Chromosoma (Berl) (1986) 94:273–278

CHROMOSOMA

Expression of 93D heat shock puff of *Drosophila melanogaster* in deficiency genotypes and its influence on activity of the 87C puff

Pradeep Kumar Burma and S.C. Lakhotia
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

Abstract. Using the overlapping deficiencies *Df(3R)GC14* and *Df(3R)e^{Gp4}* of the 93D region of *Drosophila melanogaster,* the benzamide (BM)-inducible site in polytene chromsomes was localised to the 93D6–7 region, which had earlier been identified as heat inducible. The normal developmental and BM-induced 93D6–7 puff was found to be dosage compensated since in larvae heterozygotus for a deficiency, with one dose of 93D6–7, the rate of ^{3}H-uridine incorporation in this puff was the same as in the wild type with two doses. Curiously, however, heat shock (37° C) caused regression of the 93D6–7 puff on the normal chromosome in heterozygotes. In agreement with earlier results from our laboratory, the non-inducibility of the single-dose 93D locus by heat shock in the heterozygotes, caused the 87C puff to be nearly half as active as the 87A puff at 37° C. However, in *e^{Gp4}/GC14* larvae, lacking the 93D6–7 locus on both homologues, the 87C puff was less active than 87A in some heat-shocked larvae but in other larvae 87A and 87C were equally active. Possible reasons for this inter-larval variability are discussed.

Introduction

The major heat shock puff at 93D in *Drosophila melanogaster* has several distinctive features. It can be specifically induced by agents like benzamide (Lakhotia and Mukherjee 1980) and colchicine (Lakhotia and Mukherjee 1984) and apparently has no translational product (Lakhotia and Mukherjee 1982), although a deletion of this locus affects the viability of the fly (Mohler and Pardue 1984). An equivalent of the 93D locus seems to be a component of the heat shock response in every *Drosophila* species studied (Lakhotia and Singh 1982).

The 93D heat shock site has been mapped cytologically between the distal breakpoint of *Df(3R)e^{Gp4}* and the proximal breakpoint of *Df(3R)GC14*, i.e. in the 93D6–7 region of *3R* (Mohler and Pardue 1982). Bearing in mind that 93D is also specifically induced by benzamide (Lakhotia and Mukherjee 1980) we used the overlapping deficiencies *e^{Gp4}* and *GC14* (Mohler and Pardue 1982) to test whether the benzamide trancription site corresponds cytologically to the heat shock trancription site.

Earlier observations in our laboratory had shown that any perturbation during heat shock that led to a regression of the 93D puff also resulted in unequal expression of the duplicate heat shock loci at 87A and 87C which are otherwise induced equally by heat shock (Lakhotia and Mukherjee 1980, 1984; Lakhotia and Singh 1985, Mukherjee and Lakhotia 1982). The availability of the overlapping deficiencies allowed an examination of the response of the 87A and 87C loci to heat shock in the absence of 93D locus. Our results showed that in the genotype deficient for 93D, the 87C locus was indeed less active than 87A. They also revealed that in heterozygotes, with only one dose of the 93D locus, the normal developmental as well as benzamide-induced activity was dosage compensated. However, it is intriguing that in response to heat shock the 93D locus in the heterozygotes which is normally hyperactive for dosage compensation, regressed completely, rather than becoming more active.

Offprint requests to: S.C. Lakhotia

Materials and methods

Strains. Wild-type (Oregon R$^+$) and mutant stocks carrying deficiencies of parts of the right arm of chromosome *3* of *Drosophila melanogaster* were used for these studies. The deficiency stocks were *Df(3R)GC14/TM6B* and *Df(3R)e^{Gp4}/TM6B* (described by Mohler and Pardue 1982, 1984). In *Df(3R)GC14* the deficiency spans from 93D6–7 to 93D10 while *Df(3R)e^{Gp4}*, lacks 93B11–13 to 93D6–7. These two stocks along with the wild-type stock of *D. melanogaster* were use to generate larvae homozygously and heterozygously deficient for the 93D6–7 bands which harbour the heat shock locus (Mohler and Pardue 1982). The crosses used to generate such larvae are summarised in Table 1.

Culture conditions. All *Drosophila* stocks and progeny larvae were grown on standard medium containing agar, cornmeal, brown sugar and yeast at 20°±1° C. Eggs were collected at hourly intervals in food-filled Petri dishes. Larval cultures were provided with additional yeast suspension for healthy growth.

Treatments. Freshly excised salivary glands from actively migrating late third instar larvae of different genotypes (Table 1) were used for the different treatments. The medium used for dissection and incubation of glands for the treatments was as described earlier (Lakhotia and Mukherjee 1980). For heat shock (HS), sister salivary glands from dif-

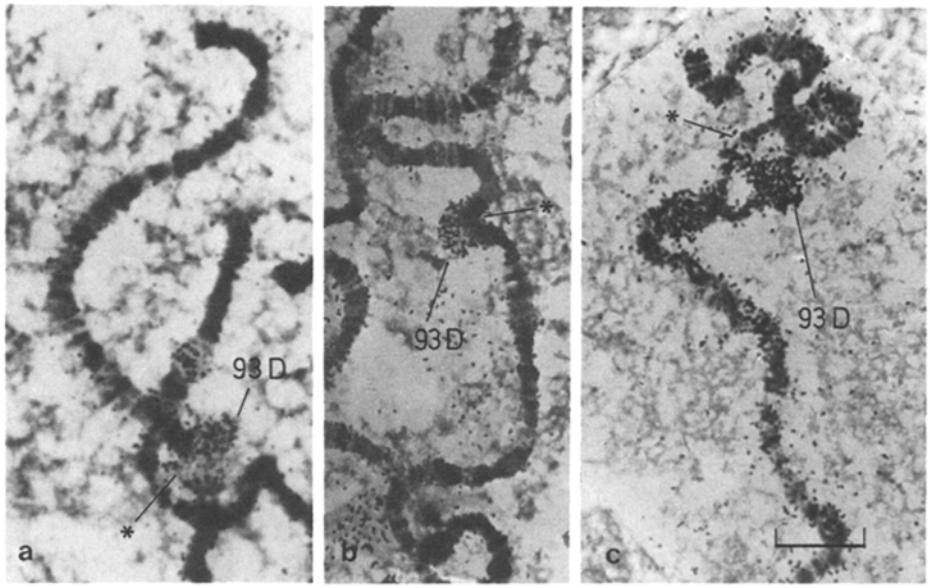

Fig. 1 a–c. Autoradiographs of segments of *3R* in $Df(3R)e^{Gp4}/+$ (**a**) and in $Df(3R)GC14/+$ (**b, c**) polytene nuclei from benzamide (BM)-treated salivary glands. The 93D region in the deficiency homologue is marked with a *star*. In **b** and **c**, the homologues ($GC14/+$) are synapsed or asynapsed, respectively. Note the BM-induced asymmetric 93D puff in **a** and **b** and the absence of an induced puff in the 93D region of the asynapsed deficiency homologue in **c**. Bar represents 10 μm

Table 1. Summary of genotypes of larvae used for experiments and the crosses generating them

Crosses	$Df(3R)GC14/TM6B$♂ × +/+ (wild-type)♀	$Df(3R)e^{Gp4}/TM6B$♂ × +/+ ♀	$Df(3R)GC14/TM6B$♂ × $Df(3R)e^{Gp4}/TM6B$♀	+/+ × +/+
F_1 progeny used for experiments	$Df(3R)GC14/+$[a]	$Df(3R)e^{Gp4}/+$[a]	$Df(3R)e^{Gp4}/Df(3R)GC14$[a]	+/+
Dosage of 93D6–7 bands	One	One	Nil	Two

[a] These larvae were easily recognised from the other F_1 progeny by being non-tubby while the other F_1 progeny were tubby due to the TM6B chromosome

ferent genotypes were separated and incubated either at 37° C (treated) or at 24° C (control) for 30 min, followed by labelling with ^{3}H-uridine (500 μCi/ml, sp. act. 15.2 mCi/mmol; BARC, Bombay) for 10 min at 37° C and 24° C, respectively. For benzamide treatment (BM), the sister salivary glands were separated and incubated either in medium freshly mixed with benzamide (1 mg/ml) or in benzamide-free medium (control) at 24° C for 10 min. Next the treated and control glands were labelled with ^{3}H-uridine as above for 10 min at 24° C either in BM medium (treated) or in BM-free medium (control).

After ^{3}H-uridine labelling, the glands were fixed, stained, squashed (one gland per slide) and processed for autoradiography with Ilford L4 or Kodak NTB2 emulsion in the usual manner. In the case of heterozygous larvae ($Df(3R)GC14/+$ or $Df(3R)e^{Gp4}/+$), glands from similarly treated wild-type (+/+) larvae were squashed on the same slide, as internal controls. The autoradiograms were exposed for 3 days in the dark at 4°–6° C and processed with D19B developer and acid fixer. After washing, the slides were stained with Giemsa.

Results

BM-inducible site at 93D6–7

Examination of ^{3}H-uridine-labelled autoradiograms of BM-treated salivary glands from $GC14/+$ or $e^{Gp4}/+$ larvae revealed that for both genotypes, whenever the homologues remained asynapsed, a BM-inducible puff in the 93D region was seen only on the normal chromosome while the homologue carrying the deficiency showed no activity in the 93D region (see Fig. 1). The number of silver grains seen in the 93D region of the deficiency chromosome in BM-treated nuclei was always less than five while on the + chromosome it was much higher (data not presented). In nuclei where the homologues were synapsed, an asymmetrical puff was usually seen (Fig. 1 a, b). In larvae carrying both deficiencies ($GC14/e^{Gp4}$) neither *3R* homologue developed a BM-inducible puff (data not presented). Since the *GC14* and e^{Gp4} chromosomes have only a deficiency of the 93D6–7 region in common, the BM-inducible site, like heat shock (Mohler and Pardue 1982), must also be located in the 93D6–7 bands.

Dosage compensation at the 93D locus

To compare the rate of ^{3}H-uridine incorporation at the 93D6–7 puff when one ($GC14/+$ or $e^{Gp4}/+$) or two doses (+/+) of this region were present, we counted the numbers of silver grains on the 93D6–7 puff and a segment of *3R* (from 94A to 99F) in control and BM-treated salivary glands. A comparison of the 93D/*3R* segment grain count ratios (Fig. 2) revealed that in all cases (control as well as BM-induced) the relative rate of transcription (^{3}H-uridine incorporation) in one dose ($GC14/+$ and $e^{Gp4}/+$) and two dose (+/+) genotypes was similar (Fig. 2). Thus transcription of 93D6–7 is dosage compensated.

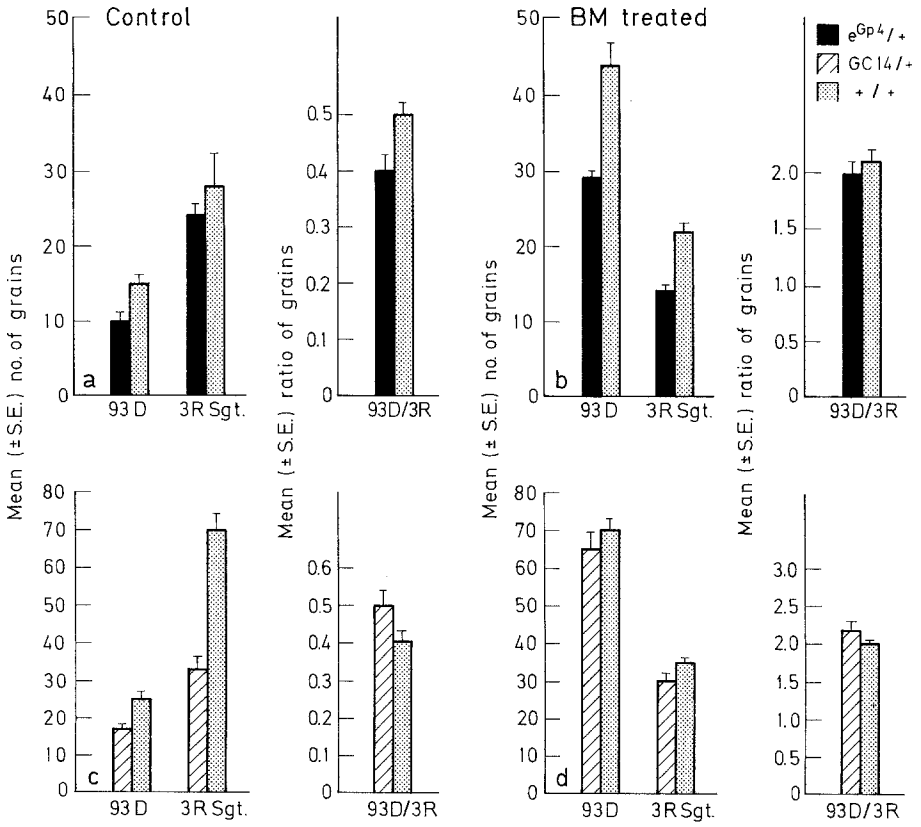

Fig. 2a–d. Incorporation of ^{3}H-uridine (mean no. of silver grains ±S.E.) on the 93D6–7 region and a segment of *3R* (94A to 99F, *3R sgt*) in control $e^{Gp4}/+$ and +/+ (**a**), benzamide (BM)-treated $e^{Gp4}/+$ and +/+ (**b**), control *GC14*/+ and +/+ (**c**) and BM-treated (*GC14*/+ and +/+ (**d**) salivary glands. The mean 93D/*3R* ratio of ^{3}H-uridine incorporation is also shown which compares the relative activity of 93D in each case. A *t*-test analysis of the 93D/*3R* ratios in deficiency/+ and corresponding +/+ genotypes in each case showed that the ratios are not significantly different ($P<0.05$). For each data point, 25–30 nuclei were scored

Heat shock fails to induce the 93D puff in larvae heterozygous for deficiency

It was intriguing that when salivary glands from *GC14*/+ or $e^{Gp4}/+$ larvae were heat shocked, the 93D6–7 region in the normal homologue did not develop the expected heat shock puff (Fig. 3). After ^{3}H-uridine labelling, silver grains on the 93D, 87A and 87C heat shock loci and a segment (from 96A to 99F) of *3R* in control and heat-shocked glands of *GC14*/+ and $e^{Gp4}/+$ larvae were compared (Fig. 4). It was seen that in *GC14*/+ and $e^{Gp4}/+$ larvae, the silver grain counts on the 93D region of the normal chromosome were actually reduced rather than elevated in heat-shocked glands. A comparison of the mean ratios of 93D/*3R* segment grain counts in control and heat-shocked glands by Student's *t*-test revealed that the values for the deficiency genotypes were significantly lower ($P<0.05$) in heat-shocked than in control glands. Thus for the *GC14*/+ or $e^{Gp4}/+$ genotype heat shock repressed rather than induced 93D activity.

The 87A and 87C heat shock loci are unequally expressed in larvae deficient for 93D

As can be seen in Figure 3 and Figure 5, in contrast to the wild-type, the heat shock induced incorporation of ^{3}H-uridine at the 87A and 87C puffs was unequal in larvae of both the *GC14*/+ and $e^{Gp4}/+$ genotypes. While the mean 87A/87C grain ratio in heat-shocked wild-type glands was close to 1.0, it was 2.0 ± 0.07 and 1.7 ± 0.07 in $e^{Gp4}/+$ and *GC14*/+ glands, respectively (Fig. 5).

A comparison of the mean grain counts on the 87A and 87C heat shock puffs in +/+ and *GC14*/+ glands labelled and autoradiographed in parallel (see Materials and methods and Fig. 5) revealed that the activity of the 87A puff was comparable in both but that of the 87C puff was significantly reduced in heterozygotes (Fig. 5). Thus the elevated 87A/87C ratio in *GC14*/+ glands was due to a reduced rate of ^{3}H-uridine uptake at the 87C puff. A similar situation appeared to exist in $e^{Gp4}/+$ glands although the overall lower labelling of all puffs in these glands precluded a direct comparison.

In $GC14/e^{Gp4}$ larvae the response of the 87A and 87C loci varied between larvae (Fig. 6), although all nuclei in glands of a given larva displayed a similar response. Data on ^{3}H-uridine labelling of the 87A and 87C puffs in heat-shocked glands from seven different $GC14/e^{Gp4}$ larvae are presented in Table 2. No significant labelling in the 93D region was seen in any of them (data not shown). In three of these larvae (A_1, A_2 and A_3), the 87A/87C grain ratio was close to 1.0, while in four other larvae (B_1 to B_4), it was greater than 1.0. A *t*-test analysis of the mean 87A/87C ratios (see Table 2) indicated that within set A and within set B, the mean 87A/87C ratios for different larvae did not differ significantly (Table 2). However, when the pooled mean of A was compared with that of B, the difference was highly significant ($P<0.001$). Thus in some *GC14*/e^{Gp4} larvae, the 87A and 87C loci were induced equally following heat shock while in others the 87C puff was induced to a lesser extent than 87A as for genotypes with a single dose of 93D (*GC14*/+ and $e^{Gp4}/+$, see above).

Discussion

Observations on ^{3}H-uridine incorporation in BM-treated salivary glands of *Df(3R)GC14*/+, $Df(3R)e^{Gp4}/+$ and $Df(3R)GC14/Df(3R)e^{Gp4}$ larvae showed that the BM-inducible transcription site at 93D lies between the distal breakpoint of $Df(3R)e^{Gp4}$ and the proximal breakpoint of

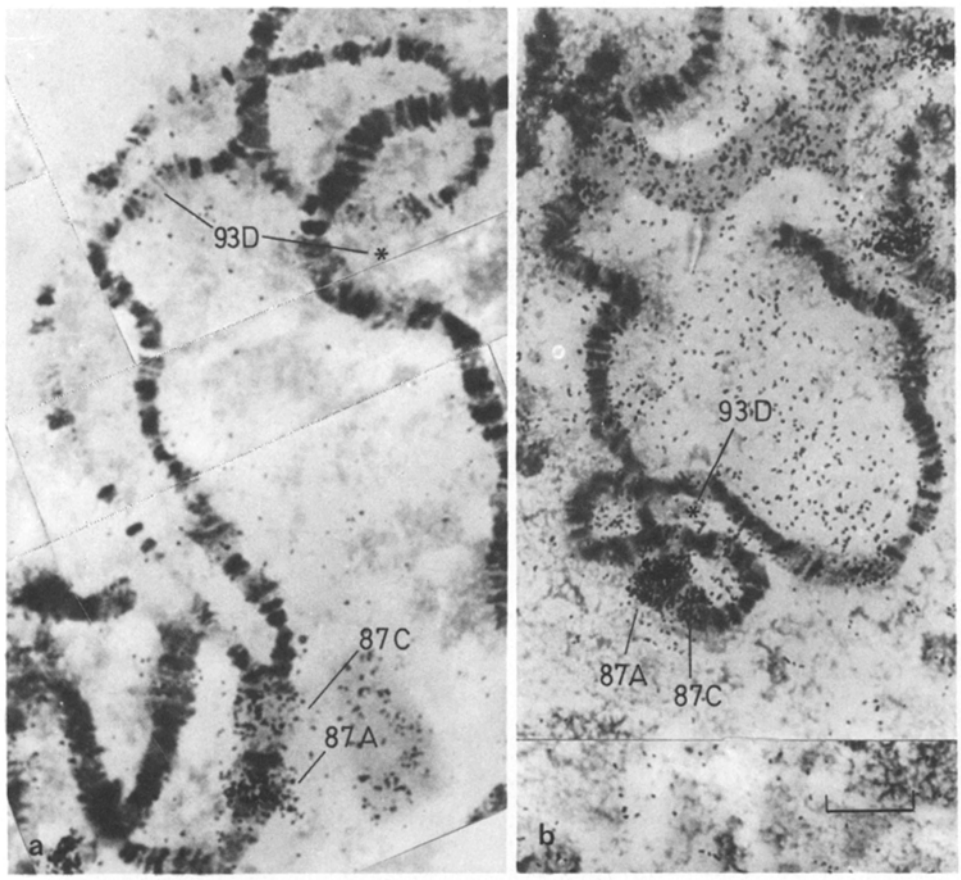

Fig. 3a, b. Autoradiographs of segments of *3R* showing ^{3}H-uridine labelling of the 87A, 87C and 93D regions following heat shock at 37° C of $e^{Gp4}/+$ (**a**) and *GC14*/+ (**b**) salivary glands. The 93D region of the deficiency homologue is marked with an *asterisk*. Note the unequal labelling of the 87A and 87C puffs and nearly completely unlabelled 93D on the wild-type as well as the deficiency homologue. Bar represents 10 μm

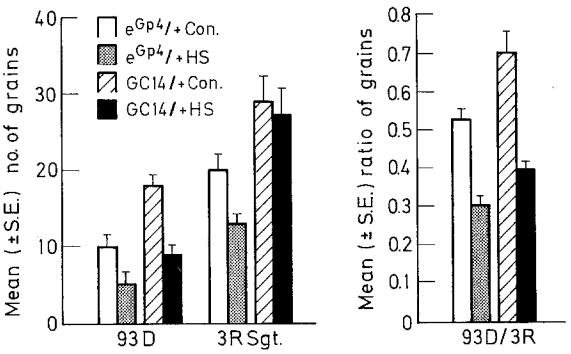

Fig. 4. Incorporation of ^{3}H-uridine (mean no. of silver grains ± S.E.) on 93D and *3R* segment (96A to 99F) from control (*Con*) and heat shocked (*HS*) $e^{Gp4}/+$ and *GC14*/+ larvae. The mean 93D/*3R* grain ratios are also shown. For each data point, 25–30 nuclei were scored

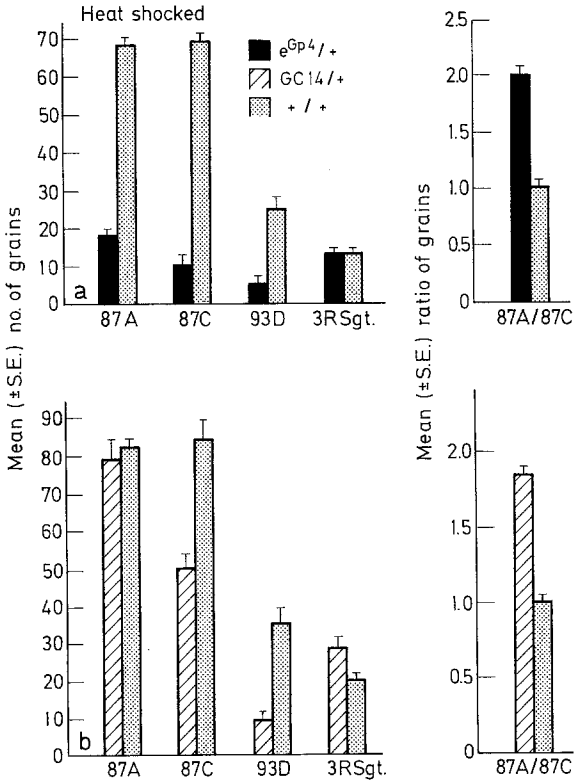

Fig. 5a, b. Incorporation of ^{3}H-uridine (mean no. of silver grains ± S.E.) on 87A, 87C, 93D and *3R* segment (96A and 99F) from heat-shocked $e^{Gp4}/+$ and +/+ (**a**) and *GC14*/+ and +/+ (**b**) salivary glands. The mean 87A/87C grain ratio in each case is shown on the right.

Df(3R)GC14, i.e. in the 93D6–7 region. The heat shock locus at 93D (Mohler and Pardue 1982) and the BM locus are, therefore, cytologically identical.

In recent years certain autosomal loci of *D. melanogaster* have been shown to be dosage compensated at the level of transcription (Devlin et al. 1982, 1984, 1985). Our results show that for developemental as well as BM-induced activity, the 93D locus is also dosage compensated and in the single dose condition becomes hyperactive. In the earlier reported cases of autosomal loci, dosage compensation was found to be operative only in whole arm trisomy and not for loci with small deficiencies or duplications (Devlin et al. 1982, 1984, 1985). Thus the finding that transcription at the 93D puff is dosage compensated even in genotypes with

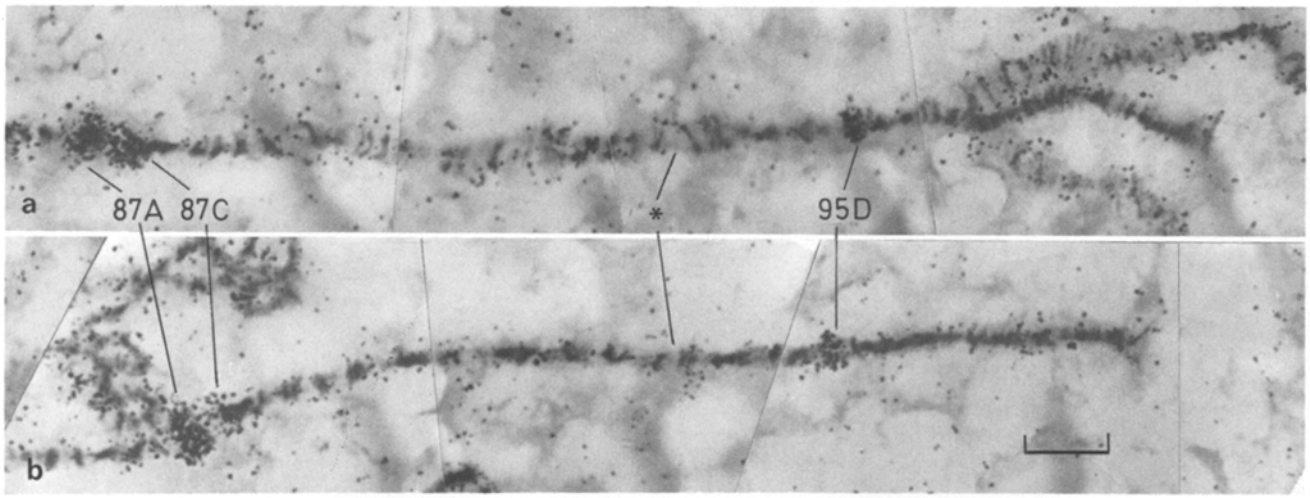

Fig. 6a, b. Autoradiographs of ^{3}H-uridine labelled segments of *3R* in *Df(3R)GC14/Df(3R)e^{Gp4}* salivary glands after heat shock at 37° C showing an equal level of incorporation at 87A and 87C puffs (**a**) or unequal (87A>87C) incorporation at the 87A and 87C puffs (**b**). The 93D region is marked with a *star*. Bar represents 10 μm

Table 2. Analysis of 87A/87C activity in individual *GC14/e^{Gp4}* larvae (for details see text)

Larva	Mean (±S.E.) no. of grains		Mean 87A/87C grain ratio (±S.E.)	*t*-test analysis between ratios of 87A/87C from different larvae		*P*-value
	87A	87C		Test between	*t*-value	
A_1	26.7±1.2 (10)	24.2±1.9 (10)	1.15±0.08 (10)	A_1–A_2	1.00	>0.3
A_2	30.0±1.4 (4)	29.2±3.8 (4)	1.04±0.05 (4)	A_1–A_3	0.47	>0.5
A_3	20.8±1.1 (7)	20.6±2.0 (7)	1.07±0.13 (7)	A_2–A_3	0.20	>0.7
B_1	24.4±1.5 (7)	18.0±2.2 (7)	1.45±0.14 (7)	B_1–B_2	2.00	>0.05
				B_1–B_3	1.33	>0.1
B_2	38.3±6.4 (6)	20.2±2.5 (6)	1.89±0.77 (6)	B_1–B_4	1.75	>0.1
				B_2–B_3	1.78	>0.05
B_3	33.3±2.7 (13)	22.1±1.7 (13)	1.57±0.07 (13)	B_2–B_4	0.41	>0.9
B_4	31.2±2.4 (6)	17.7±1.5 (6)	1.80±0.14 (6)	B_3–B_4	1.53	>0.1
Pooled A	25.4±1.0	24.0±1.3	1.10±0.06	A–B	6.55	<0.001
Pooled B	32.0±1.8	19.8±1.0	1.65±0.06			

Numbers in parantheses indicate the number of nuclei examined in each larva

small deficiencies is very significant and implies that the locus is auto-regulated at the level of transcription. Although the function of the 93D locus is not known, the existence of a dosage compensation mechanism suggests that it is of vital importance.

It is very interesting that while the developmental and BM-induced activity, of 93D in larvae heterozygous for a deficiency was found to be dosage compensated, the activity was nearly completely repressed in response to heat shock. This repression of 93D in our study appears to be contradictory to the earlier observations of Mohler and Pardue (1982) who reported that the 93D locus was active in heat-shocked glands of genotypes similar to those used by us. The reasons for these different results are not clear but two possibilities may be noted: (i) the response of 93D to specific inducers is influenced by the conditions of larval development (Lakhotia and Singh 1982) and the conditions of larvae rearing in the two laboratories may differ in some unspecified manner. (ii) A detailed comparison of the 93D activity on the normal chromosome in Mohler and Pardue's (1982) study is not available for control and heat-shocked glands and therefore, it cannot be ascertained if the ^{3}H-uridine incorporation seen on the 93D locus after heat shock was actually greater (induction) or lesser (repression) than seen in control glands. However, our results are in agreement with earlier studies on wild-type larvae in this laboratory which showed that if the 93D puff is already hyperactive (due to BM, colchicine or other treatments), heat shock causes its nearly complete regression (Lakhotia and Mukherjee 1980, 1984; Lakhotia and Singh 1985; Mukherjee and Lakhotia 1982). In the present case in *e^{Gp4}/+* or *GC14/+* genotypes, the 93D locus on the noraml

chromosome is hyperactive to achieve dosage compensation (see above) and thus responds to heat shock by regression.

It is interesting to note that the regression of 93D is seen only when heat shock is the second inducer since BM is still able to activate the single dose, hyperactive 93D in heterozygotes. This suggests that even though the heat shock and BM-inducible puffs are located at the same cytological site, 93D6–7, their RNA metabolism differs. In this context, it is already known that more than one kind of transcript is synthesised by the 93D locus (Ryseck et al. 1985; Garbe and Pardue 1986). Further analysis of the transcripts induced at the 93D locus by different treatments is required to understand the basis and significance of a hyperacitve 93D locus.

Earlier studies in our laboratory had shown that whenever heat shock was given in addition to another inducing agent (like BM, colchicine, etc), the resulting regression of the 93D puff was always accompanied by 87C becoming less or more active than 87A. On the basis of this temporal correlation, it was suggested that the transcriptional activity of the 93D locus somehow influences the 87C puff (Burma and Lakhotia 1984; Lakhotia and Mukherjee 1980, 1984; Lakhotia and Singh 1985; Mukherjee and Lakhotia 1982; also see Chowdhuri and Lakhotia 1986). However, it remained possible that the inducing agents themselves directly affected 87A/87C transcription rather than this effect being due to 93D. The present observation that in the $e^{Gp4}/+$ and *GC14*/+ heterozygotes, the non-induction of the single dose 93D locus by heat shock is also accompanied by the 87C puff being induced to only half the level of the 87A puff, is very significant. Since no other treatment was involved, the unequal expression of the 87A and 87C loci in these heterozygotes can be directly ascribed to the failure of the 93D locus to respond to heat shock.

The variability in the $e^{Gp4}/GC14$ larvae with respect to the relative activity of the 87A and 87C loci is puzzling. Only about 50% of the larvae (set B in Table 2) displayed the expected reduction in 87C activity in response to heat shock while the other larvae (set A) behaved as if they had an active 93D locus and the 87A and 87C were induced to equal levels. This difference in the two groups of larvae with apparently the same genotype becomes all the more intriguing because the 87A/87C grain ratios in all polytene nuclei in heat-shocked glands of a given $e^{Gp4}/GC14$ larva were either close to 1.0 or significantly higher than 1.0. There are two possible explanations of this variability: (i) due to a recombinational or other event in the heterozygous parent, some of the deficiency-carrying gametes may actually transmit part or all of the 93D6–7 heat-inducible sequences so that the resulting $e^{Gp4}/GC14$ larvae are not really deficient for 93D6–7 sequences and thus develop the 87A and 87C puffs equally when heat shocked. (ii) 93D transcripts may be maternally inherited and if so, it is possible that in some of the $e^{Gp4}/GC14$ larvae, the maternal 93D transcripts may be differently metabolised and remain available until late larval life so that the 87A and 87C loci in these larvae become equally induced by heat shock. At present there is no evidence to support these speculations but they remain interesting issues for further analysis.

Finally, the present study has revealed more intriguing properties of the already enigmatic 93D locus of *D. melanogaster*. The existence of a dosage compensation mechanism at this locus and the distinct effect of 93D on 87A/87C transcription provide further evidence for a vital role of this locus under normal and stress conditions.

Acknowledgements. This work was supported by a research fellowship to PKB from the Council of Scientific and Industrial Research (New Delhi) and by a Department of Atomic Energy (Government of India, Bombay) grant to SCL.

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Received February 6, 1986 / in revised form May 14, 1986
Accepted by J.H. Taylor