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Different effects of 93D on 87C heat shock puff activity in *Drosophila melanogaster* and *D. simulans*

D. Kar Chowdhuri and S.C. Lakhotia
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221005, India

Abstract. It is known that treatment of the 93D heat shock locus in *Drosophila melanogaster* with two inducers, eg. benzamide (BM) or colchicine plus heat shock, causes the 93D puff in polytene nuclei to regress and, at the same time, the 87A and 87C heat shock puffs to be expressed unequally. In view of the close phylogenetic relationship and similar banding pattern of polytene chromosomes of the sibling species *D. melanogaster* and *D. simulans*, we examined the expression of the 93D puff and its effects on the transcriptional activity of the 87A and 87C heat shock puffs in salivary glands of *D. simulans* and *D. melanogaster* × *D. simulans* hybrid larvae by autoradiography. As in *D. melanogaster*, the 93D puff was selectively induced by BM or colchicine and regressed when heat shock treatment was given in addition in *D. simulans* and in the interspecific hybrid. However, unlike in *D. melanogaster*, the relative activity of the 87A and 87C heat shock puffs on *D. simulans* chromosomes remained equal in glands exposed to heat shock in combination with BM or colchicine. In the nuclei of interspecific hybrids, the 87A and 87C loci on the two homologues responded in the same way as those of the respective parents. There was no evidence of a *transvection* effect. It is known that the 87C locus of *D. simulans* differs from that of *D. melanogaster* in not carrying the heat-inducible $\alpha\beta$ sequences and therefore, it is proposed that the 93D effect on 87C puff activity in *D. melanogaster* is mediated via the $\alpha\beta$ sequences. However, the role of possible structural differences in the 93D locus of the two species cannot be ruled out.

Introduction

The 93D heat shock locus of *Drosophila melanogaster* is unique in many respects and although it forms one of the most active puffs after heat shock, its functions remain unknown (Ashburner and Bonner 1979; Lakhotia and Mukherjee 1980, 1982; Lakhotia and Singh 1982, 1985; Walldorf et al. 1984; Mohler and Pardue 1982). However, it is known from earlier studies in our laboratory (Lakhotia and Mukherjee 1980, 1984; Mukherjee and Lakhotia 1982; Lakhotia and Singh 1985; Burma and Lakhotia 1986) that 93D affects transcription at the 87C locus, one of the two loci coding for the major heat shock polypeptide, HSP 70 (Ish-Horowicz et al. 1979). It was shown that if heat shock is given under conditions where the 93D puff is already in an induced state, it regresses, and at the same time the 87C puff becomes much more or much less active than the 87A puff, depending upon how 93D was initially induced. A more direct demonstration of the effect of 93D activity on 87C transcription is provided by Burma and Lakhotia (1986) who have shown that deficiency for the 93D heat shock locus alone affects 87C transcription.

The 87C locus of *D. melanogaster* contains, besides the HSP 70 coding sequences, heat shock inducible repetitive $\alpha\beta$ sequences which are not present at the duplicate locus at 87A (Lis et al. 1978, 1981; Ish-Horowicz et al. 1979). The organisation of the HSP 70 coding sequences in the sibling species *D. simulans* is very similar to that in *D. melanogaster* except that *D. simulans* lacks the $\alpha\beta$ sequences (Livak et al. 1978; Hellmund and Serfling 1984). This difference in the organisation of the 87C locus in *D. melanogaster* and *D. simulans* prompted us to compare the effects of 93D activity on 87C transcription in the two species. Our results showed that unlike in *D. melanogaster*, the repression of 93D activity during heat shock has no effect on 87C or 87A transcription in *D. simulans*. In view of this we presume that in *D. melanogaster* the effect is somehow related to the $\alpha\beta$ sequences.

Materials and methods

The flies and larvae of wild-type stocks of *D. simulans* and *D. melanogaster* were cultured on standard food containing agar, cornmeal, brown sugar and yeast at 20° ± 1° C. To obtain interspecific hybrid larvae, virgin females of *D. melanogaster* were aged for 6–7 days and mass mated with males of *D. simulans*. Both *D. simulans* and the hybrid progeny larvae were grown to late third instar stage in Petri dishes provided with additional yeast suspension for healthy growth.

Freshly excised salivary glands from actively migrating late third instar larvae of *D. simulans* and of the interspecific hybrid progeny were subjected to the following treatments in the salt medium described earlier (Lakhotia and Mukherjee 1980).

Temperature shock (TS). The sister salivary glands were separated and incubated either at 37° C (treated) or at 24° C (control) for 30 min. Subsequently, they were labelled with ^{3}H-uridine (500 μCi/ml, sp. act. 15.2 Ci/mM; BARC, Bombay) for 10 min at the respective temperatures.

Offprint requests to: S.C. Lakhotia

Benzamide (BM) treatment. The sister glands were separated and incubated either in medium freshly mixed with BM (1 mg/ml; treated) or in BM-free medium (control) at 24° C for 10 min. They were then labelled with ^{3}H-uridine as above for 10 min at 24° C in the presence or absence of BM, respectively.

TS followed by BM treatment. Freshly excised salivary glands from *D. simulans* and hybrid larvae were incubated at 37° C for 30 min. After 30 min the sister glands were separated and one set each of *D. simulans* and hybrid glands was incubated in medium freshly mixed with BM (1 mg/ml) at 24° C for 10 min (treated) while the other sets of sister glands were incubated in BM-free medium at 24° C for 10 min (control). Subsequently, all glands were labelled with ^{3}H-uridine as above in the presence (treated) or absence (control) of BM.

Colchicine (COLCH) treatment. The sister salivary glands were incubated either in medium containing COLCH (100 µg/ml) or in COLCH-free medium (control) at 24° C for 40 min. After the incubation, the glands were labelled with ^{3}H-uridine in the presence (treated) or absence (control) of COLCH as above.

*Colchicine at 37° C.*As in the preceding case, the sister glands were separated and incubated either in medium freshly mixed with COLCH (100 µg/ml) or in COLCH-free medium at 37° C for 30 min and then labelled with ^{3}H-uridine at 37° C for 10 min in the presence (treated) or absence (control) of COLCH.

Autoradiography. After ^{3}H-uridine labelling, the control and treated glands of the different experiments were fixed with methanol/acetic acid, 3/1, stained with aceto-carmine and squashed in 50% acetic acid. The coverslips were removed after freezing at −70° C. After dehydration, the slides were treated with 5% trichloroacetic acid at 4°–6° C for 10 min, washed in running water, dehydrated, dried and coated with Ilford L4 or K5 nuclear emulsion for autoradiography. After exposure in the dark at 4°–6° C for 2 days, the autoradiograms were developed, fixed, washed, dried and stained in the usual manner.

Results

Since most of the heat shock loci are present on the left and right arms of chromosome *3* (*3L* and *3R*, respectively), we focussed our attention on this chromosome only. The *3R* in *D. simulans* carries a long inversion from 84F1 to 93F6–7 (Ashburner 1969) and thus, compared to *D. melanogaster*, 93D and 87C appear "proximal" to 87A in *D. simulans* polytene chromosomes.

D. simulans

Induction of 93D puffing after TS, BM or COLCH treatments. A 30 min heat shock at 37° C to salivary gland chromosomes of *D. simulans* larvae caused a general inhibition of transcription and induction of the usual eight puffs on chromosome *3*, viz. 63BC, 64F, 67B and 70A on *3L* and 87A, 87C, 93D and 95D on *3R* (also see Ashburner 1970). It is interesting that unlike in *D. melanogaster* (Mukherjee and Lakhotia 1979), 93D in *D. simulans* was not found to be the most active heat shock puff (see Figs. 1 and 2) and its relative activity in different nuclei of a gland was also much less variable (data not presented). The 87A and 87C loci were nearly equally active in all nuclei of heat-shocked glands (Figs. 1 and 2).

As reported earlier for *D. melanogaster* (Lakhotia and Mukherjee 1980, 1984), BM or COLCH treatment also specifically induced the 93D puff and severely inhibited transcription in salivary gland polytene nuclei of *D. simulans* (see Figs. 1 and 2).

Effects of BM or COLCH treatment on the TS response. Previous studies from this laboratory on polytene nuclei of *D. melanogaster* showed that when BM treatment followed a TS, 93D regressed and at the same time 87C was much less active than 87A (Lakhotia and Mukherjee 1980). However, in *D. simulans* salivary glands treated with BM for 10 min following a 30 min TS at 37° C, the 93D puff regressed nearly completely but the 87A and 87C loci remained equally active as revealed by the silver grain count data (see Figs. 1d and 2).

It is also known from earlier data (Lakhotia and Mukherjee 1984) that when salivary glands of *D. melanogaster* are treated with COLCH at 37° C (COLCH+TS), 93D is not induced while the 87C locus is induced to a much greater extent than the 87A. The present results of a comparable treatment in *D. simulans* showed that while 93D was not induced in this case also, the relative activity levels of the 87A and 87C loci were not altered by COLCH+TS treatment (Figs. 1e and 2).

D. melanogaster × *D. simulans hybrid polytene nuclei*

Since *D. melanogaster* and *D. simulans* differ in the response of the 87A and 87C loci to non-induction of 93D after TS followed by BM or TS plus COLCH treatments, it was of interest to examine the response of these loci on *D. simulans* chromosomes in the presence of the 87A, 87C and 93D loci of *D. melanogaster*. Therefore, salivary glands from interspecific hybrid larvae were exposed to various treatments so that interaction, if any, between the two genomes could be ascertained. In the hybrid polytene nuclei, certain homologous chromosome regions always remained synapsed (63BC, 67B and 95D), some were always asynapsed (93D) and others were synapsed or asynapsed (87A and 87C) in different nuclei. The numbers of silver grains on the heat shock loci 63BC, 67B, 87A, 87C, 93D and 95D were counted in well-spread nuclei in the differently treated glands. Since the homologues in the 63BC, 67B and 95D regions were always synapsed, the grain counts for these reflect the cumulative activity of the two homologues. For the 93D locus, grain counts on *D. melanogaster* ($93D^m$) and *D. simulans* ($93D^s$) homologues were separately recorded. For the 87A and 87C loci, which were seen in both the synapsed or asynapsed condition, cumulative grain counts for both homologues or for *D. melanogaster* ($87A^m$ and $87C^m$) and *D. simulans* ($87A^s$ and $87C^s$) homologues were recorded depending upon the state of synapsis.

All the TS loci were induced in hybrid glands exposed to 37° C as in the parental species. The relative activity of homologous loci in *simulans* and *melanogaster* chromosomes was generally comparable (Fig. 4). BM and COLCH treatments at 24° C of salivary glands of hybrid larvae also

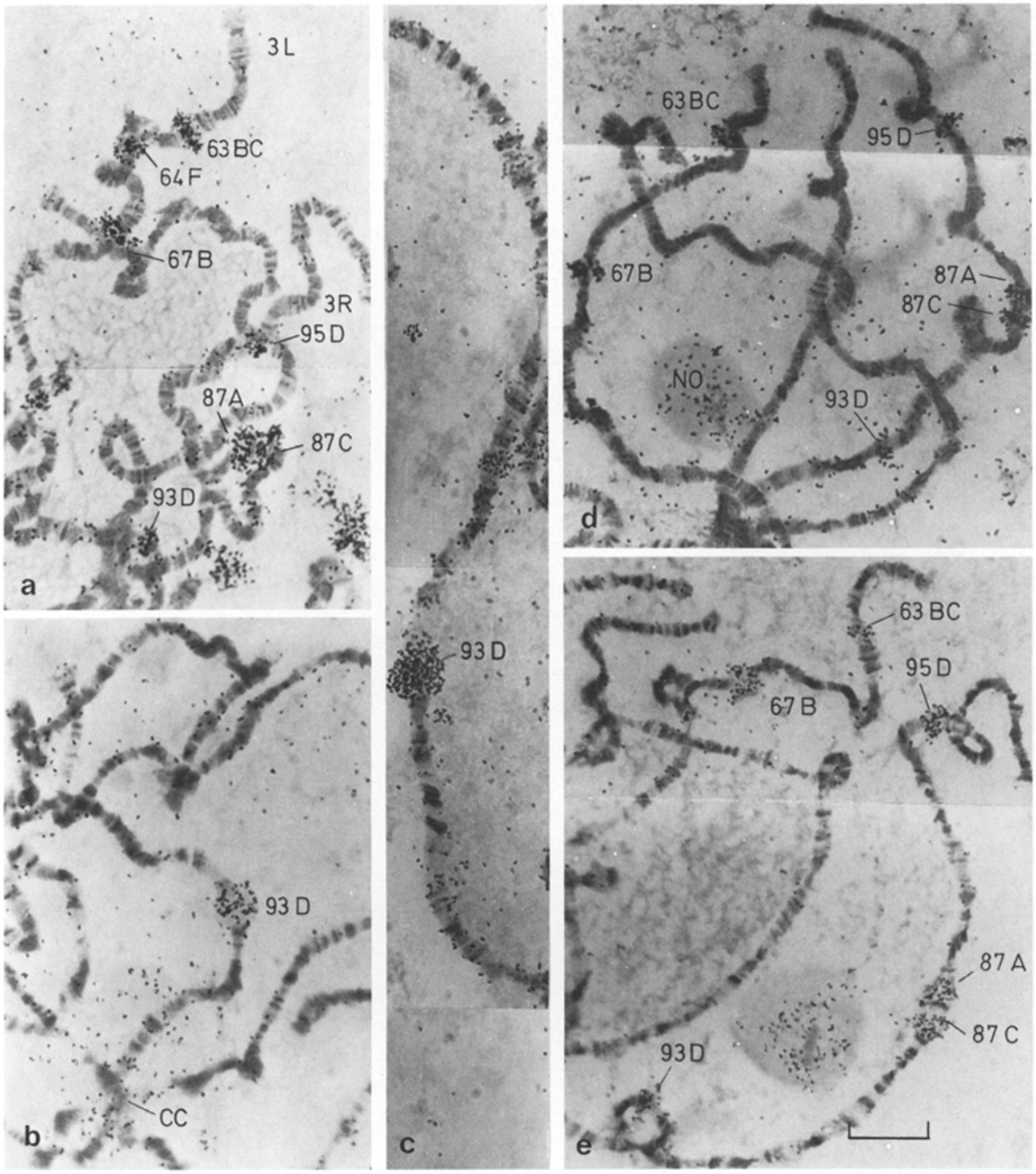

Fig. 1 a–e. Autoradiographs of polytene chromosomes of *Drosophila simulans* showing ^{3}H-uridine labelling of the major heat shock puffs after heat shock (TS) at 37° C (**a**), benzamide (BM) at 24° C (**b**), colchicine (COLCH) at 24° C (**c**), TS followed by (BM) (**d**) or COLCH at 37° C (**e**). Note the nearly equal labelling of 87A and 87C puffs in **d** and **e**. *CC* Chromocentre; *NO* nucleolus. Bar represents 10 μm

specifically induced the 93D puff equally in both homologues (data not presented). In the case of TS followed by BM or TS plus COLCH treatment of hybrid glands, however, the 87A and 87C loci of *melanogaster* and *simulans* origin behaved as for their respective species: after TS followed by BM treatment $87C^{m}$ was less induced than $87A^{m}$ (mean $87C^{m}/87A^{m}$ grain count ratio $=0.60\pm0.03$) while $87C^{s}$ and $87A^{s}$ were equally active (mean $87C^{s}/87A^{s}$ grain count ratio $=1.1\pm0.1$; see Table 1 and Figs. 3a, 4a). Similarly, after TS plus COLCH treatment, $87C^{m}$ was induced to a greater extent than $87A^{m}$ while $87C^{s}$ and $87A^{s}$ were equally induced (see Table 1 and Figs. 3b, 4b). Thus the *melanogaster* and *simulans* homologues appear to behave autonomously with respect to interaction between the 93D, 87A and 87C loci. Since the 87A and 87C puffs on the homologues of the two species remained synapsed in some nuclei, we also compared the relative activity of 87A and 87C puffs in the synapsed condition to see if pairing influenced the activity of $87C^{s}$. The mean ratio of silver grain counts on the 87C and 87A loci in nuclei showing synapsis of these loci was compared with that in nuclei showing asynapsis in this region. In the latter case, the

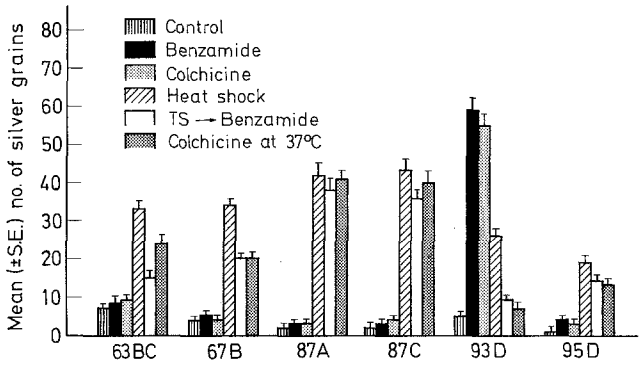

Fig. 2. Incorporation of ^{3}H-uridine (mean no. of silver grains ±S.E.) on major heat shock puffs in *D. simulans* after different treatments. For each data point, 25–35 nuclei were scored

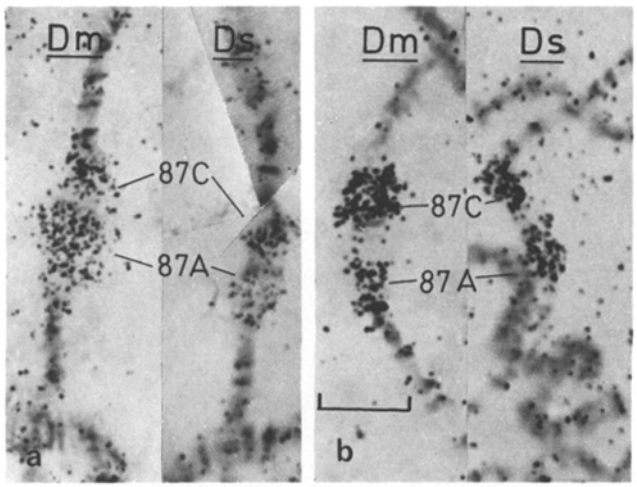

Fig. 3a, b. Autoradiographs of homologous 3R segments of *Drosophila melanogaster* (Dm) and *D. simulans* (*Ds*) origin in a given nucleus in interspecific hybrid salivary glands, labelled with ^{3}H-uridine after heat shock (TS) followed by benzamide (BM) (**a**) or TS plus colchicine (COLCH) (**b**) treatment. Note the differences in the labelling of 87A and 87C of *melanogaster* origin in each case. Bar represents 10 μm

Table 1. Effect of temperature shock followed by benzamide (TS→BM) and temperature shock plus colchicine (TS+COLCH) treatment on the relative activity of the 87A and 87C loci in *Drosophila melanogaster* and *D. simulans* homologues in interspecific hybrid polytene nuclei

Treatment	Mean (±S.E.) grain ratio		*t*-test
	$87C^m/87A^m$	$87C^s/87A^s$	
TS → BM	0.6±0.03 (43)	1.1±0.1 (40)	$P<0.001$
TS+COLCH	1.8±0.2 (23)	1.05±.04 (21)	$P<0.001$

Figures in parentheses indicate the number of nuclei examined in each case

Table 2. Comparison of activity of 87A and 87C loci in interspecific hybrid polytene nuclei subjected to heat shock followed by benzamide (TS→BM) or heat shock plus colchicine (TS+COLCH) treatment, in which the *melanogaster* and *simulans* homologues were synapsed or asynapsed

Treatment	Homologues	Mean (±S.E.) no. of silver grains		Mean (±S.E.) 87C/87A ratio	*t*-test
		87A	87C		
TS → BM	Synapsed	49.3±3.9 (25)	44.2±2.9 (25)	0.88±0.19	$P>0.3$
	Asynapsed[a]	71.7±5.1 (35)	56.0±3.6 (35)	0.81±0.03	
TS+COLCH	Synapsed	46.2±1.9 (25)	56.2±2.5 (25)	1.24±0.04	$P>0.3$
	Asynapsed[a]	67.3±3.9 (24)	86.6±3.8 (24)	1.33±0.07	

Figures in parentheses indicate the number of nuclei examined

[a] The grain counts in nuclei with asynapsis, represent the cumulative number of silver grains on the two homologues

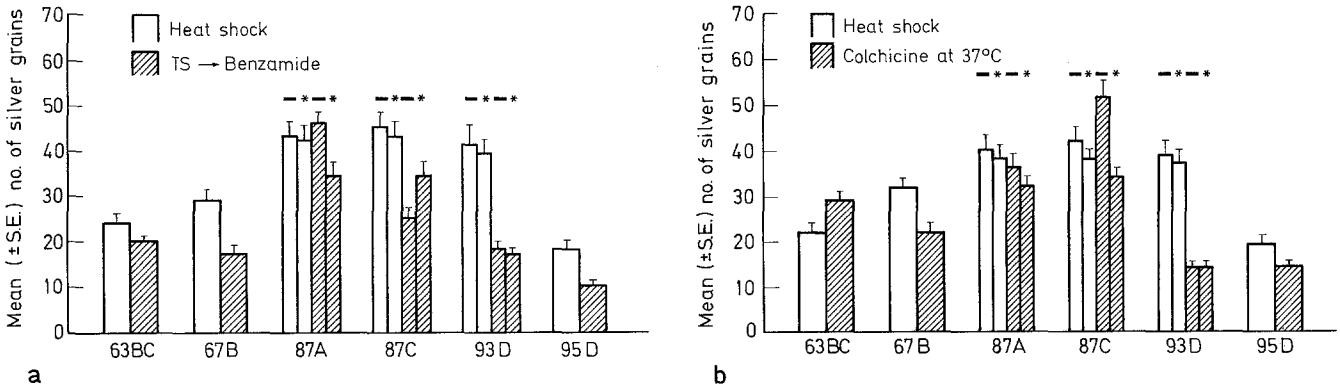

Fig. 4a, b. Incorporation of ^{3}H-uridine (mean no. of silver grains ±S.E.) on major heat shock puff sites in *Drosophila melanogaster* × *D. simulans* interspecific hybrid salivary glands after heat shock (TS) followed by benzamide (BM) (**a**) or TS plus colchicine (COLCH) (**b**) treatment. The bars marked –*–* and * represent the mean grain counts on 87A, 87C or 93D puffs of *melanogaster* or *simulans* origin, respectively

cumulative grain counts on the homologues of the two species were taken into account. The data (see Table 2) revealed that the mean 87C/87A ratios in the two sets of nuclei did not show any significant difference. Thus synapsis with $87C^m$ did not influence the activity of $87C^s$.

Discussion

As may be expected from the close phylogenetic relationship of *D. melanogaster* and *D. simulans*, the response of polytene chromosomes to TS, BM or COLCH treatments

was found to be generally comparable. However a remarkable difference between the two species was found when TS was applied in combination with BM or COLCH since unlike the earlier observations in *D. melanogaster* (Lakhotia and Mukherjee 1980, 1984), these treatments had no effect in *D. simulans* on the transcription of the 87A or 87C heat shock loci. As discussed by Burma and Lakhotia (1986), there is strong evidence that in *D. melanogaster* the 93D locus directly affects the activity of the 87C and 87A loci since whenever the 93D locus is not induced during heat shock, the 87A and 87C loci are activated to varying levels. As in *D. melanogaster*, the 93D locus of *D. simulans* was not induced when TS was given in combination with BM or COLCH. Therefore, it might be expected that in *D. simulans* also, the non-induction of 93D after TS followed by BM or TS plus COLCH treatment, should affect the relative activity of the 87A and 87C loci. This was not seen; moreover, in the interspecific hybrid nuclei, the 87A and 87C loci of the two species behaved autonomously in their response to 93D inactivity as reflected in the 87C/87A grain ratios. The autonomy of the 87A and 87C loci of *melanogaster* and *simulans* origins in interspecific hybrids is complete since even when the two homologues were synapsed in this part of *3R*, there was no evidence of a *transvection* effect (Lewis 1954) and the 87C/87A grain ratios in synapsed and asynapsed chromosomes remained similar (Table 2).

The reason for this remarkable difference between the two species may be a difference in the organisation of the 93D or 87C loci. From available data on the rapid evolutionary divergence of DNA sequences at the 93D-like heat shock loci in different species (Peters et al. 1981; Walldorf et al. 1984; Garbe and Pardue 1986) it remains possible that there has been a divergence in the 93D sequence between *melanogaster* and *simulans*. However, the lack of a 93D effect on 87C transcription in *D. simulans* may not be due only to possible differences in 93D structure in the two species for the following reasons: (i) in its response to BM, COLCH or Combination treatments, $93D^s$ behaves very similarly to $93D^m$ and (ii) if $93D^s$ fails to affect $87C^s$ because it differs structurally from $93D^m$, it would be expected at $93D^m$ would act upon $87C^s$ in the interspecific hybrid cells but this was not seen.

From the available information on the organisation of the 87C locus in *melanogaster* and *simulans* (Lis et al.1978, 1981; Livak et al. 1978; Ish-Horowicz et al. 1979; Hellmund and Serfling 1984), it is known that $87C^s$ lacks the $\alpha\beta$ sequences which are present on $87C^m$; also the $\alpha\beta$ sequences present in the chromocentre region in *simulans*, as in *melanogaster* are not heat inducible. In *D. melanogaster* the $\alpha\beta$ sequences at 87C are actively transcribed in response to TS and are under different controls from the HSP 70 coding sequences (Lengyel and Graham 1984). In view of these results, it may be suggested that the observed lack of a 93D effect on $87C^s$ is related to the absence of the $\alpha\beta$ sequences at this locus in *simulans*. In a preliminary study, the levels of the $\alpha\beta$ transcripts in *D. melanogaster* cells exposed either to TS, TS followed by BM or TS plus COLCH treatments were found to vary in accordance with increased or decreased levels of relative activity of the 87C locus (Lakhotia and Pardue 1986).

The functional significance of the 93D effect on 87C transcription in *D. melanogaster* cells remain unknown. The possible involvement of the $\alpha\beta$ sequences (see above) in this interaction makes the situation more intriguing since, like the 93D transcripts, the $\alpha\beta$ transcripts have also not been correlated with any known function. Indeed, it has been suggested that the TS inducibility of the $\alpha\beta$ sequences at 87C of *D. melanogaster* may be accidental due to their being brought by transposition close to the HSP 70 promoters (see Hellmund and Serfling 1984). Enough data are not available to make a definite statement regarding the significance or otherwise of the 93D effect on 87C in *D. melanogaster* and its absence in *D. simulans,* but we favour the view that this interaction is a significant aspect of the heat shock response in *D. melanogaster*.

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References

Ashburner M (1969) Patterns of puffing activity in the salivary gland chromosomes of Drosophila. III. A comparison of the autosomal puffing patterns of the sibling species Drosophila melanogaster and D. simulans. Chromosoma 27:64–85

Ashburner M (1970) Patterns of puffing activity in the salivary gland chromosomes of Drosophila. V. Responses to environmental treatments. Chromosoma 31:356–376

Ashburner M, Bonner JJ (1979) The induction of gene activity in Drosophila by heat shock. Cell 17:241–254

Burma PK, Lakhotia SC (1986) Expression of 93D heat shock puff of Drosophila melanogaster in deficiency genotypes and its influence on activity of the 87C puff. Chromosoma 94:273–278

Garbe JC, Pardue ML (1986) Heat shock locus 93D of Drosophila melanogaster; a spliced RNA most strongly conserved in the intron sequence. Proc Natl Acad Sci USA 83:1812–1816

Hellmund D, Serfling E (1984) Structure of hsp coding genes in Drosophila. In: Nover L(ed) Heat shock response in eukaryotic cells. Springer, Berlin, Heidelberg, New York, pp 18–27

Ish-Horowicz D, Schedl P, Artavanis-Tsakonas S, Mirault ME (1979) Genetic and molecular analysis of the 87A7 and 87C1 heat inducible loci of Drosophila melanogaster. Cell 18:1351–1358

Lakhotia SC, Mukherjee T (1980) Specific activation of puff 93D of Drosophila melanogaster by benzamide and the effect of benzamide treatment on the heat shock induced puffing activity. Chromosoma 81:125–136

Lakhotia SC, Mukherjee T (1982) Absence of novel translation products in relation to induced activity of the 93D puff in Drosophila melanogaster. Chromosoma 85:369–374

Lakhotia SC, Mukherjee T (1984) Specific induction of the 93D puff in polytene nuclei of Drosophila melanogaster by colchicine. Ind J Exp Biol 22:67–70

Lakhotia SC, Pardue ML (1986) The 93D effects on heat shock puffing at 87C in Drosophila melanogaster are due to changing levels of $\alpha\beta$ transcripts. Abstracts 9th All India Cell Biol Conference p 31

Lakhotia SC, Singh AK (1982) Conservation of the 93D puff of Drosophila melanogaster in different species of Drosophila. Chromosoma 86:265–278

Lakhotia SC, Singh AK (1985) Non-inducibility of the 93D heat shock puff in cold-reared larvae of Drosophila melanogaster. Chromosoma 92:48–54

Lengyel JA, Graham ML (1984) Transcription, export and turnover of Hsp 70 and two $\alpha\beta$ Drosophila heat shock genes sharing a 400 nucleotide 5′ upstream region. Nucleic Acids Res 12: 5719–5739

Lewis EB (1954) The theory and application of a new method of detecting chromosomal rearrangements in Drosophila melanogaster. Am Nat 88:225–239

Lis JT, Ish-Horowicz D, Pinchim SM (1981) Genomic organization and transcription of the 'alpha' 'beta' heat shock DNA in Drosophila melanogaster. Nucleic Acids Res 9:5297–5310

Lis JT, Prestidge L, Hogness DS (1978) A novel arrangement of tandemly repeated genes at a major heat shock site in D. melanogaster. Cell 14:901-919

Livak KJ, Freund R, Schwebber M, Wensink PC, Meselson M (1978) Sequence organization and transcription at two heat shock loci in Drosophila. Proc Natl Acad Sci USA 75:5613–5617

Mohler J, Pardue ML (1982) Deficiency mapping of the 93D heat shock locus in Drosophila melanogaster. Chromosoma 86:457–467

Mukherjee T, Lakhotia SC (1979) ^{3}H-uridine incorporation in the puff 93D and chromocentric heterochromatin of heat shocked salivary glands of Drosophila melanogaster. Chromosoma 74:75–82

Mukherjee T, Lakhotia SC (1982) Heat shock puff activity in salivary glands of Drosophila melanogaster larvae during recovery from anoxia at two different temperatures. Ind J Exp Biol 20:437–439

Peters FPAMN, Lubsen NH, Sondermeijer PJA (1981) Rapid sequence divergence in a heat shock locus of Drosophila. Chromosoma 81:271–280

Walldorf J, Richter S, Ryseck RP, Steller H, Edstrom JE, Bautz EKF, Hovemann B (1984) Cloning of heat shock locus 93D from Drosophila melanogaster. EMBO J 3:2499–2504

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