22.8.2 The 93D heat shock locus of *Drosophila melanogaster*: modulation by genetic and developmental factors

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The 93D locus in *Drosophila melanogaster* and the 93D-like loci in other species of *Drosophila*, collectively termed *hsr* ω (heat shock RNA omega) locus, display several unique and intriguing features: (*i*) developmental regulation and selective induction by several agents like benzamide, colchicine, thiamphenicol, vit-B_6; (*ii*) functional conservation in the genus but a very rapid DNA base sequence divergence; (*iii*) in spite of the rapid DNA sequence divergence, a strong conservation of organization (a 5′ unique region and a 3′ long tandem repeat region) and the pattern of multiple ω transcripts in the genus; (*iv*) a general nontranslatability of all the three major species of ω transcripts (an ~10-kb ω*1*, a 2.0-kb ω*2*, and a 1.2-kb ω*3* species) although some recent evidence favours translatability of a small open reading frame (~23–27 amino acid long) in the ω*3* transcript; (*v*) dispensability of the *hsr* ω locus for heat shock protein synthesis but indispensability for viability of flies. The heat shock inducibility of the 93D locus of *D. melanogaster* is selectively repressed by (*i*) combination of heat shock with another inducer of 93D; (*ii*) rearing of larvae at 10°C; (*iii*) heterozygous deficiency for the 93D region; and (*iv*) conditions that alter levels of beta-alanine. In all cases of repression of the 93D locus during heat shock, the 87A and 87C loci (the two duplicate loci harbouring multiple copies for hsp70 and the alpha–beta repeat sequences (at 87C)) develop unequal puffs. The *hsr* ω locus appears to be under a complex system of regulation involving autoregulation as well as regulation by other factors in the cell which possibly operate through different control elements on the locus.

Key words: benzamide, colchicine, beta-alanine, *hsr* ω, heat shock puffs, *Drosophila*.

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Le locus 93D chez *Drosophila melanogaster* et les locus qui ressemblent au 93D chez d'autres espèces de *Drosophila*, collectivement désignés *hsr* ω (heat shock RNA omega), présentent plusieurs caractères exclusifs qui suscitent la curiosité : (*i*) la régulation du développement et l'induction sélective par divers agents comme le benzamide, la colchicine, le thiamphénicol, la vitamine B_6; (*ii*) la conservation fonctionnelle au sein du genre, mais une divergence très rapide des séquences de bases de l'ADN; (*iii*) malgré une divergence rapide des séquences de l'ADN, une forte conservation de l'organisation (une région unique 5′ et une région 3′, longue, répétée en tandem), ainsi qu'un pattern de transcrits ω multiple au sein du genre; (*iv*) l'incapacité générale de traduire les trois espèces majeures de transcrits ω (une espèce d'environ 10-kb ω*1*, une seconde de 2.0-kb ω2 et une troisième de 1.2-kb ω3), bien que certaines évidences récentes favorisent la capacité de traduire un petit cadre de lecture ouvert (d'environ 23–27 acides aminés de longueur) dans le transcrit ω3; (*v*) la non-nécessité pour le locus *hsr* ω de synthétiser des protéines de thermochocs, mais la nécessité de les synthétiser pour la viabilité des mouches. L'induction par thermochocs du locus 93D de *D. melanogaster* est réprimée de façon sélective par (*i*) une combinaison des thermochocs avec un autre inducteur du 93D; (*ii*) l'élevage des larves à 10°C; (*iii*) une déficience hétérozygote pour la région 93D et (*iv*) des conditions qui altèrent les niveaux de bêta-alanine. Dans tous les cas de répression du locus 93D au cours des thermochocs, les locus 87A et 87C (les deux locus dupliqués porteurs de copies multiples pour la hsp70 et les séquences itératives alpha–bêta à 87°C) développent des gonflements inégaux. Le locus *hsr* ω semble relever d'un système complexe de régulation, impliquant l'autorégulation aussi bien que la régulation par d'autres facteurs dans une cellule, qui opère possiblement par différents éléments de contrôle sur ce locus.

Mots clés : benzamidine, bêta-alanine, *hsr* ω, gonflements de thermochocs, *Drosophila*.

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Introduction

The heat shock genes are a set of genes which have been found in every organism looked for and which are believed to be the cells' primary defence against a variety of stressful conditions. During the last decade, the structure and function of these generally well conserved genes have been analysed at the molecular level in diverse prokaryotes and eukaryotes (for recent reviews, see Lindquist, 1986; Schlesinger 1986; Bond and Schlesinger 1987; Bienz and Pelham 1987). The translational products of these genes, the heat shock proteins or polypeptides (hsps) are believed to provide protection to the cell against adverse effects of the stress in an as yet poorly understood manner and to also have an important role in normal development and activities of cells. In this context, the 93D heat shock locus of *Drosophila melanogaster* and its homologues in other species of *Drosophila* stand uniquely apart from the other heat shock loci in their pattern of expression, their structural organization, evolution, and possible mode of action. The uniqueness of this locus is also reflected in the fact that although transcriptionally it is one of the major heat shock loci in *Drosophila*, it has seldom received any consideration in most of the recent reviews on heat shock genes.

All species of *Drosophila* examined to date have a heat shock gene which is homologous to the 93D locus (on polytene band 93D6-7 of 3R) of *D. melanogaster*. In the absence of any protein product (see later), these loci in different species are named according to the designation of the polytene chromosome band(s) where they are localised: thus in *D. hydei* it is called 2-48C, in *D. virilis* 2-20CD, in *D. pseudoobscura* 2-58C, in *D. ananassae* 2L-2C, in *D. kikkawai* E-11E, in *D. nasuta* 2R-48A, and so on (see Lakhotia 1987). To be able to commonly address these differently named homologous loci, they have recently (Bendena et al. 1989*a*) been designated *hsr* ω (heat shock RNA omega). This term will be used here to refer collectively to the 93D locus of *D. melanogaster* or the 93D-like loci in other species. However, in specific

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cases, the individual locus name will be used.

Unique inducibility of *hsr* ω genes

The specific inducibility of the 93D heat shock locus of *D. melanogaster* by benzamide (Lakhotia and Mukherjee 1970) and of the 2-48C of *D. hydei* by vit-B_6 (Leenders et al. 1973) were among the earliest examples of singular induction of a member of the set of heat shock genes, which otherwise would be induced more like a battery of genes. During a routine heat shock also, the 93D puff was shown to behave differently from other heat shock loci (Mukherjee and Lakhotia 1979). Subsequently a variety of other treatment conditions have been reported to either selectively induce the *hsr* ω loci without simultaneously affecting other heat shock loci or to induce all heat shock loci except the *hsr* ω (reviewed by Lakhotia 1987). These observations suggested that the *hsr* ω loci are regulated differently from the other heat shock genes. Besides benzamide, the other significant selective inducers of *hsr* ω are colchicine (or colcemid), vit-B_6, and thiamphenicol. Indeed it was the common inducibility of a specific heat shock puff in each species of *Drosophila* by one or several of these agents that provided the first evidence for their evolutional homology (Lakhotia and Singh 1982). Since vit-B_6 did not induce any puff in *D. melanogaster* and since labelled RNA from vit-B_6 treated *D. hydei* cells did not hybridise *in situ* to any locus of *D. melanogaster*, it was at one time (Peters et al. 1980) concluded that a homolog of 2-48C of *D. hydei* does not exist in *D. melanogaster*. However, puffing studies with other inducers (Lakhotia and Singh 1982) revealed 93D of *D. melanogaster* to be functionally homologous to 2-48C of *D. hydei*. The lack of cross-hybridisation of RNA noted by Peters et al. (1980) between these species was due to another unique feature of the *hsr* ω loci: their DNA base sequences have diverged rather unusually rapidly.

Rapid divergence of DNA base sequence but strong conservation of organization of *hsr* ω in *Drosophila*

Molecular cloning and analysis of DNA base sequences of the *hsr* ω in species like *D. melanogaster*, *D. hydei*, and *D. pseudoobscura* (Peters et al. 1984; Walldorf et al. 1984; Garbe and Pardue 1986; Garbe et al. 1986; Ryseck et al. 1987; Bendena et al. 1989*a*, 1989*b*; M. L. Pardue, personal communication) revealed an unusually rapid divergence of the DNA base sequence but a very strong conservation of the organization of the locus. The rapid sequence divergence at this heat shock locus is specially remarkable in the context of the strong conservation known for other heat shock loci in diverse organisms extending from bacteria to higher eukaryotes (see Lindquist 1986). In all species of *Drosophila* examined so far, the *hsr* ω locus contains a 5′ unique region followed on the 3′ end, after a "spacer," by a tandem repeat of a short sequence extending over a stretch of several kilobases. In each species, the 5′ unique region contains two exons and an intron (~700 base pairs (bp)) of comparable lengths. In spite of this highly conserved organization of *hsr* ω in these species, the primary base sequence is remarkably different except for certain small stretches of strong conservation (Garbe et al. 1986; Bendena et al. 1989*a*, 1989*b*; Ryseck et al. 1987). Notable among these conserved regions are (*i*) 59 bp of perfect homology beginning 39 bp from the 3′ end of the intron between *D. melanogaster* and *D. hydei* (between *D. melanogaster* and *D. pseudoobscura*, the homology extends to 61 bp); (*ii*) the nucleotides at −1, +1, +7, +12, and +15 positions in all *hsr* ω loci are the same as in other heat shock loci of *Drosophila* (Hultmark et al. 1986); (*iii*) 14 bp around the junction of exon 1 with the intron; (*iv*) 14 bp at the polyadenylation site after the exon 2; and (*v*) a 9-bp motif in the short tandem repeats on the 3′ end; it is interesting that although the unit lengths of *hsr* ω repeats vary between species (115 bp in *D. hydei* and 280 bp in *D. melanogaster*), the 9-bp motif occurs at more or less comparable locations in the overall repeat region (Garbe et al. 1986). The sequence conservation around the exon–intron junctions in *hsr* ω is apparently not for splicing of the heat shock RNA per se, since the only other heat shock RNA (the hsp83 RNA) known to require splicing in *Drosophila* does not share any of these conserved sequences (Blackman and Meselson 1986; Garbe et al. 1986).

Inducer-specific pattern of ω transcripts with limited coding function

The *hsr* ω loci in all species produce three major populations of transcripts: (*i*) *ω1* species which is ~9–11 kb long and contains the 5′ unique as well as the 3′ repeat regions; (*ii*) *ω2* species, ~2 kb long and comprising the 5′ unique region (exons 1 and 2 with intron); and (*iii*) *ω3* species, the spliced product of *ω2* (Ryseck et al. 1985, 1987; Hovemann et al. 1986; Garbe and Pardue 1986; Garbe et al. 1986; Bendena et al. 1989*a*, 1989*b*). The *ω1* and *ω2* transcripts are intranuclear, whereas the *ω3* transcripts are cytoplasmic and sediment with monosome–disome peaks in polysome gradients (Bendena et al. 1989*a*). It appears unlikely that the *ω1* transcripts are precursor to the other two, although all of them start from the same point (Garbe et al. 1986; Ryseck et al. 1987).

It is remarkable that different inducers of the 93D locus affect the cellular levels of the three species of ω RNA differently. Heat shock elevates the levels of all ω transcripts; colchicine and benzamide cause a large increase of *ω1* transcripts; in addition, colchicine also elevates to a smaller extent the *ω2* levels (Bendena et al. 1989*b*). In contrast, cycloheximide and other inhibitors of protein synthesis specifically elevate *ω3* levels in treated cells (Bendena et al. 1989*a*, 1989*b*). Since these inhibitors of protein synthesis do not induce puffing at 93D and since prior treatment with actinomycin D does not affect the increase in *ω3* levels caused by cycloheximide, it is apparent that the protein synthesis inhibitors stabilise the *ω3* RNA which otherwise turns over very rapidly (Bendena et al. 1989*a*, 1989*b*). Thus the cellular levels of the three major ω transcripts can be independently regulated by controlling their rates of synthesis, processing, and (or) turnover.

Earlier studies on the absence of synthesis of a new polypeptide in relation to specifically induced high transcriptional activity of the 93D puff in *D. melanogaster* led Lakhotia and Mukherjee (1982) to suggest that this locus does not have a coding function. A similar conclusion was reached by Mohler and Pardue (1982, 1984) on not finding an effect of 93D deficiency on heat shock protein synthesis. This conclusion, based on indirect evidences, has in general been confirmed by the recent data on DNA sequences of *hsr* ω loci: none of the *hsr* ω sequences show any appreciable lengths of open reading frames and thus these loci cannot have the usual coding functions (Ryseck et al. 1985, 1987; Hovemann et al. 1986; Garbe et al. 1986). However, evidence has been obtained recently

(Bendena et al. 1989*a*) to suggest that one of the short open reading frames (the *ORF* ω) in the ω*3* transcript, beginning near position +120, is translatable and is possibly translated: the first four amino acids (not nucleotide sequence) in this ORF are conserved in *D. melanogaster, D. hydei*, and *D. pseudoobscura* but the rest are not, although the length of this ORF is comparable in three species (27 amino acids in *D. melanogaster*, 23 in *D. hydei*, and 24 in D. pseudoobscura); stable transformation of cultured cells of *Drosophila* with recombinant DNA containing the CAT sequence fused at various locations with the ω*3* sequence revealed that when the CAT sequence is fused in frame to the *ORF* ω, the CAT-fusion protein is found abundantly but when the CAT is separated from the *ORF* ω by 30 bp, the CAT protein is not detectable (Bendena et al. 1989*a*). These results thus suggest that the *ORF* ω is translated in cells and that its translation inhibits any more translation downstream.

hsr ω locus is developmentally regulated and is very sensitive to altered cell physiology

The *hsr* ω loci in all species of *Drosophila* form developmentally regulated puffs during the post-ecdysone, late third instar stage (Ashburner 1970; Lakhotia and Singh 1982; Burma and Lakhotia 1985; Lakhotia 1987). Levels of *hsr* ω transcripts at different embryonic and larval stages are also developmentally regulated (Ryseck et al. 1985; Bendena et al. 1989*a*): early embryos show a low level of ω*3* but very little of ω*1* and ω*2* while middle and late embryos have increased levels, mainly of ω*1* and ω*3* species; likewise during larval development, second instar stages have low levels of ω transcripts while third instar and pupal stages show much higher levels of all the three transcript species. Imaginal disks from *D. melanogaster* larvae lacking both copies of the *hsr* ω locus owing to overlapping deficiencies in the 93D6-7 region fail to respond to ecdysone-induced *in vitro* evagination and differentiation (Lakhotia 1987). Moreover, these larvae also suffer a high rate of mortality at the pupal stage and the few that manage to emerge are very weak and die shortly after (Mohler and Pardue 1982; P. Burma and S.C. Lakhotia, unpublished observations). These observations suggest a role for ecdysone in regulation of the *hsr* ω locus. In agreement with this, it was found that treatment of cultured cells of *D. melanogaster* with ecdysterone (10^{-6} M) caused a slow induction of *hsr* ω transcripts which continued for at least 24 h (Bendena et al. 1989*a*, 1989*b*).

In view of the very rapid and specific induction of high transcriptional activity at the *hsr* ω locus by a variety of apparently unrelated agents like benzamide, colchicine, thiamphenicol, vit-B_6 (Lakhotia 1987), it appears that besides being developmentally regulated, this locus also sensitively monitors diverse alterations in the cell environment. The sensitivity of regulation of *hsr* ω is also manifest in the rapid regression of this locus in the continued presence of the same inducer, e.g., treatment of larval salivary glands with benzamide for longer than 20 min leads to its partial inhibition and similarly during heat shock the *hsr* ω puff is the first to regress.

In striking contrast to the ready inducibility of the *hsr* ω locus by diverse inducers, there are certain conditions that make this locus selectively refractory to induction by heat shock. The conditions that selectively repress the *hsr* ω in response to heat shock can be grouped into the following three categories: (*i*) heat shock in combination with another specific (like benzamide or colchicine) or general inducer of *hsr* ω (like recovery from anoxia, for review see Lakhotia, 1987); (*ii*) certain genotypes; and (*iii*) certain conditions of development of wild type larvae.

The heat shock induced selective repression of *hsr* ω in certain genotypes of *D. melanogaster* or under certain conditions of development of wild type larvae is intriguing. We had earlier shown that *D. melanogaster* larvae heterozygously deficient for 93D6-7 region failed to show the heat shock induced puff at the remaining single copy of 93D (Burma and Lakhotia 1986). Rearing of wild type larvae of *D. melanogaster* at 10°C also rendered the 93D locus selectively refractory to heat shock induction (Lakhotia and Singh 1985). Recent studies in our laboratory have revealed an interesting effect of the unusual amino acid, beta-alanine, on heat shock puffing of the 93D locus (S. C. Lakhotia, D. Kar Chowdhuri, and P. Burma, in preparation). Among the several mutations that affect beta-alanine levels in *D. melanogaster* (see Wright 1987 for review), *ebony* (*e*, very close to the *hsr* ω, Mohler and Pardue 1984; Caizzi et al. 1987) and *black* (*b*, on second chromosome) share the same phenotype, i.e., light pupae and dark adults, but have contrasting levels of beta-alanine in their haemolymph: *e* individuals have higher levels because of non-utilisation of beta-alanine for sclerotization, whereas *b* have little of it because of defects in synthesis of beta-alanine (Wright 1987). Our studies, details of which will be published elsewhere (S. C. Lakhotia, D. Kar Chowdhuri, and P. Burma, in preparation), show that *D. melanogaster* larvae carrying mutant alleles either at the *e* or *b* locus fail to show heat shock induced puffing at the 93D locus (see Table 1). As the summary of our observations on heat shock inducibility of the 93D puff (monitored in [^{3}H]uridine transcription autoradiograms) in the different genotypes listed in Table 1 shows, in every genotype that had a mutant allele(s) at the *e* or *b* locus, the 93D puff instead of being induced was actually repressed by heat shock. In addition, it was also seen that the noninducible phenotype of the 93D puff cosegregated with the *e* mutant allele. Since mutations at both *e* or *b* loci affect beta-alanine metabolism (see above), we also tested the effect of beta-alanine on heat shock puffing in salivary glands of wild type larvae. When salivary glands from wild type larvae were heat shocked either following 1 h incubation in beta-alanine medium (100 mM) or in the presence of beta-alanine, the 93D puff was repressed; similarly, rearing of wild type larvae since the first instar on food supplemented with beta-alanine (100 mM) or feeding third instar wild type larvae on food containing beta-alanine (500 mM) for 18 h rendered the 93D locus to be selectively uninducible by heat shock. The repression of heat shock puffing by beta-alanine in salivary glands of wild type larvae confirms that the similar puffing phenotype in *e/e* (or *e/+*) and *b/b* larvae too is related to the altered metabolism of beta-alanine in these genotypes.

Our above finding (S. C. Lakhotia, D. Kar Chowdhuri, and P. Burma, in preparation) of the effect of beta-alanine on *hsr* ω puffing is very promising from the viewpoint of understanding the regulation and role of *hsr* ω in cell metabolism, since, unlike most of the other specific chemical inducers, beta-alanine is a normal metabolite in the cell and affects several metabolic pathways like phosphorylation of glucose, degradation of phenylalanine, tyrosine, uracil, aspartate, sclerotization of the cuticle, and possibly several others (Jacobs 1968, 1970; Wright 1987). It is intriguing that beta-alanine has a similar effect on 93D puffing whether its level

TABLE 1. Summary of heat inducibility of 93D, 87A, and 87C puffs in different genotypes

Genotype	Treatment	No. of mutant alleles		Heat shock puff induction	
		e	*b*	93D	87A/87C ratio
+/+ (Oregon R)	TS	0	0	Induced	1.0
T(1;3)e^{H2}/+	TS	0	0	Induced	1.0
In(3R)C, 1(3)a/+	TS	0	0	Induced	1.0
In(3R)C, 1(3)a/red	TS	0	0	Induced	1.0
In(3R)C, Sb e 1(3)e/e	TS	2	0	Repressed	>1.0
In(3R)C, Sb e 1(3)e/e^s	TS	2	0	Repressed	>1.0
e/e	TS	2	0	Repressed	>1.0
e^s/e^s	TS	2	0	Repressed	>1.0
e/+	TS	1	0	Repressed	>1.0
e^s/+	TS	1	0	Repressed	>1.0
In(3R)C, Sb e 1(3)e/ In(3R)C, 1(3)a	TS	1	0	Repressed	>1.0
In(3R)C, 1(3)a/e^s	TS	1	0	Repressed	>1.0
red/red e	TS	1	0	Repressed	>1.0
b/b	TS	0	2	Repressed	>1.0
In(3R)C Sb e 1(3) e/+	TS	1	0	Repressed	<1.0
+/+	Larvae fed beta-Ala > TS[a]	0	0	Repressed	>1.0
+/+	*In vitro* beta-Ala > TS[b]	0	0	Repressed	>1.0
+/+	*In vitro* beta-Ala + TS[c]	0	0	Repressed	~1.0

NOTE: For details of genetic markers, etc., see Lindsley and Grell (1968). Data in table are summarized from S. C. Lakhotia, D. Kar Chowdhuri, and P. Burma, in preparation.

[a]Larvae fed on food supplemented with beta-alanine (100 mM for entire larval life or 500 mM for the last 18 h of third instar stage) and then subjected to heat shock (TS).

[b]Salivary glands from normally grown larvae incubated first in beta-alanine (100 mM) containing medium for 1 h and then subjected to TS for 30 min.

[c]Salivary glands from normally grown larvae heat shocked in presence of beta-alanine (100 mM).

is lower (as in *black* larvae) or higher (as in *ebony* larvae or in *+/+* larvae provided with exogenous beta-alanine) than the normal. This may suggest that instead of acting directly, beta-alanine levels influence some other event in the cell which in turn modulates the 93D response. In view of the wide spectrum of inducers already known for the *hsr* ω locus, it is likely that a further search would reveal other genetic or developmental perturbations that affect this locus.

Multiple regulation of *hsr* ω

The selective inducibility of *hsr* ω by several chemical agents and its selective noninducibility by heat shock under certain conditions mentioned earlier show that this locus is subject to multiple controls at the level of transcription itself. The simplest control mechanism to explain its regression when the inducer is present for a longer period would be autoregulation, possibly a negative regulation, so that accumulation of ω transcripts beyond a threshold level leads to inhibition of further transcription. The inducers of *hsr* ω presumably exert a positive regulation by promoting active transcription. The induction of different ω transcripts in varying proportions by the different inducers (see above) suggests that there is more than one site (enhancers?) that can modulate the movement of RNA polymerase II on this locus. All heat shock genes have been found to carry a heat shock control element (HSE) consensus sequence (CT-GAA--TTC-AG), within the first 400 bp upstream of the gene (Bienz and Pelham 1987). In view of the independent regulation and variable inducibility of the 93D locus during heat shock, it is not surprising that the putative HSE of the 93D locus, positioned at about 300 bp upstream of the start point, has only a weak homology (CACGAATTCTTAGA) to the typical HSE (Hovemann et al. 1986). The other inducers of *hsr* ω (colchicine, benzamide) do not appear to operate through this putative HSE, since first, in different genotypes where heat shock fails to induce the 93D locus, it remains inducible with benzamide, colchicine, and second, benzamide, colchicine do not affect other heat shock loci at 24°C. At present it is not known whether the different inducers act directly on these putative regulatory elements or act via other special effectors in the cell which are triggered by the specific inducers.

The conservation of singular inducibility of *hsr* ω by the specific inducers in the genus *Drosophila* implies conservation of the concerned regulatory elements too. This conserved inducibility appears paradoxical in view of the rapid sequence divergence noted at this locus. Moreover, Ryseck et al. (1987) did not find any significant homology between *hsr* ω loci of *D. melanogaster* and *D. hydei* upstream of the TATA-box over a length of 800 bp. Instead, the 3′ flanking region appeared more strongly conserved. Whether the stronger conservation of DNA base sequence in the 3′ flanking region in *D. melanogaster* and *D. hydei* noted by Ryseck et al. (1987) has any relevance to these putative regulatory elements remains to be examined. Comparative analysis of base sequence of the flanking regions in different species will be useful in this context.

The repression of the 93D locus in response to a combination of heat shock and another inducer in salivary glands of wild type larvae may be due to autoregulation and (or) due to a possible blockage to any transcription at this locus when the heat shock control element and a second regulatory element identified by the other inducer are simultaneously occupied. On the other hand, the selective noninduction of 93D by heat shock in salivary glands of certain genotypes (like those carrying *e* or *b* mutant alleles), or in beta-alanine treated wild type salivary glands, could be due to a specific blockage of its heat

shock regulatory element itself caused by the particular cell physiological changes associated with these genotypes, etc.

In addition to these varying themes of control at the transcriptional level, the ω transcripts are also subject to regulation at the levels of processing (splicing), transport, and (or) turnover. The developmentally regulated $\omega 1$ and $\omega 2$ transcripts turn over rapidly while the $\omega 1$ are more stable (Bendena, et al. 1989*b*). As noted earlier, the conservation of base sequences at the intron–exon junctions in ω transcripts is not for splicing of heat shock RNA per se. These conserved sequences may be specific signals, unique to ω transcripts, which would determine the rate or extent of their splicing under different conditions. Dangli et al. (1983) found ω transcripts to be associated with specific intranuclear proteins and thought these to have a storage function. These nuclear proteins may control splicing and (or) transport of ω transcripts to other cellular locations. Stabilization, induced by inhibitors of protein synthesis, of the $\omega 3$ transcripts is also known (Bendena et al. 1989*a*). Besides, the possible binding of the various ω transcripts to other macromolecules in the cell (as part of their "functions") may also modify the status of autoregulation by modulating the levels of "available" ω transcripts.

93D affects puffing of 87A and 87C heat shock loci in *D. melanogaster*

A very curious effect of the activity status of the 93D puff during heat shock in salivary gland polytene nuclei of *D. melanogaster* is on relative activity of the 87A and 87C puffs which code for hsp70. During the course of several studies in our laboratory, it was consistently seen that whenever the 93D puff failed to be induced by heat shock (see above), the 87A and 87C loci formed unequal puffs rather than equal-sized puffs as during the normal heat shock response (Lakhotia 1987). During our more recent studies on *e* or *h* genotypes or on beta-alanine treated wild type larval salivary glands, we noted a strong correlation between heat shock induced repression of 93D and unequal puffing of the 87A and 87C loci (see Table 1 for summary; details will be published elsewhere, S. C. Lakhotia, D. Kar Chowdhuri, and P. Burma, in preparation). However, in another recent report (Hochstrasser 1987) it was found that in heat-shocked polytene nuclei from midgut of *D. melanogaster* larvae, the 87A and 87C loci were unequally puffed although the 93D puff was well induced. In addition to such exceptions is the fact that among the instances of unequal puffing of the 87A and 87C loci when 93D is not puffed, are situations where either 87A is more puffed or 87C is more puffed. These variables raise doubts on the suggestion that noninducibility of 93D during heat shock is the causative factor for unequal puffing of 87A and 87C loci (Hochstrasser 1987). However, two points must be considered in this context: (*i*) the variability in greater puffing of 87A or 87C is mostly *between* different conditions of treatment or genotypes; in most cases, the relative response of 87A and 87C loci to a given condition of treatment is remarkably reproducible; (*ii*) since the 93D locus produces at least three species of transcripts and since their relative proportions can be varied independently, mere "puffing" of the 93D locus does not tell us everything about the underlying molecular events. We presume that the unequal puffing of 87A and 87C loci during certain conditions of heat shock is due to variations in the specific *hsr* ω transcripts being made or being available in the cell so that even if the 93D puff is well developed but is not producing a particular set of ω transcripts, it may be equivalent to no puff at 93D as far as the relative inducibility of 87A and 87C is concerned. However, in addition to the ω transcripts, other conditions in the cell may also directly modulate puffing at 87A and 87C so that sometimes they may respond differentially to heat shock in spite of the apparently "normal" puffing of 93D.

The nature and significance of the unequal puffing at 87A and 87C remain little understood. It is not known whether the unequal puffing is due to unequal rates of transcription or turnover of the RNA from the puff site. The transcription autoradiograms also do not permit a distinction between transcription of hsp70 mRNA and the heat-inducible alpha–beta repeat sequences resident at the 87C locus of *D. melanogaster*. The altered puffing has no effect on hsp70 synthesis (Lakhotia 1987; S. C. Lakhotia and P. Burma, unpublished observations). However, taking advantage of the fortuitous absence of alpha–beta repeat sequences at the 87C locus of *D. simulans* (Lis et al. 1981), Kar Chowdhuri and Lakhotia (1986) suggested the unequal puffing of 87A and 87C loci to be somehow related to the alpha–beta repeat sequences since in the interspecific hybrid polytene nuclei, only the 87A and 87C loci of *D. melanogaster* origin were affected by nonpuffing of 93D of both *D. melanogaster* as well as *D. simulans* origins. Unfortunately, since the function, if any, of the alpha–beta repeat sequences in heat shock response or otherwise is not known (Lengyel and Graham 1984), the observed effect of 93D on 87A and 87C puffing remains just one more curiosity.

Future prospects

The *hsr* ω loci in *Drosophila* have continued to be enigmatic. Hopes of resolving their function, generated first by discoveries of their selective inducibility and later by their molecular cloning, were belied by the unexpected and puzzling complexities revealed. In spite of intensive efforts, the present research work has continued to only add more unconventional properties to the list of oddities already known to be associated with *hsr* ω locus. However, the hope continues that ultimately these isolated oddities will fall in place to provide a comprehensive understanding of an unusual gene exerting its action in a novel fashion. In view of its conservation in the genus and the lethality associated with its deficiency, the *hsr* ω locus has a definite and vital role to play in the life of an organism. This also makes it unlikely that the *hsr* ω is merely an aberration of the eukaryotic genome that happened in *Drosophila*. However, till now the search for a heat shock locus in other dipterans having inducible properties similar to the *hsr* ω of *Drosophila* have yielded only negative results (Singh and Gupta 1985; Lakhotia 1987; Nath 1988; Barettino et al. 1988). Nevertheless, the presence of a functional homologue of *hsr* ω in other dipterans cannot yet be ruled out, since one of the major heat-inducible loci in some species of *Chironomus* has certain molecular properties that are strongly reminiscent of the *hsr* ω of *Drosophila* (Carmona et al. 1985; Lakhotia 1987; Barettino et al. 1988). Thus a more direct approach to look for a heat shock gene in other dipterans which shares some or all properties of *hsr* ω of *Drosophila* may yield positive results. Immunological and (or) immunocytochemical methods in conjunction with other molecular techniques may be specially rewarding in this direction. The functional significance of *hsr* ω will have to be sought in the protein-binding functions

of the different ω transcripts. If the *ORF* ω is indeed translated in cells, its distribution in the cell and its possible binding with other micromolecules will also be important issues for studying. The extraordinary power of combined molecular and cytological methods that are feasible now should provide answers to many of these questions in the near future.

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