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Mutations affecting β-alanine metabolism influence inducibility of the 93D puff by heat shock in *Drosophila melanogaster*

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Abstract. Effect of mutations at the ebony or black locus on induction of heat shock puffs in polytene nuclei of salivary glands of *Drosophila melanogaster* larvae were examined by [^{3}H]uridine autoradiography. The levels of β-alanine in the body are known to be increased by mutation at the ebony locus but decreased by mutation at the black locus. The presence of mutant allele/s at either locus in the homo- or heterozygous condition prevented induction of the 93D puff by heat shock. Elimination of the mutant allele at the ebony or black locus by recombination or by reversion of a P element insertion mutant allele of ebony restored the heat shock inducibility of the 93D puff. In vivo or in vitro administration of excess β-alanine to salivary glands of wild-type larvae also resulted in the 93D site being refractory to heat shock induction. In agreement with earlier results, non-induction of the 93D puff during heat shock due to the β-alanine effect was accompanied by unequal puffing of the 87A and 87C loci. The selective inducibility of the 93D puff by benzamide was not affected by ebony or black mutations or by excess β-alanine in wild-type larvae

Introduction

Among the heat-inducible genes of *Drosophila melanogaster*, the gene located at the 93D region of polytene chromosomes is unique in many ways (reviewed in Lakhotia 1987, 1989). Upon heat shock, the 93D locus (also called *hsr ω*, Bendena et al. 1989a), produces three major species of RNA, viz., ω1 (~10 kb), ω2 (1.9 kb) and ω3 (1.2 kb), each with different turnover rates; other specific inducers of the 93D locus, such as benzamide or colchicine, also induce these three transcripts but in different proportions (Bendena et al. 1989b). The ω1 and ω2 transcripts are nuclear while ω3, a spliced product of ω2 RNA, is cytoplasmic and one of its short open reading frames may possibly be translated into a very fast turning over polypeptide of 27 amino acids (Fini et al. 1989). The functions of these transcripts are not known although the locus is also expressed constitutively in a developmentally regulated manner and is essential for survival of the organism (Mohler and Pardue 1984; Lakhotia 1987; Bendena et al. 1989a, b).

One of the "effects" of the 93D locus is that if during heat shock the 93D puff does not develop for some reasons the 87A/87C puffs, which are duplicate loci for the most abundant Mr = 70,000 heat shock polypeptide (hsp), become unequally active (see Lakhotia 1987 for review). To study further the relationship between puffing of 93D and 87A and 87C, a translocation chromosome, *T(1; 3)eH^2, red e^v* (see Henikoff 1980), was used in which the 93D region is translocated to the centromeric heterochromatin on the right arm of the *X* chromosome. The present study was initiated to examine if the 93D heat shock puff site showed position effect variegation in this translocation chromosome and, if it did, what effect this had on the 87A/87C puffing in individual cells where the 93D locus was puffed after heat shock to a greater or lesser degree because of the position effect variegation. While trying to eliminate any effects of background genetic factors, it was observed that certain genetic markers on the non-translocation homologue influenced the 93D heat shock puffing activity on the translocated homologue as well. This paper presents the results of studies on the effects of the translocation on 93D puffing and a cytogenetic analysis of some of the other genetic factors that affect 93D activity. An unanticipated but very significant observation was that mutations at the ebony locus, in the homo- or heterozygous condition, prevent puffing at 93D after heat shock on both homologues (*trans* effect) and this also leads to unequal puffing at the 87A and 87C heat shock loci. Since the ebony mutation causes accumulation of high levels of β-alanine in the haemolymph (reviewed in Wright 1987), we also examined the effect of excess β-alanine on heat shock puffing at the 93D locus in wild-type larvae. In addition, we further analysed heat shock

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puffing in salivary glands of black mutant larvae, which are deficient in β-alanine because of a defect in its endogenous synthesis (see Wright 1987). Our results show that both elevated and lowered levels of β-alanine make the 93D puff non-inducible by heat shock. Interestingly, unlike heat shock, benzamide specifically induced 93D in spite of mutations at the *e* or *b* locus.

Materials and methods

All flies and larvae of different genotypes of *D. melanogaster* were reared on standard food at 20° ± 1° C. Some of the genetic markers/rearrangements used in this study are briefly described below. For details of the others, see Lindsley and Grell (1968).

1. *T(1;3)eH²*, *red* e^v. This is a translocation of the distal half of the right arm of chromosome *3* (*3R*), from polytene band 92D1 to 100F2, to centromeric heterochromatin on the right arm of the *X* chromosome (Henikoff 1980). The translocation brings the ebony and 93D6-7 heat shock loci close to the centromeric heterochromatin of *X*.

2. *In(3R)C*. This inversion on *3R* has its left breakpoint at 92D1-E1, and right breakpoint at 100F2-F5. Two different *In(3R)C* chromosomes, one marked with *Sb e l(3)e* and the other with *l(3)a*, were used (Lindsley and Grell 1968). The 93D heat shock locus on *In(3R)C* chromosome is close to the tip of *3R*.

3. $l(3)A_{63}O_5231?$, *e*. This is a recessive lethal mutation at the ebony locus resulting from P element insertion described by Cooley et al. (1988). With ebony mutant alleles, it shows the ebony phenotype.

4. *Sp/CyO*; *Sb ry P*[ry^+ *Δ2-3*]*/TM6*. This is a "jump-starter" stock which was used following essentially the scheme of Cooley et al. (1988) to mobilize the P insertion in the $l(3)A_{63}O_5231?$, *e* chromosome to revert it to e^+. The three revertants that were obtained in these crosses were named e^{r+1}, e^{r+2} and e^{r+3}.

Treatments. Eggs of the desired genotypes (either from the stocks or generated by appropriate crosses) were collected over a 1–2 h period in food-filled Petri dishes and allowed to develop at 20° ± 1° C. Additional yeast suspension was provided for healthy growth of larvae. Salivary glands from actively migrating late third instar larvae were excised in Poels' salt solution (Lakhotia and Mukherjee 1980) and subjected to temperature shock or benzamide treatment. For the latter, excised glands were incubated in Poels' salt solution containing 1 mg/ml benzamide at 24° C for 10 min following which the glands were pulse labelled with [³H]uridine (500 μCi/ml; specific activity 12.4 Ci/mmol, BARC, Bombay) for 10 min in the presence of benzamide. For temperature shock, the excised glands were incubated at 37° C for 30 min and then pulse labelled with [³H]uridine as above for 10 min. The [³H]uridine-labelled glands were fixed, squashed and processed for autoradiography in the usual way (Lakhotia and Mukherjee 1980).

In one set of experiments, salivary glands of wild-type larvae were exposed in vivo or in vitro to an excess of β-alanine prior to heat shock. For in vivo treatment, either the wild-type larvae were grown at 20° ± 1° C from hatching on food containing 100 mM β-alanine or the mid third instar larvae were transferred to food containing 500 mM β-alanine for 18 h. For in vitro treatment, salivary glands of normally grown wild-type larvae were either incubated for 1 h at 24° C in Poels' salt solution containing 100 mM β-alanine prior to a 30 min temperature shock at 37° C (β-alanine followed by temperature shock) or were heat shocked at 37° C for 1 h in the presence of 100 mM β-alanine (β-alanine plus temperature shock); in both cases sister glands were heat shocked in the absence of β-alanine to serve as controls. The glands were labelled after the treatments with [³H]uridine and processed for autoradiography as above.

Quantitation of puffing. The autoradiograms were scored for labelling on the 87A, 87C and 93D puff sites. In addition, the labelling of a non-puffed segment of *3L* (61A to 63A) or of *3R* (96A to 99F) was also examined to monitor the general transcription rate. The number of grains scored on each puff site was divided by that on the reference chromosome segment (*3L* or *3R* segment) of the same nucleus to obtain the relative activity of the puff. The activity of 87A relative to that of the 87C puff was expressed as the 87A/87C grain ratio, obtained by dividing the number of grains on the 87A puff by that on the 87C puff of a nucleus.

In certain cases, the activity levels of the 87A, 87C and 93D puffs after temperature shock were estimated from orcein-stained squash preparations by measuring the puff size as the ratio of the maximum puff width to the width of a reference band (band 87D for the 87A and 87C puffs and 93E for the 93D puff).

Results

Heat shock puffs in genotypes carrying mutant alleles at the ebony locus

The general patterns of induction (relative activities) of the 93D, 87A and 87C heat shock puffs in several different genotypes are presented in Table 1. A 93D/*3L* ratio of >1.0 indicates an induced 93D while a ratio of 1.0 or <1.0 indicates non-induction of 93D. An 87A/87C ratio of <0.80 indicates 87C to be transcriptionally more active than 87A while a ratio between 0.8 and 1.25 indicates equal activity at these two loci. Finally a ratio of >1.25 indicates the 87A locus to be more active than 87C. The data presented in Table 1 reveal remarkable differences in the inducibility of the three major heat shock loci in specific genotypic combinations. As known from earlier studies (Lakhotia and Mukherjee 1980), heat shock to wild-type (+/+) larval salivary glands causes 93D, 87A and 87C to form large puffs with the sizes and numbers of silver grains on 87A and 87C puffs being nearly equal (mean 87A/87C grain ratio 0.99 ± 0.04; see genotype no. 1 in Table 1). Translocation of the 93D puff to *XR* heterochromatin in the *T(1;3)eH²*, *red* e^v chromosome led to relatively less induction of the translocated puff (position effect) by heat shock in comparison with that on the non-translocated homologue. However, the difference in 93D activity on the translocation and the other homologue varied depending upon the nature of the latter (see genotypes 2 and 3 in Table 1 and Fig. 1): with the *In(3R)C, Sb e l(3)e* homologue, the induced activity of the translocated 93D was significantly less than on the *In(3R)C* chromosome (on Student's *t* test, $t = 2.94$, $P < 0.01$) while in combination with a wild-type (+) homologue, the difference between the two 93D loci was less marked ($t = 1.51$ and $P > 0.3$).

The 87A and 87C puffs were relatively equally active after heat shock in *T(1;3)eH²*, *red* e^v/+ salivary gland polytene nuclei (mean 87A/87C grain ratio = 1.07 ± 0.04), but in the *T(1;3)eH²*, *red* e^v/*In(3R)C, Sb e l(3)e* glands the 87A puff was much larger with almost twice as much [³H]uridine labelling on it as on the 87C puff (mean 87A/87C grain ratio = 2.07 ± 0.15, see Table 1 and Fig. 1).

Heat shock to salivary glands of *In(3R)C, Sb e l(3)e*/+ larvae failed to induce the 93D puff altogether

Table 1. Relative activities of the 93D, 87A and 87C puffs in different genotypes after heat shock

Genotype[a]		Relative activity (mean (±S.E.) grain ratio)				Number of mutant alleles at the *ebony* locus
		93D/*3L*[b]	87A/*3L*	87C/*3L*	87A/87C	
1.	+/+ (wild type) (23)	5.69±0.36	5.72±0.32	6.10±0.38	0.99±0.04	0
2.	*T(1;3)e^{H2}, red e^{v}/ In(3R)C, Sb e l/(3)e* (28)	4.38±0.4^{T} 5.89±0.53^{I}	3.66±0.26	1.89±0.16	2.07±0.15	1
3.	*T(1;3)e^{H2}, red e^{v}/+* (22)	3.19±0.32^{T} 3.81±0.36^{+}	4.93±0.43	4.66±0.47	1.07±0.04	0
4.	*In(3R)C, Sb e l(3)e/+* (35)	0.99±0.1	5.11±0.35	7.89±0.48	0.77±0.03	1
5.	*red/In(3R)C, l(3)a* (31)	3.30±0.18	2.74±0.19	2.75±0.16	0.99±0.03	0
6.	*red/In(3R)C, Sb e l(3)e* (23)	0.56±0.04	3.66±0.13	2.36±0.15	1.61±0.06	1
7.	*e/e* or *e^{s}/e^{s}* (90)	0.44±0.02	3.37±0.2	2.00±0.06	1.75±0.05	2
8.	*e/+* or *e^{s}/+* (36)	0.50±0.08	3.80±0.13	2.93±0.1	1.63±0.07	1
9.	*e^{s}/In(3R)C, Sb e l(3)e* (34)	0.50±0.06	5.46±0.35	3.41±0.24	1.49±0.06	2
10.	*e^{s}/In(3R)C, l(3)a* (25)	0.56±0.08	5.57±0.2	3.61±0.19	1.67±0.08	1
11.	*red e/In(3R)C, l(3)a* (19)	0.55±0.03	3.96±0.24	3.03±0.23	1.36±0.06	1
12.	*red e/In(3R)C, Sb e l(3)e* (25)	0.45±0.05	4.05±0.23	2.55±0.15	1.66±0.07	2
13.	*red/red e* (30)	0.50±0.03	4.34±0.21	2.78±0.14	1.60±0.05	1
14.	*In(3R)C, l(3)a/In(3R)C, Sb e l(3)e* (28)	0.35±0.02	3.60±0.15	2.38±0.13	1.52±0.05	1
15.	*mwh ry e* (29)	0.50±0.06	5.62±0.35	3.79±0.25	1.48±0.04	2
16.	*mwh^{+} ry^{+} e* (24)	0.62±0.03	5.49±0.4	3.38±0.3	1.62±0.07	2
17.	*mwh ry e^{+}* (30)	4.40±0.21	3.56±0.18	3.51±0.14	1.02±0.03	0
18.	*e^{r+1}* (*e^{+}* revertant) (34)	4.70±0.15	4.09±0.17	4.06±0.15	0.99±0.02	0

[a] Figures in parentheses indicate the number of nuclei examined. Nos. 16 and 17 were derived by recombination from no. 15
[b] The superscripts, T, I or + indicate puffs on the translocated, inverted or normal homologues of *3R*, respectively

(mean 93D/*3L* grain ratio = 0.99±0.1) on either of the homologues while 87A in this case displayed significantly less ($t = 4.26$, $P < 0.001$) [^{3}H]uridine labelling in comparison with 87C (mean 87A/87C grain ratio = 0.77± 0.03; see Table 1 and Fig. 2). Since the *In(3R)C, Sb e l(3)e* chromosome appeared to influence the heat shock inducibility of the 93D, 87A and 87C puffs on the *T(1;3)eH^{2}* as well as on a wild-type (+) homologue, further analysis was carried out to see if it was the inversion per se or some of the markers associated with this balancer chromosome that was/were responsible for the above effects on the 93D, 87A and 87C loci.

A series of different genotypes were analysed for inducibility of 87A, 87C and 93D puffs in their larval salivary glands by heat shock. A comparative analysis of the data on [^{3}H]uridine labelling of these heat shock puffs in different genotypes presented in Table 1 (also see Fig. 2) showed that *In(3R)C* by itself did not influence 93D puffing since in salivary glands of *red/ In(3R)C, l(3)a* larvae (genotype 5 in Table 1, Fig. 2), 93D was well induced on both homologues following heat shock while in salivary glands of *red/In(3R)C, Sb e l(3)e* larvae (genotype 6 in Table 1), 93D was not induced on either homologue. It was interesting that the presence of the *e* or *e^{s}* mutant allele in the homozygous (*e/e* or *e^{s}/e^{s}*) or heterozygous condition (*e/+* or *e^{s}/+*,

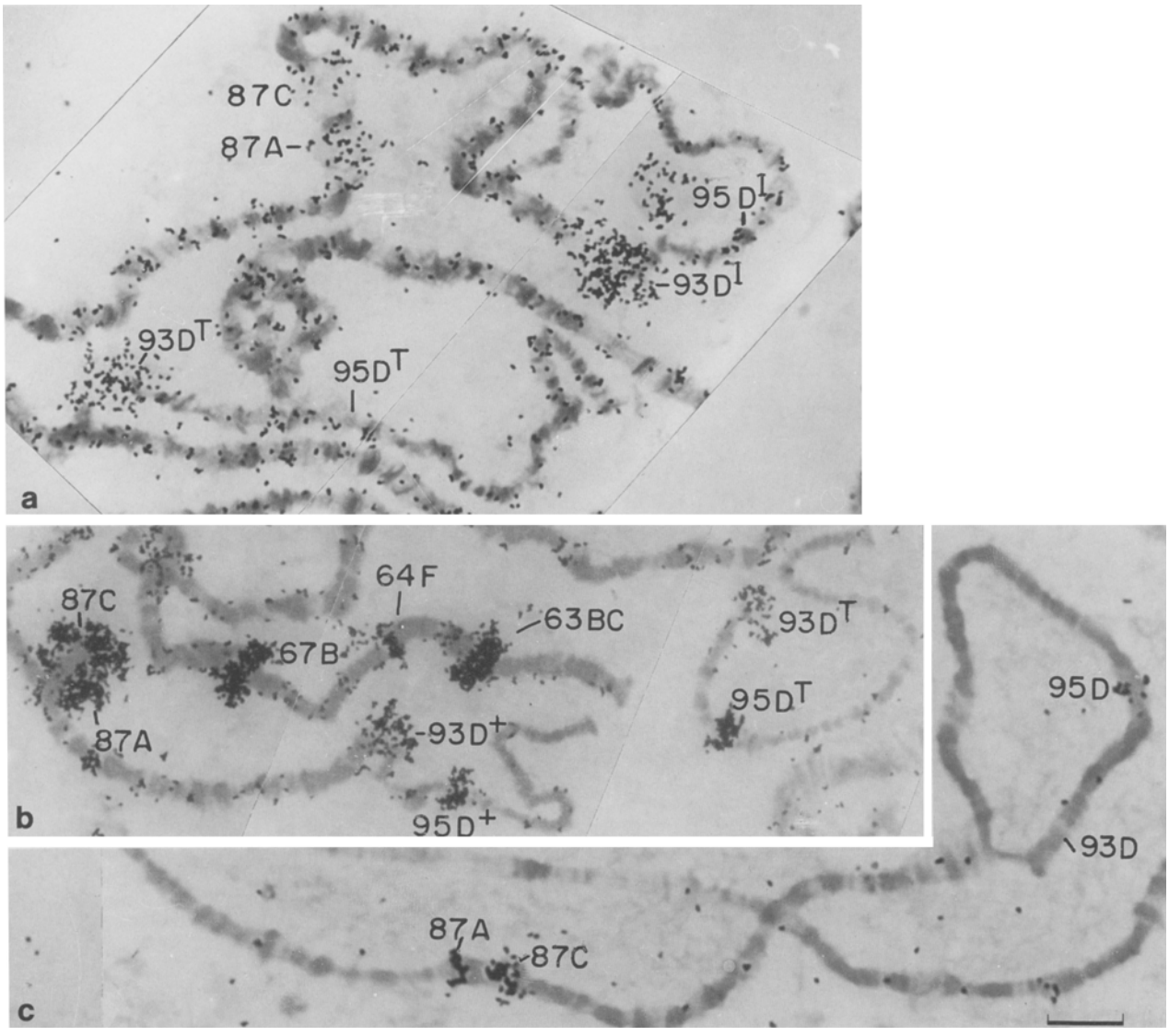

Fig. 1a–c. Autoradiograms of [^{3}H]uridine-labelled polytene chromosomes of heat-shocked salivary glands from **a** *T(1;3)eH^2, red e^v/In(3R)C, Sb e l(3)e*, **b** *T(1;3)eH^2, red e^v/+* and **c** *In(3R)C, Sb e l(3)e/+* larvae. Major heat shock puff sites are indicated. The superscripts, *T*, *I* or + in **a** and **b** indicate puffs on translocated, inverted or normal homologues of *3R*, respectively. Bar represents 10 μm

genotypes 7 and 8 in Table 1) without any associated inversion or other markers, made the 93D puff non-inducible by heat shock. Similarly in genotypes 9 to 14 in Table 1, all of which carried a mutant allele at the ebony locus on one or both homologues, the 93D puff was not induced by heat shock: in all these cases, no morphological puff was seen at 93D nor was any appreciable labelling noticeable in the autoradiograms (Table 1 and Fig. 2).

Thus the results of heat shock on genotypes 4 to 14 in Table 1 suggested that a mutant allele at the ebony locus was somehow affecting inducibility of the 93D heat shock puff. To map this effect more specifically to the ebony locus, two additional approaches were made: in one approach e^+ recombinants were selected from *mwh ry e/+ + +* heterozygotes and in another, an ebony mutant resulting from P element insertion *(l(3)A$_{63}$O$_5$231?, e*, see Cooley et al. 1988) was reverted to e^+ using the jump-starter strategy (Cooley et al. 1988). The objective in both cases was to see if the e^+ recombinant or revertants displayed a heat shock inducible puff at 93D.

As the data in Table 1 (genotypes 15–17) show, in the parental (*mwh ry e*) and in the *mwh*$^+$ *ry*$^+$ *e* recombinants, the 93D puff was not inducible by heat shock. However, in all larvae of the six *mwh rye*$^+$ recombinant lines that were examined, a well-developed active puff was seen at 93D after heat shock (93D/*3L* grain ratio = 4.4, see genotype 17 in Table 1).

Heat shock induced puffing was examined in orcein-stained preparations of salivary glands of *l(3)A$_{63}$O$_5$231?, e/+* and *l(3)A$_{63}$O$_5$231?, e/ry* heterozygous and *e^{r+}* (revertant) homozygous larvae. The data on puff size measurement presented in Table 2 clearly

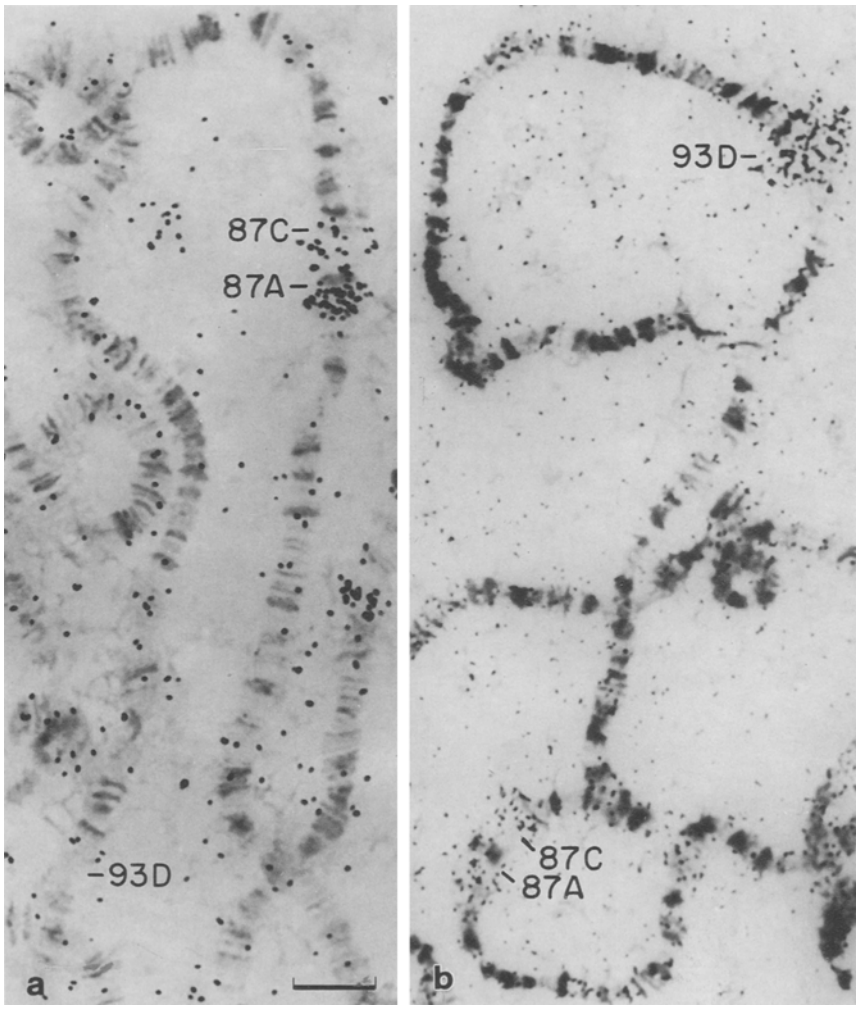

Fig. 2a, b. Autoradiograms of [^{3}H]uridine-labelled polytene chromosomes of heat-shocked salivary glands from **a** *In(3R)C, Sb e l(3)e/In(3R)C, l(3)a* and **b** *In(3R)C, l(3)a/red* larvae. Note the high induction of the 93D puff in **b**. Bar represents 10 μm

Table 2. Heat shock induced activity of the 93D, 87A and 87C puffs in salivary glands of larvae with a P element insertion mutation of the ebony locus and revertants

Genotype[a]	Puff activity index[b]		
	98D	87A	87C
1. *l(3)A$_{65}$O$_5$231?, e/ry* (25)	1.11±0.02	2.64±0.03	1.60±0.05
2. *l(3)A$_{65}$O$_5$231?, e/+* (23)	1.13±0.03	2.67±0.07	1.57±0.03
3. e^{r+1}/e^{r+1} (25)	2.74±0.06	2.57±0.04	2.66±0.04

[a] Figures in parentheses indicate the number of nuclei examined in each case

[b] Puff activity index is the ratio of the maximum puff diameter to that of the reference band (see Materials and methods) in temporary squash preparations examined under phase optics

show that larvae heterozygous for the P insertion ebony mutant locus did not develop a 93D puff after heat shock but all the revertants (e^{r+}) displayed a typical heat shock puff at 93D (Fig. 3). [^{3}H]uridine labelling of the salivary glands of the revertant also confirmed good induction of the 93D puff after heat shock (see genotype 18 in Table 1). It should be mentioned that although data on puff induction are presented for only one of the revertants (e^{r+1}), similar results were obtained for the other two revertants also.

The data in Table 1 also show that in all those genotypes where at least one mutant allele was present at the ebony locus (see right-hand column in Table 1) and 93D was not induced by heat shock, [^{3}H]uridine labelling at the 87A and 87C puffs was unequal. In all these cases, except in the *In(3R)C, Sb e l(3)e/+* genotype, the 87A puff was always significantly more active than that of 87C. In other genotypes that displayed a good heat shock puff at 93D and did not have a mutant allele at the ebony locus, the 87A/87C grain ratio was not significantly different from 1.0 (see Figs. 1–3).

Heat shock puffs in β-alanine-treated wild-type larvae

In vitro treatment with 100 mM β-alanine for 1 h at 24° C did not induce transcription at any specific locus nor was ongoing transcription inhibited (data not presented). Although, β-alanine by itself did not alter the transcriptional activity of nuclei in any detectable way,

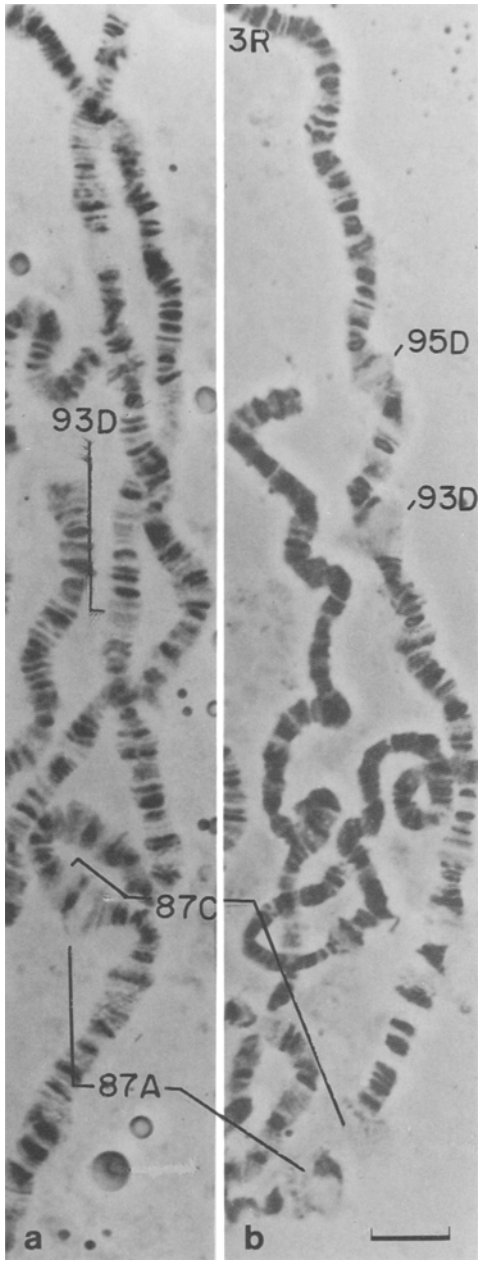

Fig. 3a, b. Phase contrast photomicrographs of polytene chromosomes of heat-shocked salivary glands from **a** *l(3)A*$_{63}$*O*$_{5}$*231?, e/ry* and **b** e^{r+1} (revertant) larvae. Note the well-developed puffs at 87A, 87C and 93D in **b**. Bar represents 10 μm

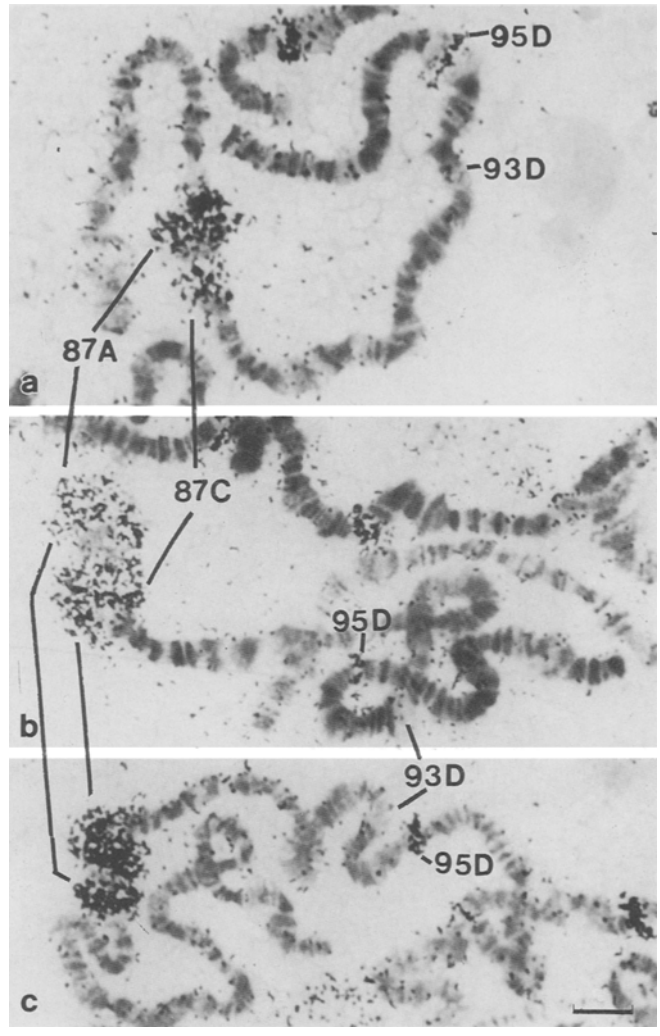

Fig. 4a–c. Autoradiograms of [^{3}H]uridine-labelled polytene chromosomes of salivary glands of wild-type larvae heat shocked following a 1 h in vitro treatment with 100 mM β-alanine at 24° C. Note no labelling of the 93D site in all three cases with different patterns of labelling of 87A and 87C puffs, viz., **a** 87A > 87C, **b** 87A = 87C and **c** 87A < 87C. Bar represents 10 μm

the data presented in Table 3 show that heat shock in the presence of β-alanine (in vitro) or following β-alanine treatment (in vitro or in vivo) resulted in specific transcriptional changes. In this set of data the puff activity was compared with a segment of *3R*. Since the *3R* segment (96A to 99F) was larger than the *3L* segment used as reference for the data in Table 1, the numerical values of relative puff activity were generally smaller in this case. Therefore, in this set of data (Table 3) a 93D/*3R* grain ratio < 0.30 was taken to indicate no induction of the 93D puff. In control glands (without β-alanine) that had been heat shocked for 30 min or for 1 h, all the heat shock puffs were well induced (Table 3), while in wild-type salivary glands heat shocked following an in vitro β-alanine treatment or heat shocked in the presence of β-alanine, the 93D puff was not induced (nos. 2 and 3 in Table 3; Fig. 4).

Heat shock to salivary glands from wild-type larvae reared on 100 mM β-alanine-containing food or fed on 500 mM β-alanine for the last 18 h also failed to induce the 93D puff as evidenced by the very low 93D/*3R* grain ratio noted in both cases (see nos. 4 and 5 in Table 3).

The mean 87A/87C grain ratios in heat-shocked and β-alanine-treated wild-type glands were significantly higher than 1.0 except in glands heat shocked in the presence of β-alanine in which the mean ratio was close to 1.0 (Table 3). A significant point to note is that in β-alanine-treated and heat-shocked glands, the 87A/87C grain ratio varied considerably in individual nuclei such that in some the 87A/87C ratio was < 0.75, in some it was > 0.75 but < 1.25 and in others it was > 1.25 (see Table 4 and Fig. 4). Except in glands heat shocked

Table 3. Heat shock induced relative activities of 93D, 87A and 87C puffs in β-alanine-treated wild-type larval salivary glands and in salivary glands of *black* larvae

Genotype[a]	Treatment[b]	Relative activity (Mean (±S.E.) grain ratio)			
		93D/*3R*	87A/*3R*	87C/*3R*	87A/87C
1. +/+	TS (20)	1.19±0.08	1.31±0.08	1.49±0.07	0.90±0.04
2. +/+	β-ala in vitro →TS (27)	0.21±0.03	1.90±0.14	1.30±0.16	1.50±0.09
3. +/+	β-ala in vitro +TS (33)	0.20±0.03	2.30±0.16	2.46±0.13	0.97±0.05
4. +/+	100 mM β-ala fed →TS (20)	0.20±0.05	2.00±0.31	1.68±0.33	1.42±0.14
5. +/+	500 mM β-ala fed →TS (28)	0.14±0.04	2.40±0.32	1.73±0.17	1.42±0.14
6. *b pr cn*	TS (20)	0.17±0.03	1.41±0.14	1.19±0.19	1.50±0.17
7. *b*	TS (33)	0.20±0.03	3.50±0.30	2.68±0.24	1.33±0.04
8. b^+ *pr cn*	TS (18)	1.24±0.07	1.33±0.31	1.44±0.34	0.97±0.03

[a] Genotypes 7 and 8 were obtained by recombination from 6
[b] Figures in parentheses indicate the number of nuclei examined in each case. TS, temperature shock as described in Materials and methods. β-ala, β-alanine

Table 4. The 87A/87C grain ratios in different nuclei following heat shock to β-alanine-treated wild-tyoe salivary glands

Genotype	Treatment[a]	Mean 87A/87C ratio	Nuclei (%) in the range		
			<0.75	>0.75<1.25	>1.25
1. +/+	TS (20)	0.90±0.04	15.0	85.0	0.0
2. +/+	β-ala in vitro →TS (27)	1.50±0.09	3.7	25.9	70.4
3. +/+	β-ala in vitro +TS (33)	0.97±0.05	30.0	46.7	23.3
4. +/+	100 mM β-ala fed →TS (20)	1.42±0.14	25.0	15.0	60.0
5. +/+	500 mM β-ala fed →TS (28)	1.42±0.14	21.4	25.0	53.6

[a] Figures in parentheses indicate the number of nuclei examined in each case. TS, temperature shock; β-ala, β-alanine

in the presence of β-alananine the 87A/87C grain ratio was >1.25 in the majority of nuclei and therefore, the mean value was also >1.25 (Table 4). It should be mentioned that in none of the genotypes listed in Table 1 did the 87A/87C grain ratios vary in individual nuclei as seen in the β-alanine-treated wild-type salivary glands.

Heat shock puffing in black mutant larvae

Heat shock experiments were also carried out on another body colour mutant, black, of *D. melanogaster* which though phenotypically similar to ebony, is physiologically different from it: whereas ebony has excessive β-alanine, black is deficient in it (see Wright 1987). As the black mutant line used in this study was isolated by recombination from a parental *b pr cn* stock, the heat shock response was also studied in the parental *b pr cn* as well as the other recombinant strain, *pr cn*.

Analysis of the autoradiograms of heat-shocked glands revealed that while in *pr cn*, 93D was well induced by heat shock, in *b pr cn* and *b*, 93D remained uninduced (see nos. 6–8 in Table 3; Fig. 5). The 93D/*3R* ratio in heat-shocked *pr cn* nuclei was comparable to that in heat-shocked wild-type nuclei (compare nos. 1 and 8 in Table 3).

The non-induction of the 93D puff by heat shock in black larvae (*b*/*b* or *b pr cn*/*b pr cn*) was also accompanied by unequal labelling of the 87A and 87C puffs with 87A being more labelled (Table 3). The b^+ *pr cn*/br^+ *pr cn* recombinants, like the +/+ larvae, displayed equally labelled 87A and 87C heat shock puffs. In the

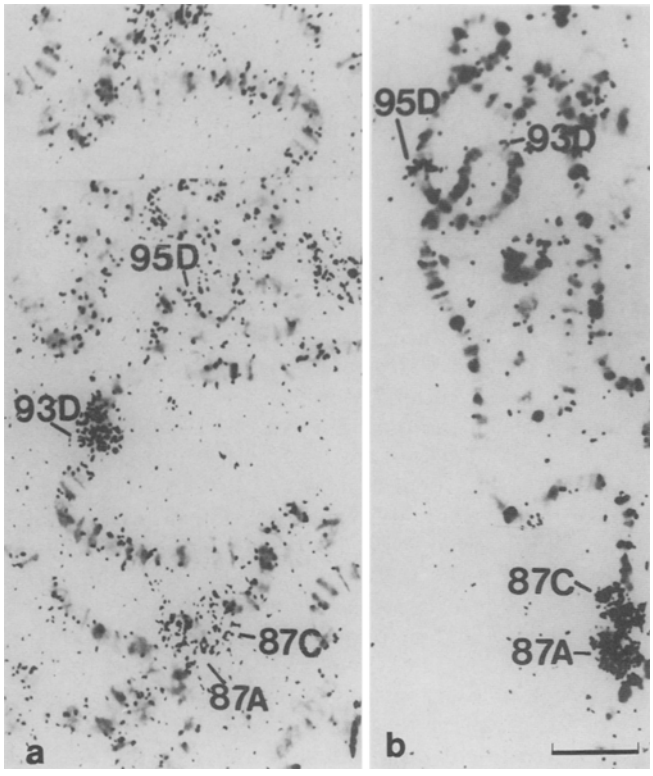

Fig. 5a, b. Autoradiograms of [^{3}H]uridine-labelled polytene chromosomes of heat-shocked salivary glands of **a** b^+ *pr cn* and **b** *b pr cn* mutant larvae. Note the high induction of 93D in **a**. Bar represents 10 μm

case of *b* larvae, the 87A/87C ratios did not vary as much as in the β-alanine-treated wild-type larvae since no nucleus was seen in these glands in which the 87A/87C ratio was <0.75.

Induction of 93D by benzamide in different genotypes

Benzamide is known to induce the 93D puff selectively in salivary gland polytene nuclei of wild-type *D. melanogaster* (Lakhotia and Mukherjee 1980). In the present study, benzamide was found to induce the 93D puff selectively in all genotypes (+/+; *T(1;3)eH*^{2}, *red* e^v/*In(3R)C, Sb e l(3)e*; *T(1;3)eH*^{2}, *red* e^v/+; e^s/e^s or e/e; e^s/+ or e/+; *In(3R)C, Sb e l(3)e*/+; *l(3)*$A_{63}O_5$*231?, e/ry*; *l(3)*$A_{63}O_5$*231?, e*/+; e^{r+}/e^{r+} and *b*/*b*) that were tested (data not presented). The 93D puff was also selectively induced by benzamide in salivary glands of wild-type larvae that had been exposed to β-alanine in vitro or in vivo (data not presented).

Discussion

Data presented in Tables 1 and 2 convincingly demonstrate that in the presence of mutant allele/s at the ebony locus, the 93D puff is not induced by heat shock. While genotypes 4 to 14 in Table 1 provide circumstantial evidence for this correlation, the results with the last four genotypes in Table 1 and with those in Table 2 permit the direct mapping of this effect on heat shock puffing at 93D to the ebony locus. Thus all the e^+ recombinants examined from *mwh ry e*/+ + + heterozygotes, displayed a typical heat shock puff at 93D while the parental type (*mwh ry e*) or the other class of recombinant, mwh^+ ry^+ *e*, failed to show heat shock puffing at 93D. Our results with the wild-type revertants (e^{r+}) of the P element insertion mutant *e* allele (*l(3)*$A_{63}O_5$*231?, e*) are more definitive. The heterozygous larvae (*l(3)*$A_{63}O_5$*231?, e*/+ or *l(3)*$A_{63}O_5$*231?, e/ry*) failed to show the heat shock puff at 93D on either homologue while the revertants always developed a typical 93D puff after heat shock. Since mutation due to P element insertion and its reversion are fairly specific events (Cooley et al. 1988), these results provide conclusive evidence for involvement of the ebony locus in affecting heat shock puffing at 93D.

Results with *e* heterozygotes show that the ebony locus exerts a *trans* effect on 93D either directly through its product or resulting from the action of its product. A well-known effect of the ebony mutation in *D. melanogaster* is the accumulation of excessive levels of β-alanine due to non-utilization of this unusual amino acid in sclerotization of cuticle (Jacobs 1978, 1980; Hodgetts 1972). The ebony locus codes for the enzyme β-alanyl dopamine synthetase (see Wright 1987) which conjugates β-alanine to dopamine. A mutation at the ebony locus prevents this conjugation and the non-utilized β-alanine accumulates. The fact that exogenous β-alanine phenocopied the ebony effect, as far as the heat shock inducibility of 93D was concerned, confirms that the *trans* effect of the ebony mutation on the 93D heat shock locus is related to elevated levels of β-alanine in individuals with dysfunction of the e^+ gene. Moreover, our observation that the *e*/+ heterozygotes also failed to show the 93D heat shock puff agrees with an earlier observation that the *e*/+ heterozygotes have a higher β-alanine level than the +/+ wild-type individuals (Jacobs and Brubaker 1963).

Our observation that mutation at the black locus also affected heat shock inducibility of the 93D puff in a manner comparable to that of the ebony mutation was rather surprising since mutation at the black locus causes a reduction in β-alanine levels due to a defect in metabolic pathway/s leading to its endogenous synthesis (Wright 1987). Apparently, reduced levels of β-alanine affect the heat shock inducibility of 93D puff as do its increased levels. In another study in our laboratory (S.C. Lakhotia and K. Arundhati, unpublished) it has been observed that *b*/*b* larvae grown on β-alanine-supplemented food display a typical 93D puff when heat shocked. This confirms that the absence of a heat shock puff at 93D in black larvae was due to the lowered levels of β-alanine in their body. Taken together, our present results revealed that reduced as well as elevated levels of β-alanine in larvae of *D. melanogaster* lead to non-inducibility of the 93D puff by heat shock.

In view of the effect of the ebony mutation on 93D puffing, our results with *T(1;3)eH*^{2}/*In(3R)C, Sb e l(3)e* larvae are rather intriguing since as in other ebony heter-

ozygotes, one would expect the 93D locus in these larvae also not to be inducible by heat shock. However, the 93D puff in these larvae was actually very strongly induced following heat shock. A few other observations are relevant in this context. In their study with 93D deficiency heterozygotes, Mohler and Pardue (1982) found a good induction of the 93D heat shock puff on the *In(3R)C, e* chromosome when placed against a homologue deficient for the 93D6-7 region (viz., *Df(3R)e*^{Gp4} or *Df(3R)GC14)*. Interestingly, however, when the same deficiency homologues were placed against a wild-type homologue (e^+), the 93D puff was not induced by heat shock on the + homologue (Burma and Lakhotia 1986). Moreover, it has also been seen that placing these 93D deficiency chromosomes against an *e*-marked standard homologue also leads to a good induction of 93D heat shock puff on the homologue bearing the *e* mutant allele (S.C. Lakhotia and M. Mutsuddi, unpublished). Thus the effect of the ebony mutant allele/s on heat shock inducibility of the 93D puff seems to depend upon the organization of the 93D locus itself so that when an *e*-bearing chromosome is placed against a homologue deficient for the 93D locus, the inhibitory effect of ebony is not manifest. It is possible that the close proximity of the 93D locus to chromocentric heterochromatin in the *T(1;3)eH*^{2} chromosome leads to certain changes (e.g. position effect variegation) which in turn modify the ebony effect. Thus the puffing of 93D following heat shock in *T(1;3)eH*^{2}*/In(3R)C, Sb e l(3)e* larvae does not contradict the inhibitory effect of ebony mutant alleles on its puffing in other genotypes, rather its stronger induction in this genotype than in *T(1;3)eH*^{2}*/+* larvae reflects a different aspect of the ebony effect.

Earlier studies from this laboratory had shown that non-induction of the 93D puff during heat shock is accompanied, in nearly all cases, by an unequal puffing of the 87A and 87C loci (see Lakhotia 1987). In agreement with these results, in the present study we also found that the non-induction of 93D heat shock, either due to mutation at the *e* or *b* loci or to excess β-alanine, was accompanied by unequal puffing at the 87A and 87C loci (Tables 1–4). Conversely, a typically induced 93D heat shock locus was associated with equally active 87A and 87C puffs. However, the following apparent exceptions to these correlations were also noted: (i) *T(1;3) eH*^{2}*/In(3R)C, Sb e l(3)e* nuclei showed unequal puffing at 87A and 87C although the 93D puffs were well induced; and (ii) in the case of wild-type salivary glands heat shocked in the presence of β-alanine, the mean 87A/87C grain ratio was close to 1.0 in spite of 93D not being induced. However, the ratio in individual nuclei was often very different from 1.0, unlike during the typical wild-type heat shock response (compare nos. 1 and 3 in Table 4). Similarly, in other cases of β-alanine and heat shock treatments (see Table 4), individual nuclei showed variations in induction at 87A and 87C, though the mean 87A/87C ratio was about 1.5 (Table 4, nos. 2, 4, 5).

Exceptions to the typical heat shock induced puffing patterns of 93D, 87A and 87C loci have also been found in a few studies (Hochstrasser 1987; Myohara and Okada 1988). Based on the observations that under certain conditions even with a well-induced 93D puff, the 87A and 87C loci were unequally puffed, Hochstrasser (1987) questioned the inference that activity of the 93D locus has some effect on heat shock induced puffing of the 87A and 87C loci. In the context of these apparent exceptions to correlation between the pattern of 93D puffing and the relative activity of the 87A and the 87C puffs noted in our earlier (Lakhotia 1987, 1989) and present studies, the relation between puffing and actual transcript patterns needs to be considered. While in general, the size and [^{3}H]uridine labelling of a puff reflect its rate of transcription (Beermann 1972), there are other determinants of the puff size as well. Thus Simon et al. (1985) noted that the length of transcript made by the locus is a strong determinant of puff size. The rate of transport of the transcripts from a puff also determines its size (Bonner and Pardue 1977). The 93D locus is known to produce a variety of transcripts whose size and turnover rates are strongly influenced by the nature of the inducer and by other physiological conditions of the cell (Bendena et al. 1989a, b). We presume that the 87A to 87C activity ratio is influenced by the particular set of 93D transcripts that are available following the specific treatment conditions. If this be so, the mere puffed state of the 93D locus may not guarantee that an appropriate set of 93D transcripts is indeed available. Thus in those situations where in spite of a strong 93D puff, the 87A and 87C puffs were unequally induced, the set of 93D transcripts actually available in the cell may have been different from those under typical heat shock conditions. This may also be relevant to the fact that under different conditions of non-inducibility of the 93D heat shock puff, 87A may be less or more active than 87C in a condition-specific manner (see Lakhotia 1987, 1989). The variable 87A/87C activity ratios noted in β-alanine-heat shock treatments may reflect a variable effect of these treatments on 93D transcript patterns in individual cells or this unusual amino acid may also directly affect 87A and 87C puffing in some way. These aspects are being studied further.

It is significant that benzamide continued to induce 93D specifically even under conditions where the locus was refractory to heat shock induction. Obviously, the regulatory pathways involved in these two inducer responses are different. The different patterns of 93D transcripts seen in response to benzamide and heat shock (Bendena et al. 1989b) also suggest differences in regulation.

The present study has thus revealed a strong effect of β-alanine on heat shock induced puffing of the 93D locus. The reasons for and mechanism of this effect are not clear but this effect further shows that among the heat shock loci 93D is unusually sensitive to a variety of physiological conditions of the cell. Since β-alanine is a normal cell metabolite, our finding is a significant step forwards in the direction of understanding this enigmatic locus. Finally, the present results further confirm that non-inducibility of the 93D locus during heat shock affects relative puffing at the 87A and 87C loci.

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