

The 93D heat shock locus in *Drosophila*: a review

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Abstract. One of the major heat shock induced genes of *Drosophila melanogaster* is located in the 93D6-7 bands of polytene chromosomes. The 93D locus shows many unique and intriguing features which are reviewed here. This locus is induced selectively and independently of the other heat shock loci by very diverse agents. The heat shock inducibility of this locus shows a remarkable dependence on the developmental history of the organism, its genetic background and other specific inducers of 93D. In all species of *Drosophila* so far tested, one of the major heat shock loci is also selectively induced by the same set of 93D inducers and thus this locus is conserved in the genus. However, cloning and molecular characterisation of the primary DNA base sequence of the 93D of *D. melanogaster* and the 93D-like locus of *D. hydei* (the 2-48C locus) reveal that except for certain small stretches, the primary DNA base sequence of this locus has diverged rapidly. Interestingly, the structural organisation of the 93D of *D. melanogaster* and 2-48C of *D. hydei* is remarkably similar—both have a 5' unique sequence followed on the 3' end by a long stretch of a species-specific repetitive sequence apparently unique to the locus. Heat shock in both cases induces 3 major transcripts: a long 10–12 kb RNA covering the 5' unique and 3' repeat part, a 1.9–2.0 kb RNA complementary to the 5' unique part and a 1.2 kb transcript which lacks the 0.7 kb "intron" of the 2 kb RNA. None of the transcripts in either species has any appreciable open reading frames, and other evidence also suggests that the 93D or 2-48C loci do not code for any polypeptide. Functions of 93D or 93D-like loci remain uncertain although the locus is essential for ecdysone-induced differentiation of imaginal disks and viability of flies. Strong evidence exists that the activity pattern of the 93D heat shock locus in *D. melanogaster* distinctly influences heat shock puffing of the 87C locus, one of the two duplicate loci carrying the coding sequences for the major 70 kd heat shock polypeptide; presently available evidence suggests that this effect on 87C puffing is mediated via the mid-repetitive and heat-inducible $\alpha\beta$ sequences residing at the 87C locus. The strong conservation of its inducible properties, of the organisation rather than the primary DNA base sequence and of the pattern of heat shock transcripts of 93D locus in the genus *Drosophila* point to important but hitherto little understood functions of this locus. The structure of its transcripts may have vital regulatory roles.

Keywords. Heat shock genes; 93D heat shock locus; *Drosophila*.

1. Introduction

Cellular response to sudden elevation in environmental temperature is manifest at different levels of gene activity, and is collectively termed the *heat shock response*. First observed in *Drosophila* (Ritossa 1962, 1964), the heat shock response is now known to be ubiquitous in all organisms and occurs in response to a variety of other stress conditions (Ashburner and Bonner 1979; Schlessinger *et al*

1982; Nover 1984; Atkinson and Walden 1985). The major events of the heat shock response are: activation of a specific set of "heat shock genes" and a rapid translation of their transcripts into the heat shock polypeptides (HSP) with concurrent inhibition of ongoing transcriptional and translational activities. In *Drosophila melanogaster*, exposure of larvae or their isolated salivary glands to 37°C for a brief period results in induction of 9 heat shock puffs (Ashburner 1970). The expression and regulation of these heat shock loci in *Drosophila* have been studied extensively to permit correlation between many of these heat shock genes and their transcriptional and translational products (see recent reviews in Schlessinger *et al* 1982; Nover 1984; Atkinson and Walden 1985).

The heat shock puff induced in the 93D region on the right arm of chromosome 3 in polytene nuclei of *D. melanogaster* is one of the largest and is most active in transcription (Ashburner 1970; Mukherjee and Lakhotia 1979). However, in spite of its high transcriptional activity, it appears that it does not code for any of the known HSP. Besides, this locus shows several other unusual features in its inducible properties in different species of *Drosophila*. These intriguing features of this locus have attracted our attention for some time and the present review attempts to provide a state of the art report on this locus.

2. Inducibility of the 93D heat shock locus

The 93D region forms a developmental puff in salivary gland polytene nuclei of late 3rd instar larvae of *D. melanogaster*, being an "early" ecdysone puff. The 93D locus is also inducible in Kc cells in culture in response to ecdysone (W Bendena, M L Pardue, personal communication). The 93D locus is a member of the set of heat shock puffs induced by temperature shock, carbon dioxide, recovery from anoxia, 2-4-dinitrophenol, arsenic compounds etc. (Ritossa 1964; Burdette and Anderson 1965; Ashburner 1970; Ashburner and Bonner 1979; Mukherjee and Lakhotia 1982; Lakhotia and Singh 1985). An accidental observation by Lakhotia and A S Mukherjee (1970) that the 93D puff is specifically induced by a 10 min *in vitro* treatment of salivary glands of late 3rd instar larvae of *D. melanogaster* with benzamide (1 mg/ml), provided the first instance of singular induction of this locus. Subsequent observations on inducibility of the 93D locus made it clear that its activity was regulated independently of other heat shock loci and thus the concept that heat shock genes formed a "battery" of co-ordinately regulated genes was questioned. Bonner and Pardue (1976a) found that upon heat shock in aged Graces' medium, the activity of the 93D was much higher than that of other heat shock loci in larval tissues like salivary glands, imaginal disks etc. During a routine heat shock also, the level of ³H-uridine uptake at the 93D puff was not correlated with the levels at other heat shock loci which were active in a more or less co-ordinated fashion (Mukherjee and Lakhotia 1979). Using an *in vitro* system, Compton and McCarthy (1978) found that incubation of isolated polytene nuclei in the cytoplasm of heat-shocked Kc cells resulted in induction of heat shock puffs. However, the relative induction of the 93D puff was less than that of the other loci. On the other hand, Mukherjee and Lakhotia (1981, also see Singh and Lakhotia 1983) observed that incubation of intact salivary glands rather than isolated polytene nuclei in a homogenate of heat-shocked salivary glands or brain ganglia of

whole larvae resulted in an increased ^3H -uridine uptake by the 93D puff while other loci remained unaffected. Among the other specific inducers of the 93D puff are thiamphenicol (Behnel 1982) and colchicine or colcemid (Lakhotia and Mukherjee 1984). In the context of inducibility of 93D by colchicine or colcemid, Singh and Lakhotia (1984) tested a variety of microtubule poisons (see table 1) but none of them was effective in inducing the 93D or any other heat shock locus.

A common feature of the induction of 93D by heat shock, benzamide, colchicine etc. is that all these treatments severely inhibit general chromosomal (RNA polymerase II dependent) but not the nucleolar (RNA polymerase I dependent) transcription. This is interesting since like the rest of the chromosomal but unlike nucleolar transcription, RNA synthesis at the 93D puff seems to be dependent upon RNA polymerase II as evidenced by indirect immunofluorescent localisation studies (Bonner and Kerby 1982). How the RNA polymerase II escapes inhibition at the 93D locus is not known.

A very intriguing aspect of 93D induction is its near complete regression when two 93D-inducing agents are applied together or consecutively: recovery from anoxia at 37°C leads to 93D being totally inactive (Mukherjee and Lakhotia 1982); likewise, combinations of heat shock and benzamide (Lakhotia and Mukherjee 1980) or heat shock and colchicine (Lakhotia and Mukherjee 1984) also inhibit activity of the 93D puff (also see later).

Using small deficiencies in the 93D region (*Df(3R)e^{Gp4}* and *Df(3R)GCI4*), Burma and Lakhotia (1986a) made the very interesting observation that the developmentally regulated as well as the benzamide induced activity of the 93D heat shock locus was dosage compensated. In the heterozygous deficiency genotypes with only one dose of the 93D6-7 locus, ^3H -uridine uptake at this puff was equivalent to the two doses in wild type larvae. This observation is significant since in all the cases of autosomal gene dosage compensation known so far (Devlin *et al* 1982, 1984, 1985), whole arm duplication or deficiency is required to elicit dosage compensation. Apparently, the 93D locus is auto-regulated so that as soon as it is present in single dose, it becomes hyperactive. In the context of the peculiar property of 93D of being inhibited in response to a second stimulus (see above), Burma and Lakhotia (1986a) also noted that heat shock failed to induce the 93D6-7 locus on the normal chromosome in these 93D-deficiency heterozygotes: this was interpreted to mean that the single dose 93D heat shock locus in these genotypes was already hyperactive (to achieve dosage compensation) and the heat shock therefore, acted as a second inducer, and in agreement with earlier observations, caused regression rather than induction of 93D.

Another interesting feature is the influence of larval rearing conditions and certain genotypes on the inducibility of the 93D locus. Lakhotia and Singh (1985) found that the 93D locus is completely refractory to induction by its specific inducers like heat shock, benzamide, colchicine etc. in salivary glands from wild type larvae grown since hatching at 10°C rather than at 20°C . Furthermore, it appears that the ingredients of food on which the larvae grow may also influence the inducibility of the 93D puff since in several laboratories (M L Pardue and I S Gubenko, personal communications) benzamide or colchicine have not been found to be as effective inducers of 93D puff as in the Varanasi lab. This particular effect does not seem to be related to the genetic background since a similar situation was found in several wild type and mutant strains. However, the inducibility of 93D

Table 1. Inducibility of the 93D heat shock locus of *D. melanogaster*

Treatment	Larvae grown at (°C)	Induction properties		Reference
		93D	other TS loci	
Heat shock (37°C)	24	Induced	93D activity levels vary independently of other TS loci	Mukherjee and Lakhotia (1979)
Heat shock	10	No induction	Normal induction, except 87A/87C (see figure 1)	Lakhotia and Singh (1985)
Warm shock (24°C)	10	Relative activity level elevated	No induction	Lakhotia and Singh (1985)
Cold shock (10°C)	24	Relative activity level elevated	No induction	Lakhotia and Singh (1985)
Recovery from anoxia at 24°C	24	Induced	Induced	Mukherjee and Lakhotia (1982)
Recovery from anoxia at 37°C	24	No induction	Normal induction, except 87A/87C (see figure 1)	Mukherjee and Lakhotia (1982)
Benzamide 24°C	24	Singular induction	No induction	Lakhotia and Mukherjee (1970) Lakhotia and Mukherjee (1980)
Colchicine/colcemid/benzamide	24	Singular induction	No induction	Lakhotia and Mukherjee (1984)
Colchicine/colcemid	10	No induction	No induction	Lakhotia and Singh (1985)
Thiamphenicol	24	Singular induction	No induction	Behnel (1982)
Heat shock in aged Graces' medium	24	Relative activity level more	Induced	Bonner and Pardue (1976a)
Vit-B ₆	24	No induction	No induction	Lakhotia and Singh (1985) Ashburner (1970) Derksen <i>et al</i> (1973)
Na-cacodylate	24	Induced	Induced	Lakhotia and Singh (1985)
Na-cacodylate	10	Less induced	Induced	Lakhotia and Singh (1985)
Microtubule poisons:	24			
a. Griseofulvin		No induction	No induction	Singh and Lakhotia (1984)
b. Oncodazole		No induction	No induction	Singh and Lakhotia (1984)
c. Vinblastine		No induction	No induction	Singh and Lakhotia (1984)
d. Chloralhydrate		No induction	No induction	Singh and Lakhotia (1984)
Isolated polytene nuclei in heat-shocked cytoplasm	24	Less induced	Induced	Compton and McCarthy (1978)

(Contd. next page)

(Table 1 *contd.*)

Treatment	Larvae grown at (°C)	93D	Induction properties other TS loci	Reference
Homogenate of heat-shocked salivary glands/larvae	24	Relative activity increased	No induction	Mukherjee and Lakhotia (1981) Singh and Lakhotia (1983)
Nuclear or cytoplasmic extract of heat-shocked cells	24	Relative activity increased	No induction	Singh and Lakhotia (1983)
Homogenate of heat-shocked cells of heterologous species	24	No induction	No induction	Singh and Lakhotia (1983)
Paracetamol	24	Singular induction	No induction	Srivastava and Bangia (1985)
Cycloheximide	24	No induction	No induction	P Burma and S C Lakhotia (unpublished)
Cycloheximide	Kc cells	93D transcripts elevated	No induction	W Bendena (personal communication)

puff is influenced by certain genetic conditions. Kar Chowdhuri and Lakhotia (1986b) have found that translocation of the 93D region to heterochromatin of the X-chromosome (in T(1;3)20, 93D of Henikoff 1980), leads to variegation at this puff and the extent of variegation is influenced by the nature of the homologue. Thus when the T(1;3) 20, 93D chromosome is present against the In(3R)C homologue, the variegation of 93D is much more pronounced than when against a normal (+) homologue. The 93D locus also fails to respond to heat shock in salivary glands from In(3R)C/+ larvae (Kar Chowdhuri and Lakhotia 1986b).

A difference between 93D and other heat shock puffs was also manifest in the association of cGMP with the puff sites after induction: Spruill *et al* (1978) found that after heat shock, the 93D puff had maximum cGMP as revealed by indirect immunofluorescence.

3. Cytogenetic localisation of the inducible site in 93D region

Using a series of overlapping deficiencies, Mohler and Pardue (1982a,b) localised the heat shock inducible site in the 93D6-7 bands defined by the proximal and distal breakpoints of *Df(3R)GCI4* (from 93D6-7 to 93D10) and *Df(3R)e^{Gp4}* (from 93B11-13 to 93D6-7) deficiencies, respectively. Using the same set of deficiencies, Burma and Lakhotia (1986a) have localised the benzamide and colchicine inducible sites also to the 93D6-7 region. A detailed mutational analysis of the 93D region by Mohler and Pardue (1984) revealed only one complementation unit (*er3*), defined solely by lack of complementation between the *GCI4* and *e^{Gp4}* deficiencies which corresponds to the heat shock locus in the 93D6-7 band. No apparent point mutations mapping at 93D6-7 could be detected. This lack of "mutable" sites in the 93D locus may now be understood by comparison of the 93D sequence with that of

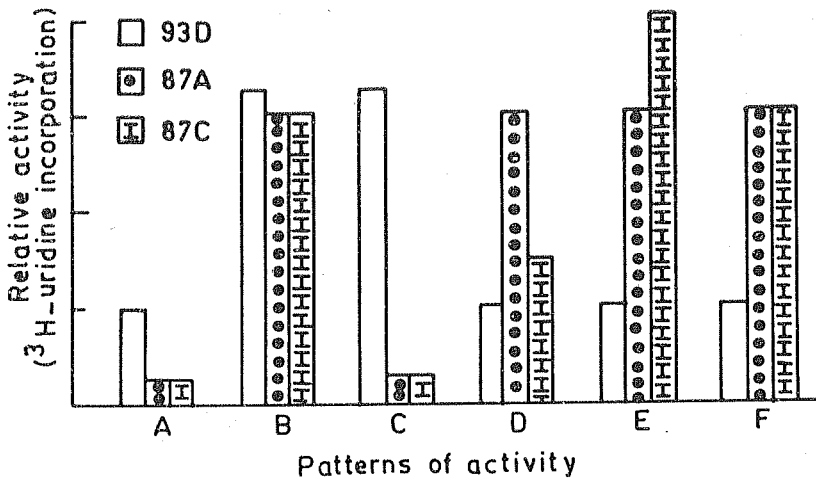


Figure 1. Patterns of relative activity of 93D, 87A and 87C heat shock loci in polytene nuclei of salivary glands of *D. melanogaster* larvae under different conditions:

Patterns	Examples
A. 93D moderately active; 87A and 87C inactive	Normal development
B. 93D, 87A and 87C highly induced (93D > 87A = 87C)	Normal temperature shock (TS) response in 24°C reared larvae
C. Only 93D induced; 87A and 87C inactive	93D-specific inducers in 24°C reared larvae
D. 93D not induced beyond normal developmental level or inactive; 87A and 87C induced but 87A > 87C (87A/87C ratio varies)	<ul style="list-style-type: none"> i) Benzamide (BM) at 24°C followed by TS (Lakhotia and Mukherjee 1980) ii) TS followed by BM at 24°C (Lakhotia and Mukherjee 1980) iii) TS to glands from <i>Df(3R)e^{Gp4}/+</i> or <i>Df(3R)GC14/+</i> larvae (Burma and Lakhotia 1986a) iv) TS to glands from <i>Df(3R)e^{Gp4}/Df(3R)GC14</i> larvae (also pattern 'F' in ~ 50% nuclei) (Burma and Lakhotia 1986a)
E. 93D as in 'D', but 87C > 87A (87C/87A ratio varies)	<ul style="list-style-type: none"> i) TS to glands from 10°C reared larvae (Lakhotia and Singh 1985) ii) Anoxia, recovery from anoxia at 24°C or at 37°C (Mukherjee and Lakhotia 1982) iii) Colchicine at 37°C (Lakhotia and Mukherjee 1984)
F. 93D as in 'D', but 87A = 87C	<ul style="list-style-type: none"> i) Na-Cacodylate at 24°C to glands from 10°C reared larvae (Lakhotia and Singh 1985) ii) TS to glands from <i>Df(3R)e^{Gp4}/Df(3R)GC14</i> (in ~ 50% nuclei) (Burma and Lakhotia 1986a)

its equivalent 2-48B locus of *D. hydei*: the general structure of the transcripts is conserved but many base changes have been tolerated (see later).

4. 93D Homologue in other species of *Drosophila*

Since the heat shock response is ubiquitous and fairly well-conserved (Schlesinger *et al* 1982), existence of a 93D-like locus as member of the heat shock genes in other species could be expected. However, initial studies suggested that this is not the case. Other than *D. melanogaster* the heat shock response has been studied in some detail in *D. hydei*. In this species, one of the major heat shock loci, the 2-48C puff, was known to show unusual characteristics which would compare with those of the 93D. In a series of investigations, Berendes and his group (Berendes 1972; Leenders *et al* 1973; Derksen 1975; Lubsen *et al* 1978) found the 2-48C puff in polytene cells of *D. hydei* to be singularly induced with vit-B₆ and to accumulate characteristic large RNP particles. A comparable vit-B₆ puff was also seen in several other species like *D. virilis* (see Ashburner and Bonner 1979). However, vit-B₆ does not induce any puff in *D. melanogaster* (Ashburner 1970; Lakhotia and Singh 1982); Derksen (1975) also claimed absence of the large RNP particles characteristic of the 2-48C puff in *D. melanogaster* cells. Moreover, Peters *et al* (1980) found that the heat shock induced intranuclear or polysomal RNA from *D. melanogaster* did not hybridise *in situ* with 2-48C puff of *D. hydei* and thus concluded that an equivalent of 2-48C of *D. hydei* is not present in *D. melanogaster*. However, a systematic search by Lakhotia and Singh (1982) revealed that the 2-48C of *D. hydei* and the 93D of *D. melanogaster* were indeed functionally homologous since both were singularly inducible with benzamide, homogenate of heat shock glands (autologous) and also with colchicine. Furthermore, in all species, close as well as distant relatives of *D. melanogaster*, that were tested, one of the major heat shock puffs was singularly inducible with vit-B₆, benzamide, colchicine, homogenate of autologous heat shock glands (see table 2). A functional homology between these 93D-like loci in different species of *Drosophila* is also manifest in the accumulation of the large RNP particles after induction (Derksen *et al* 1973; Swift 1965; Dangli *et al* 1983). Dangli *et al* (1983) also found that a monoclonal antibody against chromosomal proteins, Q18, bound specifically to 93D of *D. melanogaster*, 2-48C of *D. hydei* and 20CD of *D. virilis*. Interestingly, some other monoclonal antibodies which also bound specifically to 93D of *D. melanogaster* did not bind with the 93D-like loci in other species.

5. 93D Homologue in other Diptera

Since the 93D heat shock locus is functionally conserved in the genus *Drosophila*, it may be expected that a 93D-like heat shock locus would be present in other Dipterans also. In their original study with benzamide, Sirlin and Jacob (1964) did not notice any singularly inducible puff in polytene nuclei of *Smittia*. Singh and Gupta (1985) looked for a benzamide inducible puff in *Melanogromyza obtusa* but did not find any. B B Nath and S C Lakhotia (unpublished) looked for benzamide, colchicine and vit-B₆ inducible puff in a tropical species of *Chironomus* and in this

Table 2. 93D Homologue in other species of *Drosophila*

Treatment\Map position	Species							
	<i>D.m.</i>	<i>D.s.</i>	<i>D.a.</i>	<i>D.k.</i>	<i>D.p.</i>	<i>D.v.</i>	<i>D.h.</i>	<i>D.n.</i>
	93D	93D	2L-2C	E-11B	2-58C	20CD	2-48C	48A
Benzamide	+	+	+	+	+	ns	+	+
Colchicine	+	+	ns	ns	+	+	+	+
Vit-B ₆	-	ns	+	+	ns	+	+	+
Heat shock gland homogenate (autologous)	+	ns	+	ns	ns	ns	+	+
Presence of large RNP particles at puff	+	ns	ns	ns	ns	+	+	ns
Binding with Q18 monoclonal antibody	+	ns	ns	ns	ns	+	+	ns
Binding with P11 monoclonal antibody	+	ns	ns	ns	ns	-	-	ns

D.m. = *D. melanogaster*; *D.s.* = *D. simulans*; *D.a.* = *D. ananassae*; *D.k.* = *D. kikkawai*; *D.p.* = *D. pseudoobscura*; *D.v.* = *D. virilis*; *D.h.* = *D. hydei*; *D.n.* = *D. nasuta*

+ = positive response; - = negative response; ns = not studied

case too, the results were negative since none of the heat shock or any other locus responded to any of the 93D-inducers. All these results thus indicate that a 93D-like locus, as far as identifiable by inducibility with colchicine, benzamide etc. is absent outside the genus *Drosophila*. However, a molecular characterisation of the heat shock loci in *Chironomus thummi* has revealed that one of the heat shock induced terminal Balbiani ring (TBR-III) has properties which are remarkably reminiscent of the 93D locus: the transcription products of TBR-III (Santacruz *et al* 1984) resemble in their size, shape and general disposition those seen at 93D or 93D-like loci (Derksen *et al* 1973; Dangli *et al* 1983); the DNA sequence at TBR-III shows internal repetition, absence of long open reading frames and a very rapid sequence divergence (Carmona *et al* 1985) as known for the 93D locus (see below). In view of these, it appears very likely that in spite of the failure of benzamide, colchicine etc. to induce any of the heat shock loci in *Chironomus* or other Dipterans, one of the heat shock loci in these species too may function as a counterpart of 93D. Further molecular studies are needed to elucidate this aspect.

6. Molecular biology of 93D

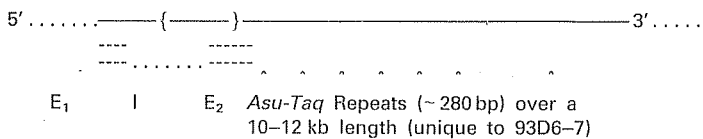
The studies referred to earlier utilised transcription autoradiography to obtain information on the inducibility and activity patterns of the 93D locus. The first insights into the unusual properties of its transcription products were provided by *in situ* hybridisation studies utilising ³H-labelled heat shock RNA. Spradling *et al* (1977) isolated ³H-labelled cytoplasmic poly A⁺ RNA from heat-shocked Schneider's cells and hybridised *in situ* to polytene chromosomes of *D. melanogaster*: none

of the electrophoretically separated mRNA fractions hybridised specifically to the 93D region. A more detailed analysis was undertaken by Lengyel *et al* (1980) using saturation and competition *in situ* hybridisation of poly A⁺ and poly A⁻ nuclear and cytoplasmic heat shock RNA fractions. The following interesting features were revealed: i) the length of the transcribed region at 93D was about 9.6 kb; ii) a major fraction of the 93D transcripts remained intranuclear; iii) 93D transcripts were found both as poly A⁺ and poly A⁻ fractions; and iv) surprisingly, the 93D complementary RNA in cytoplasm was predominantly poly A⁻ and was only 28–58% of the length of the DNA transcribed at 93D.

A major advance in the study of the 93D locus was its cloning using a variety of strategies (Walldorf *et al* 1984; Garbe and Pardue 1986). Genomic as well as cDNA clones are available and these have revealed some novel features of its sequence organisation and transcripts. A summary of the current information is presented in figure 2.

Basically, the 93D heat shock locus consists of a 5' unique sequence followed on the 3' end by a 10–12 kb stretch comprising about 280 bp long *Asu-Taq* repeats (Walldorf *et al* 1984; Garbe and Pardue 1986). The picture of 93D transcripts which emerges from the application of northern analysis (Ryseck *et al* 1985; Hovemann *et al* 1986; Garbe and Pardue 1986) confirms the initial results of Lengyel *et al* (1980) and shows the 93D locus to be very different from the other heat shock loci. Heat shock induces at least three major transcripts at 93D: a long 10–12 kb and two shorter 1.9 kb and 1.2 kb transcripts. The 10–12 kb transcript is complementary to the unique as well as the repeat part of 93D whereas the 1.9 and 1.2 kb transcripts are complementary to the unique part only. All the three transcripts are transcribed in the same direction (centromere to telomere) and apparently use the same start point (Ryseck *et al* 1985; Hovemann *et al* 1986; Garbe and Pardue 1986). A comparison of the 1.2 kb and 1.9 kb transcripts and the sequence of the genomic

Genomic DNA:



Heat shock induced transcripts:

A. Major :—

10–12 kb RNA <---{ }----->

1.9 kb <---{ }----->
poly A⁺ RNA (relative level increases upon heat shock)

1.2 kb <---{ }----->
poly A⁺ RNA (spliced?)

B. Minor :—

<----->
Less abundant and mostly poly A transcripts in
entire size range (degradation products?)

Figure 2. Diagrammatic representation of the organisation of the 93D6-7 heat shock locus of *D. melanogaster* [based on information from Walldorf *et al* (1984); Ryseck *et al* (1985); Hovemann *et al* (1986); Garbe and Pardue (1986); Garbe *et al* (1986); Pardue *et al* (1987)]. Not drawn to scale.

and cDNA clones revealed the existence of an intron (0.7 kb) in the 1.9 kb RNA. The 10 kb and 1.9 kb transcripts are limited to the nucleus and are relatively more abundant than the 1.2 kb transcript. In addition to these major transcripts, other less abundant transcripts of an entire size range are also found which suggest that the termination and/or processing is variable (Hovemann *et al* 1986; Garbe and Pardue 1986; Garbe *et al* 1986; Pardue *et al* 1987). The significance of these various transcripts is not known. Also not known is whether the populations of 93D transcripts vary with specific inducers.

Earlier results of Peters *et al* (1980) indicated a rapid sequence divergence at the 2-48C locus of *D. hydei* since the heat shock RNA from *D. melanogaster* did not hybridise *in situ* with 2-48C and similarly, the heat shock RNA from *D. hydei* did not hybridise with vit-B₆ inducible heat shock puff present in *D. repleta* or *D. virilis* although hybridisation was specifically seen within the members of the *hydei* subgroup. Subsequent cloning of the 2-48C of *D. hydei* (Peters *et al* 1984) has permitted a more specific comparison of the DNA sequence organisation between 93D and 2-48C (Garbe and Pardue 1986; Hovemann *et al* 1986; Garbe *et al* 1986; Pardue *et al* 1987). This comparison reveals very interesting parallels in the structural organisation of these loci: both have a 5' unique sequence followed by a long stretch of repeat sequence (~115 bp repeat unit in 2-48C and ~280 bp repeat unit in 93D); the unique part in both carries an intron at an analogous position. However, in spite of this similar structure, the DNA base sequence itself shows high divergence in unique as well as the repeat part except for some regions which are remarkably highly conserved. These regions of strong conservation include: a 59 bp sequence around the 3' splice site, a 16 bp sequence at the 5' splice site and a 14 bp sequence preceding the polyadenylation site (Garbe *et al* 1986). These regions of strong conservations are apparently not involved in processing of heat shock RNA since the hsp82 gene which also has an intron does not share any of these conserved sequences (Garbe *et al* 1986; Blackman and Meselson 1986). Although the repeat parts at 2-48C and 93D are highly diverged, a short 9 bp long sequence (ATAGGTAGG), present once every 115 bp repeat unit in *D. hydei* and twice every 280 bp repeat unit in *D. melanogaster*, seems strongly conserved. Thus the concentration and distribution of this 9 bp sequence would be nearly similar in the two RNA and if the 9 bp sequence has some role in recognition function, the 2-48C and 93D RNA might be equivalent in spite of the overall sequence divergence between them (Garbe *et al* 1986; Pardue *et al* 1987).

7. Functions of 93D

7.1 Role of 93D in normal development

The 93D heat shock locus is known to be inducible by ecdysone and forms a prominent puff in salivary glands of 3rd instar larvae soon after the release of ecdysone in haemolymph (Ashburner 1967). In other species also the 93D-like loci puff during normal development (Lakhotia and Singh 1982). 93D transcripts are also detectable, at low levels, during embryonic development and at some other larval and adult stages (Hovemann *et al* 1986; Garbe and Pardue 1986). These suggest that the 93D locus has some function in normal development. Genotypes

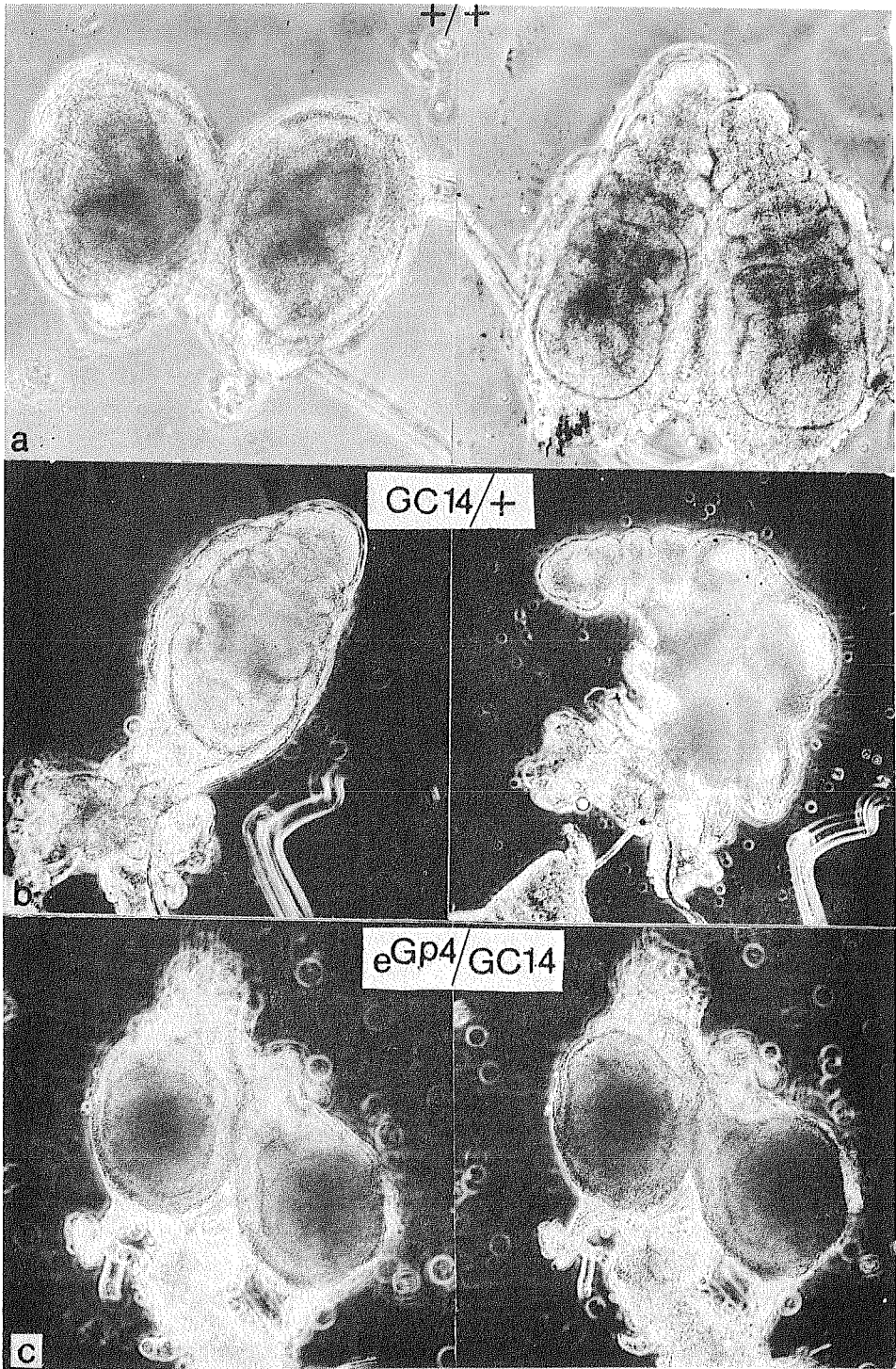
with complete deficiency of the 93D6-7 heat shock locus (as in *Df(3R)e^{Gp4}/Df(3R)GCI4* trans-heterozygotes) have only 20–30% viability and even of these, the majority die at the late 3rd instar stage and the few that emerge as adults are weak and die soon (Mohler and Pardue 1982b, 1984). These observations suggest a vital but unknown role for this locus in normal development. In an attempt to identify the role of 93D in development, wing and leg imaginal disks from late 3rd instar larvae deficient for the 93D6-7 locus (*e^{Gp4}/GCI4*) were cultured *in vitro* in Robb's medium supplemented with ecdysone; disks from wild type and deficiency heterozygotes (with one copy of the 93D6-7 locus) were also cultured simultaneously: the 93D-deficient disks failed to show any differentiation *in vitro* while the disks from wild type and deficiency heterozygote larvae differentiated normally (S C Lakhotia and M Sandhu, unpublished, see figure 3). Bonner and Pardue (1976b) also noted that the imaginal disk RNA was rich in 93D transcripts.

These results together with the known ecdysone responsiveness of the 93D heat shock locus suggest that one of the functions of this locus may be to mediate ecdysone-induced cellular responses. How this is achieved is not known.

7.2 Search for translational product of 93D transcripts

Since the 93D is one of the major heat shock loci and the other heat shock gene transcripts are very rapidly translated into discrete HSP, it was expected that the abundantly transcribed 93D locus would also code for an HSP. However, so far no translational product of 93D transcripts has been identified and hence it appears that the 93D does not have the usual protein-coding function. Lakhotia and Mukherjee (1982) compared the newly synthesised polypeptides in larval salivary glands treated with benzamide with those in untreated glands with a view to identify any novel polypeptide(s) whose synthesis could be correlated with the abundant transcripts induced at 93D by benzamide treatment: no novel polypeptides were formed in the treated glands and thus they concluded that unlike the other heat shock loci, 93D transcripts are not translated. Mohler and Pardue (1982b, 1984) found that all the regular HSP were synthesised in response to heat shock in glands deficient for 93D heat shock locus. Such indirect evidence together with the unusual properties of 93D transcripts (Lengyel *et al* 1980) strengthened the view that 93D does not have a coding function. This was substantiated by examination of the DNA base sequence of the 93D genomic as well as cDNA clones (Garbe and Pardue 1986; Hovemann *et al* 1986; Garbe *et al* 1986). Analysis of the coding function of the DNA sequence of the 93D locus revealed that the largest reading frame in this locus can code for short peptides not exceeding 35 amino acids. A specific search for such small peptides failed to give any positive result (Garbe and Pardue 1986). In view of all these results, it appears certain that the 93D heat shock locus does not have a protein-coding function.

Some earlier studies "identified" apparent translational products of the 93D of *D. melanogaster* (Scalenghe and Ritossa 1977) or the 2-48C of *D. hydei* (Brady and Belew 1981; Belew and Brady 1981). Scalenghe and Ritossa's (1977) paper was subsequently withdrawn. Brady and Belew (1981) and Belew and Brady (1981) observed that the vit-B₆ induced activity of the 2-48C puff in *D. hydei* was correlated with a significant elevation of tyrosine-amino-transferase (TAT), whose level was also found to go up after heat shock (also see Leenders and Knoppien



1973). However, in view of the DNA base sequence data now available for 93D as well as the 2-48C (see above), it appears certain that the TAT is not a translational product of the 2-48C transcripts. Nevertheless, since vit-B₆ is a coenzyme for TAT, the elevation of TAT levels and the induction of the 2-48C puff by vit-B₆ may be somehow causally related.

7.3 Role of 93D in HSP synthesis and thermotolerance

As mentioned above, Mohler and Pardue (1982b, 1984) found that the HSP synthesis was not affected by deficiency for the 93D heat shock locus. Mohler and Pardue (1982b, 1984) were unable to demonstrate a decreased thermotolerance in animals lacking the 93D locus. Results of a more recent study in our laboratory (S C Lakhotia and P K Burma, unpublished) confirm the conclusion of Mohler and Pardue that the HSP synthesis remains unaffected by presence or absence of 93D locus. However, our studies reveal a significant effect of 93D-deficiency on acquisition of thermotolerance. In these experiments early 3rd instar larvae of different genotypes (+/+, *TM6B*/+, *TM6B/Df(3R)e^{Gp4}* or *TM6B/Df(3R)GC14*, and *Df(3R)e^{Gp4}/Df(3R)GC14*) were exposed to 33°, 37°, 40°, 33° followed by 40°C or 37° followed by 40°C and the survival was monitored as the proportion of exposed larvae pupating. Briefly, the results were: the +/+ (Oregon R) larvae show better survival if the 40°C severe heat shock is preceded by a milder shock at 33°C rather than at 37°C; on the other hand, larvae of genotypes *TM6B*/+ or *TM6B/Df(e^{Gp4} or GC14)* survive the 40°C heat shock better when pre-exposed to 37°C rather than to 33°C. The survival of the 93D-deficient larvae (*e^{Gp4}/GC14*) is significantly poorer when compared to their *TM6B*-carrying sibs whether the 40°C severe shock is preceded by 33°C or 37°C milder shock. Interestingly, the relative viability of the 93D-deficient larvae after 37°C followed by 40°C heat shock treatment compares with that of the similarly treated but poorly thermoadapted +/+ larvae. Mohler and Pardue (1982b, 1984) did not compare the effect of 37°C pre-exposure on survival after 40°C in these genotypes and thus could have failed to notice the poorer thermotolerance of the 93D-deficient larvae.

7.4 Effect of 93D on activities of 87A and 87C heat shock loci

In a series of investigations, Lakhotia and his group have suggested an effect of 93D activity on the relative rates of transcription at the 87A and 87C heat shock loci. The 87A and 87C loci are duplicate sites coding for the major HSP 70 (Ish-Horowicz *et al* 1977; also see Hellmund and Serfling 1984, for a recent review of the structure and organisation of these loci) and after a routine heat shock to larval salivary glands, show nearly equal rates of ³H-uridine incorporation. However, Lakhotia and group (see figure 1) have shown that if during heat shock the 93D activity is

Figure 3. Ecdysone induced *in vitro* differentiation of imaginal disks from larvae of different genotypes: a. prothoracic leg disks from wild type (+/+) larvae; b. mesothoracic leg disk from *Df(3R)GC14*/+ larvae; c. prothoracic leg disks from *Df(3R)e^{Gp4}/Df(3R)GC14* larvae. The left panel in each case shows the disks at 0 hr of explantation and the right panel after 12 hr of ecdysone exposure *in vitro*. (a) Under positive phase-contrast, (b) and (c) under negative phase-contrast optics. Note the failure of any development in (c) while the disks in (a) and (b) show normal differentiation capacity.

modified for some reasons, the 87A and 87C loci are induced unequally with 87C being significantly less or more active than 87A puff. The different treatments and the patterns of response of 87A, 87C and 93D loci are illustrated in figure 1. It was noted that in all these cases, the level of 87C went up or down, while the rate of ^3H -uridine incorporation at the 87A puff remained at the expected level. It is interesting to mention that the conditions used by Bonner and Pardue (1976a) and Compton and McCarthy (1978) to induce the heat shock puffs resulted in atypical induction of 93D (see table 1); significantly, a careful examination of the photomicrographs published by these authors reveals that the 87A and 87C puffs were unequally induced in these studies too. Since the increased or decreased level of 87C activity in all these cases was associated with a modified pattern of induction of 93D, it was suggested that the 93D activity had some effect on transcription at the 87C locus. Burma and Lakhotia (1984, 1986b) noted that in *D. pseudoobscura*, the equivalents of 93D and 87C of *D. melanogaster* share a common cytological site and they considered this to be evolutionarily significant in view of the apparent interaction between 93D and 87C of *D. melanogaster*.

Kar Chowdhuri and Lakhotia (1986a) made an interesting observation that this effect of 93D on 87C is absent in *D. simulans*, a sibling species of *D. melanogaster*. They also noted that in interspecific hybrid nuclei, the 87A and 87C puffs of *D. melanogaster* origin only were affected in specific patterns by combinations of heat shock and benzamide or colchicine and heat shock while those of *D. simulans* origin remained unaffected. Kar Chowdhuri and Lakhotia (1986a) suggested that the 93D effect on 87C is perhaps mediated via transcription of heat-inducible *alpha-beta* sequences present at the 87C site of *D. melanogaster* but absent in *D. simulans* (see Hellmund and Serfling 1984). Lakhotia and Pardue (1986) also presented preliminary evidence that the level of the *alpha-beta* transcripts went up in Schneider cells when treated with colchicine at 37°C, a treatment that is known to cause the 87C puff in salivary glands to be more active (figure 1, this work; Lakhotia and Mukherjee 1984).

It is also to be noted, however, that not all conditions which cause 93D to remain uninduced when other TS loci are induced, lead to an unequal expression of the 87A and 87C puffs (see figure 1). Thus the interaction between the 93D and 87A/87C heat shock loci is complex and an understanding of their relationship and its significance would have to await a better knowledge of the populations of 93D transcripts present/induced under different conditions. The significance of multiple copies of the *hsp70* coding and of the *alpha-beta* sequences at the 87C locus also needs to be better understood.

7.5 Nature of 93D inducers

Some idea of 93D function could possibly be obtained from a knowledge of how the various 93D-inducers affect the cell. Unfortunately, however, the three main inducers of 93D or 93D-like heat shock puffs, viz., benzamide, colchicine and vit-B₆, appear to have little in common with respect to their hitherto known cellular effects. Benzamide has been found to have varied effects in different systems: i) to delay or prevent pupation when fed to *Drosophila* larvae by affecting the ring gland metabolism (Abd-el-Wahab and Sirlin 1959); ii) to specifically inhibit chromosomal transcription without affecting nucleolar RNA synthesis in polytene nuclei (Sirlin

and Jacob 1964; Lakhotia and Mukherjee 1970); iii) to depolymerise contractile fibrils in *Physarum* (Korohoda and Wohlfarth-Bottermann 1976); iv) to affect the mitotic spindle and to act as a clastogenic agent in mammalian cells (Babu *et al* 1980); and finally v) to specifically block poly-ADP-ribosylation in mammalian and other cells (see reviews in Cleaver *et al* 1985; Painter 1985). Apparently, these varied effects of benzamide do not provide a common denominator for the basis of 93D induction. The effect of benzamide on poly-ADP-ribosylation may be important. However, a more potent inhibitor of poly-ADP-ribosylation is 3-amino-benzamide, a derivative of benzamide and the results of D Kar Chowdhuri and S C Lakhotia (unpublished) show that although 3-amino-benzamide affects chromosomal transcription in polytene cells in much the same way as benzamide, it fails to induce 93D at different concentrations ranging from 100 $\mu\text{g/ml}$ to 1 mg/ml . This negative result with 3-amino-benzamide makes it doubtful that the 93D induction by benzamide is a direct consequence of altered poly-ADP-ribosylation in the treated cells.

The best known effect of colchicine on cells is that on polymerisation of tubulins to form microtubules (Dustin 1978). As mentioned above, benzamide also may affect the cytoskeletal components, and together these may suggest that the 93D induction by benzamide or colchicine may be related in some way to their effects on the cytoskeleton. However, a variety of other microtubule poisons failed to induce 93D (see table 1, this work, also Singh and Lakhotia 1984) and this casts doubts on any correlation between the two effects of colchicine, viz., depolymerisation of microtubules and 93D induction. In view of the other effect of colchicine on meiocytes known earlier (Hotta and Shepard 1973; Salonen *et al* 1982), Lakhotia and Mukherjee (1984) speculated that the polytene cells may have a colchicine-binding protein, distinct from microtubules, and this may mediate 93D induction by colchicine and benzamide.

The basis for vit-B₆ induced activity of 93D-like loci in other *Drosophila* species is also not known. As mentioned earlier, the reported correlation between increased TAT activity and 2-48C induction in *D. hydei* (Brady and Belew 1981; Belew and Brady 1981) is unlikely to be due to this locus being the structural gene for TAT.

Put together, the specific inducers of 93D have so far failed to provide any insight into the 93D function. The apparently unrelated nature of the various inducers of 93D and 93D-like loci in other species makes it very difficult to find a unifying basis although a unifying mechanism must exist since in all *Drosophila* species tested, these agents have a singularly common effect.

8. Concluding remarks

The structure as well as the products of the 93D heat shock locus have revealed themselves to be very unusual and intriguing. The functions of 93D, although apparently of vital importance to the organism, continue to remain uncertain. However, one thing that appears almost certain is that whatever function 93D may have, these are carried out directly by its transcripts themselves since they are untranslatable. The rapid sequence divergence along with strong conservation of certain sequences within its transcripts indicate that rather than the primary

structure, the secondary structure of these RNA species is of consequence. An obvious and strong possibility is that these transcripts have a regulatory function but what they regulate remains unknown. They may have a protein-binding function but answers to all questions like how, why, where, and what for, remain unknown. Caizzi and Ritossa (1983) reported that a species of RNA remains covalently bound to subunit I of glutamine synthetase in *D. melanogaster* and that different *ebony* mutant strains show electrophoretic variants of this subunit. Since *ebony* is very close to the 93D6-7 heat shock locus (Mohler and Pardue 1982), could this observation have a bearing on a possible role for 93D RNA?

In any case, studies on this most unusual heat shock locus continue to be exciting and challenging. One hopes that the apparently confusing wealth of data available for this gene would be sorted out in the near future into more ordered and meaningful information.

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Note added in proof: D Kar Chowdhuri and S C Lakhota (to be published) have found that mutant alleles of the e^+ locus strongly influence the heat shock inducibility of the 93D puff: in the presence of e (or other mutant allele) in homo- or heterozygous (e/\pm) conditions, the 93D loci on either homolog fail to puff in response to heat shock but still respond to benzamide.

W G Bendena, S C Lakhota, J G Garbe and M L Pardue (*J. Cell Biol.*, 1987 suppl., in press) have found that the relative proportions of the three 93D transcripts (10, 1.9 and 1.2 kb) induced after heat shock, benzamide, colchicine or cycloheximide vary: benzamide strongly induces the 10 and 1.2 kb species, colchicine induces the 10 and 1.9 kb transcripts while cycloheximide has a strong bias for the 1.2 kb RNA.