h,l,o,p and q) or by beta-gal staining of different tissues of *951lacZ* (b,e,i,k,m) and *498lacZ* (c,f,j,n,) lines. Prominent *hsr-omega* activity can be seen in the notum of wing discs (a,b,c), the presumptive ommatidia of eye antennal discs (d,e,f), optic lobes of brain ganglia of third (h,i,j) or second (k) instar larvae; note the blue stained thoracic and abdominal ganglia of the ventral ganglion in the *951lacZ* (i,k) but not in the *498lacZ* line (j). A very strong localization of *hsr-omega* transcripts is seen in all tissues with polytenised nuclei like different parts of the larval gut (g), Malpighian tubules (g), prothoracic gland (h) and salivary glands (l); polytene nuclei from larval salivary glands (o), Malpighian tubules (p) and prothoracic glands (q) are shown at higher magnification to reveal the very strong hybridization to a specific region (arrow).

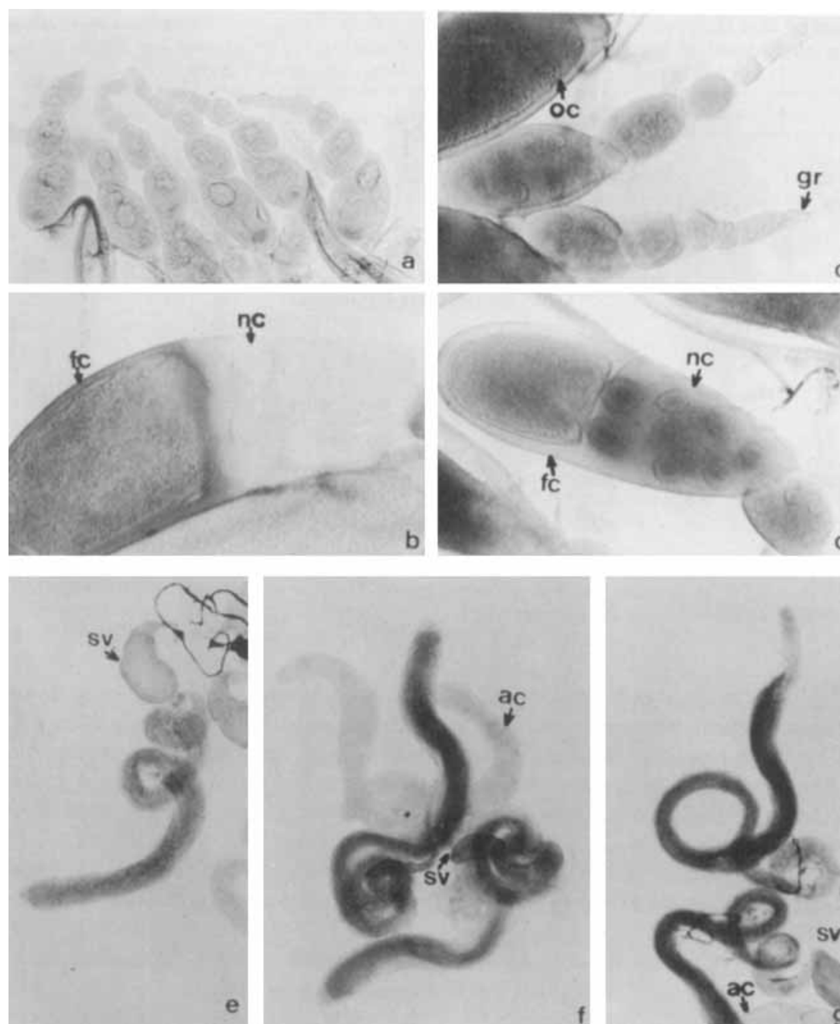

Fig. 4. *hsr-omega* expression in reproductive systems of adult flies seen through localization of *hsr-omega* transcripts by RISH (a,e) and through reporter gene activity in *951lacZ* (c,d,f) and *498lacZ* (b,g) lines in ovarioles (b,c,d) and testes (f,g). In the *951lacZ* line (c,d), a strong reporter gene activity is seen in nurse cells (nc) and oocytes (oc) but not in the germarium (gr) or follicle cells (fc) but in the *498lacZ* line only the follicle cells enclosing the oocyte are strongly stained (b). In males, a strong activity is seen in most of the testes, except the germarium; the seminal vesicles (sv) are negative in e and g but they show a strong reporter gene activity in the *951lacZ* line (f); the accessory glands (ac) are negative in all cases.

gions (Table 1), were examined and found to give similar patterns of the reporter expression (not shown). This ensured that the reporter gene activity was indeed driven by the *hsr-omega* promoter and not by other nearby enhancer.

As may be seen in Figs. 3–4, the pattern of X-gal staining in all the larval and adult tissues examined in the transgenic lines carrying either the 844bp promoter or the 498bp distal promoter was, with some exceptions (see below), similar to the pattern of *in situ* localization of *hsr-omega* transcripts in the respective tissues. The X-gal staining in all the three transgenic lines (Table 1) carrying the 498bp distal promoter was, however, generally weaker in imaginal discs (see Figs. 3c,f).

The reporter gene expression was also noted in wing blades and the optic lobes of brain ganglia of adult flies with the junction in between the retina and the lamina being more strongly stained (not shown).

Some notable differences between the patterns of *in situ* hybridization to *hsr-omega* transcripts and X-gal staining in larval brain and adult male and female reproductive systems in the two sets of transgenic lines are listed in Table 2 (see also Figs. 3–4).

Nucleotide Sequence of the Promoter Region

The complete base sequence of the 844bp upstream region of the *hsr-omega* locus (including the 580bp described earlier [Hovemann *et al.*, 1986; Garbe *et al.*, 1986] and 265bp sequence determined in the present

TABLE 2. Comparison of the Expression of *hsr-omega* in Adult Male and Female Reproductive Organs and in Larval Brain Ganglia as Revealed by RNA *In Situ* Hybridization (RISH) and by X-gal Staining in the *951lacZ* and *498lacZ* Germline Transformed Lines

	Third Instar Larval Brain Ganglia					
	Prothoracic glands	Brain hemispheres	Ventral ganglion	Thoracic & Abdominal ganglia		
RISH	+ + +	+ +	+ +	−		
951lacZ	+ + +	+ +	+ +	+ + +		
498lacZ	+ + +	+	+	−		
	Male Reproductive Organs					
	Germarium	Mid	Distal	Seminal vesicle	Accessory gland	Ejaculatory duct
RISH	+	+ + +	+	−	−	+ +
951lacZ	+	+ + +	+	+ +	−	+
498lacZ	+	+ + +	−	−	−	−
	Ovarioles					
	Germarium	Nurse cells	Oocyte	Follicle cells		
RISH	−	+ + +	−	−		
951lacZ	−	+ + +	+	−		
498lacZ	−	−	+	+ + +		

(− = no expression; ± = weak, + = moderate, + + = strong and + + + = very strong expression)

study) is shown in Fig. 2. An analysis of the −844 promoter sequence revealed two sequence motifs at −740bp (5′TGACAT3′) and at −483bp (5′TGAACA3′) which closely match the consensus ecdysone receptor element half site (5′TGAC/ACPy3′) identified from a comparison of the promoter regions of *hsp27* and *Eip28/29* genes of *D. melanogaster* [Cherbas *et al.*, 1991]. Two putative *GAGA* factor binding motifs are seen at −68bp and −496bp.

DISCUSSION

hsr-omega is Widely Expressed in Different Cell Types

Our present study using whole organ *in situ* hybridization to cellular *hsr-omega* transcripts and reporter gene assay in different intact larval and adult organs complements and supplements the earlier study of Bendena *et al.* [1991] on developmental expression of this gene using northern hybridization or *in situ* hybridization to transcripts in histological sections. The stronger nuclear localization of *hsr-omega* transcripts agrees with the fact that both the primary transcripts, viz., the large (>10kb) *hsr-omega-n* and the 1.9kb *hsr-omega-pre-c* [Hogan *et al.*, 1994], are exclusively intranuclear with only the 1.2kb spliced product, the *hsr-omega-c*, being transported to the cytoplasm; the spliced out intron is also known to accumulate at the site of synthesis [Lakhotia and Sharma, 1995a]. The stronger hybridization signal in a small region of all polytene nuclei in different cell types corresponds to the location of this gene since *in situ* hybridization to transcripts on polytene chromosome spreads has shown a high level of transcript accumulation at the *93D* site [Lakhotia and Sharma, 1995a].

Compared to diploid cell types, *hsr-omega* transcripts were more abundant in all endoreplicating tissues like larval salivary glands, prothoracic gland, midgut, Malpighian tubules, nurse cells, etc. It is notable that kinetics of response of the *hsr-omega* to various inducers is also different in the large endoreplicated and smaller diploid cell types. Thus while in the larval salivary gland polytene nuclei benzamide induces the *hsr-omega* within minutes [Lakhotia and Mukherjee, 1980], in cultured diploid cells a much longer treatment is required for the induced accumulation of its transcripts [Bendena *et al.*, 1989]. Likewise in response to ecdysone release, the *93D* locus in larval salivary glands puffs out before the *67B* locus [Ashburner, 1972] which houses the genes for smaller hsps [Corces *et al.*, 1980], whereas in ecdysone treated diploid cell lines the transcripts for smaller hsps are detected before *hsr-omega* transcripts [Bendena *et al.*, 1991]. Such variations in the relative amounts and kinetics of induction of *hsr-omega* transcripts in polytenised versus non-polytenised tissues may suggest that the large polytenised cells have a differential requirement of these non-coding transcripts and that their metabolism is dependent on the architecture of cell [see Lakhotia and Sharma, 1995b].

The *hsr-omega* is expressed in almost all the larval and adult tissues that were examined. However, in each case, the pattern of expression is spatially specific since certain areas in a given tissue either do not express or express at varying levels. The regulated expression of the *hsr-omega* locus in widely different tissues of *Drosophila* agrees with this locus being essential for the viability of flies [Mohler and Pardue, 1982] in spite of the fact that it does not code for any protein [Lakhotia and Mukherjee, 1982; Fini *et al.*,

1989]. The presence of *hsr-omega* transcripts in all the imaginal discs of late third instar stage is interesting in the context of an earlier observation [see Lakhotia, 1987] that imaginal discs from larvae homozygously deficient for the *hsr-omega* locus fail to respond to ecdysone. Apparently, the *hsr-omega* gene has important functions in the cascade of differentiation events triggered by the moulting hormone. This is further supported by recent observations in our laboratory [T.K. Rajendra and Lakhotia, unpublished] that the few *hsr-omega* deficient escapee flies that survive, have a variety of pattern abnormalities.

A very high level of expression of *hsr-omega* in prothoracic gland, the site of ecdysone synthesis [Hadorn, 1937], is significant. It is notable that benzamide, a strong inducer of *hsr-omega* gene activity [Lakhotia and Mukherjee, 1980, Bendena *et al.*, 1989], also affects RNA metabolism in the prothoracic gland and delays pupation [Wahab and Sirlin, 1959]. Further analysis of the role of these transcripts in prothoracic gland will be rewarding.

The patterns of *in situ* hybridization to cellular transcripts and the reporter gene expression suggest important functions for the *hsr-omega* locus in spermatogenesis as well as oogenesis since in both cases, this gene was significantly more active during the developing stages (late primary spermatocytes to maturing sperms in males and the nurse cells in females). Earlier studies in our laboratory showed an effect of *hsr-omega* transcript on transport and/or turnover of hsp70 and alpha-beta transcripts from their sites of synthesis in heat shocked cells [Sharma and Lakhotia, 1995]. In view of this, it seems attractive to speculate that the abundant presence of the non-coding *hsr-omega* transcripts during gametogenesis and in many other non-stressed cells may be related to these RNAs having roles in transport and turnover of other cellular transcripts as well [see also Lakhotia and Sharma, 1995b].

Cis-acting Regulators of Developmental Activity of the *hsr-omega* Locus

Combination of whole organ *in situ* hybridization with reporter gene assay using promoter deletion variants has allowed us to identify putative regulatory upstream regions that may control the spatial and temporal pattern of expression of the *hsr-omega* gene in different tissue types. The germ line transformed lines carrying transgene with the 951bp of *hsr-omega* (844bp upstream and 107bp 5′ transcribed) sequence fused to the *lacZ* reporter gene showed beta-galactosidase expression almost identical to the expression of the resident *hsr-omega* gene. A major difference was with respect to the oocytes: while the *hsr-omega* transcripts were absent in oocytes, the 844bp transgenic line showed beta-galactosidase activity in the oocyte. This difference, however, seems to be related to possible differences in kinetics of turnover of the reporter gene product and the *hsr-omega* transcripts: the *lacZ* mRNA or the beta-galactosidase may be transported to the oocyte from the nurse cells in transgenic lines while the *hsr-omega* transcripts seem to either lack the signals necessary (Ding and Lipshitz, 1993] for translocation or may have specific signals that prevent its movement to oocyte.

In view of the general similarity of the reporter gene activity, most of the cis-acting sequences that regulate the developmental expression of *hsr-omega* gene in different larval and adult tissues should reside within the −844bp promoter region. The other transformed lines lacked the region from −346bp to +107bp of the *hsr-omega,* yet expressed the reporter gene in parallel to that in the lines carrying 951bp (from −844bp to +107bp) of *hsr-omega* except for a weaker expression in all imaginal discs and for certain other specific differences noted in Table 2. This suggests that most of the cis-acting elements controlling developmental expression of the *hsr-omega* gene are within the −346bp to −844bp interval. The proximal upstream region (from −346bp to transcription start point), however, appears necessary for a normal expression of the *hsr-omega* gene in ovarioles: this region perhaps carries a negative regulator for follicle cell expression and a positive regulator for nurse cell expression so that the absence of this region in the *498lacZ* line results in an ectopic expression of the reporter gene in follicle cells and its absence in the nurse cells. This can be more directly confirmed by examining the reporter gene expression in a construct carrying the −346 to +107bp region.

The reporter gene activity in the various accessory reproductive organs of adult males in the two promoter-deletion variant transgenic lines was not in agreement with the normal distribution of the *hsr-omega* transcripts (Table 2). Such variations indicate that additional regulatory sites, not covered by the 844bp promoter region analyzed in this study, are also involved. The absence of reporter gene activity in the thoracic and abdominal ganglia in late third instar larvae of the *498lacZ* lines resembles the patterns of *hsr-omega* expression as seen by *in situ* hybridization. Presence of reporter gene activity in these ganglia in the 844bp lines was, therefore, unexpected and needs further analysis. Apparently, the developmental regulation of the *hsr-omega* gene is under multiple regulatory elements having distinct tissue-specific roles. Similar tissue specific regulatory sequences are already known for some of the smaller heat shock proteins of *Drosophila* [Glaser and Lis, 1990; Cohen and Meselson, 1985].

Sequence analysis of the upstream region revealed the presence of GAGA-factor binding CT or GA repeats [Biggin and Tjian, 1988] at −495bp and at −68bp within the −844bp upstream sequence. Since the consensus GAGA-factor binding site has three GA or CT repeats (Granok *et al.*, 1995), we believe that the sites at −495 and at −68bp may function as GAGA-factor binding sites. Tsukiyama *et al.* [1994] have recently

reported the distribution of GAGA-factor on polytene chromosomes of *Drosophila:* although the authors have not specifically mentioned, an examination of the illustrations in that paper suggests that the GAGA-factor indeed binds at the *93D* site. Presence of the GAGA-factor at CT repeats is well known to facilitate efficient binding of other trans-acting factors [Lu *et al.*, 1992, 1993; Granok *et al.*, 1995]. These CT-repeats may therefore, be involved in the widespread expression of this gene as also in its ready inducibility with a variety of agents [Lakhotia, 1989]. The promoter region (from −346bp to −844bp) in the *498lacZ* line includes only the distal CT repeats and this may be responsible for the weaker reporter gene activity in this line.

Since like other small heat shock genes, the *hsr-omega* is also induced by ecdysone, we examined the promoter sequences for ecdysone receptor element (EcRE) consensus sequence [Cherbas *et al.*, 1991]: sequence motifs bearing close homology to the consensus ecdysone receptor half element as defined by Cherbas *et al.*, [1991] were seen at −740 and at −483bp. A definitive functional role of these regions can, however, be ascertained only after a more direct analysis. A comparison of the *hsr-omega* promoter sequence of *D.melanogaster* with that of the sequence available for *D.hydei* [Ryseck *et al.*, 1987] revealed conservation at a few places like the TATA box and the proximal GAGA binding site at −68bp. We could not check for putative EcREs as only 363bp of *D.hydei* 5′ sequence of *hsr-omega* promoter sequence is available in literature.

The wide spread developmental expression of this gene together with lethality and abnormal differentiation in escapee *hsr-omega* deficient flies emphasize important functions of the *hsr-omega* gene. In the absence of a translational product [Lakhotia and Mukherjee, 1982; Fini *et al.*, 1989], the *hsr-omega* transcripts may regulate transport and/or turnover of other transcripts [Lakhotia and Sharma, 1995b], possibly by participating in the formation of a local macromolecular assembly [Ding and Lipshitz, 1993]; at least some of the *hsr-omega* transcripts are known to be present as very large sized RNP particles [Dangli *et al.*, 1983]. A few other genes like the *H19* [Wrana, 1994] and the *Xist* genes of mammals [Goldman, 1992], and the fertility genes on Y chromosome of *Drosophila* [Hennig *et al.*, 1989], like the *hsr-omega*, also do not have typical protein products. Function of these genes, all of which seem to participate in local macromolecular assemblies, may be dependent on the secondary structure of RNA rather than the base sequence. Further studies on the molecular mechanism of such genes which function through RNA will provide exciting insights into novel cellular mechanisms and the exact role played in development by the *hsr-omega* gene.

ACKNOWLEDGMENTS

This work was supported by a grant from the Department of Science & Technology, Govt. of India, New Delhi to SCL. MM was supported by a research fellowship of the Council of Scientific & Industrial Research, New Delhi. We thank Dr. B. Hovemann for providing the *p5A* clone and Prof. C. Thummel for the *pCaSpeR* vectors.

NOTE ADDED IN PROOF

We have recently examined the reporter gene activity in ovaries from the rare surviving *951lacZ2; Df(3R)e^{GP4}/Df(3R)GC14* flies which lack both copies of the resident *93D* locus due to the overlapping deletion of the 93D6-7 band [Mohler and Pardue, 1982]. The ovarioles in these flies were not normally differentiated since the egg chambers contained a more or less disorganized mass of cells without any differentiation of the nurse cells or the oocyte. The smaller ovarioles were devoid of the *lacZ* reporter gene activity while in the more enlarged ovarioles, all cells, except the outermost layer of cells (presumptive follicle cells) particularly on the distal end, showed a uniform β-galactosidase activity. The disruption of normal ovarian organization in these *93D*-deficient flies further substantiates important roles of the *hsr-omega* transcripts in ovarian development.

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