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Genetic mapping of the amide response element(s) of the *hsr*ω locus of *Drosophila melanogaster*

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Abstract. Small chromosomal deletions [*Df(3R)e*$^{R-1}$ and *Df(3R)e*^{P}] with intact *hsr*ω transcription units but with variable deletions of the upstream region were used to map the upstream regions that regulate heat shock and amide responsivity of the 93D puff (*hsr*ω locus) in salivary glands of late third instar larvae of *Drosophila melanogaster*. The *Df(3R)e*^{P} deletion, generated by a P-element mobilization screen, removed the 93B6–7 to 93D3–5 cytogenetic region. [^{3}H]uridine-labeled transcription autoradiograms revealed that normal developmental and heat shock-induced expression of the 93D puff remained unaffected in both the deficiency chromosomes. However, the amide responsivity of the 93D site was lost on the *Df(3R)e*^{P} homolog while the *Df(3R)e*$^{R-1}$ homolog responded normally to amides. Southern hybridizations with a series of upstream probes mapped the distal breakpoint of the *Df(3R)e*^{P} deletion between −22 kb and −23 kb of the *hsr*ω transcription unit. Since the distal breakpoint of *Df(3R)e*$^{R-1}$ is at about −45 kb upstream of the *hsr*ω gene it is inferred that the amide response element(s) that modulate the specific transcriptional activation of the 93D puff following treatment of salivary glands with a variety of amides is/are located in the −22 kb to about −45 kb upstream interval. The *Df(3R)e*^{P} and *Df(3R)e*$^{R-1}$ deletions also abolished dosage compensation at the 93D locus as well as the effect of β-alanine levels on its heat shock inducibility.

Introduction

The *hsr-omega* (*hsr*ω) locus at the 93D cytogenetic region of *Drosophila melanogaster* is a non-protein-coding gene that is developmentally expressed in almost all stages and cell types (Mutsuddi and Lakhotia 1995), is a member of the heat shock gene family and is also singularly induced, independent of the other heat shock or other genes, by amides such as benzamide, 3-amino-benzamide, colchicine, formamide, acetamide, or nicotinamide (Lakhotia and Sharma 1996; Tapadia and Lakhotia 1997). A series of earlier studies from our laboratory had clearly shown that a variety of conditions can affect heat shock inducibility of the 93D puff without affecting its benzamide or colchicine inducibility, suggesting that heat shock and amides exert their effects on this locus through different pathways (reviewed in Lakhotia 1989; Lakhotia and Sharma 1996). Using *promoter-lacZ* reporter gene fusion constructs for germline transformation, it was further confirmed (Mutsuddi and Lakhotia 1995; Lakhotia and Mutsuddi 1996) that the upstream regulatory elements for heat shock and amide inducibility were distinct since only the developmental and heat shock regulatory elements of the *hsr*ω gene were localized within the −840 bp upstream region; the response to amides was not elicited by this upstream region. In the present study, we have used two deficiencies that delete small chromosomal segments upstream of the *hsr*ω locus, viz., *Df(3R)e*$^{R-1}$ (Eisenberg et al. 1990) and *Df(3R)e*^{P} (generated in this study) to map genetically the amide response elements (AREs) in the upstream region of the *hsr*ω gene. On the basis of molecular mapping of the distal breakpoints of these deletions, we suggest that the AREs are located between about −22 kb and −45 kb upstream of the *hsr*ω gene. This far-upstream region also seems to affect dosage compensation (Burma and Lakhotia 1986) at this locus and to eliminate the effect of β-alanine levels (Lakhotia et al. 1990) on heat shock inducibility of this gene.

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Materials and methods

Fly stocks and rearing conditions. Flies were reared at 21°±1° C on standard food containing agar, maize powder, sugar and yeast. The larval cultures were grown in petri dishes supplemented with yeast.

Generation of Df(3R)e^{P} *by P-element mutagenesis.* Flies of a homozygous viable P-insertion line, *P(lacZ w*$^{+}$*)93B6–8*, with the *P(lacZ*

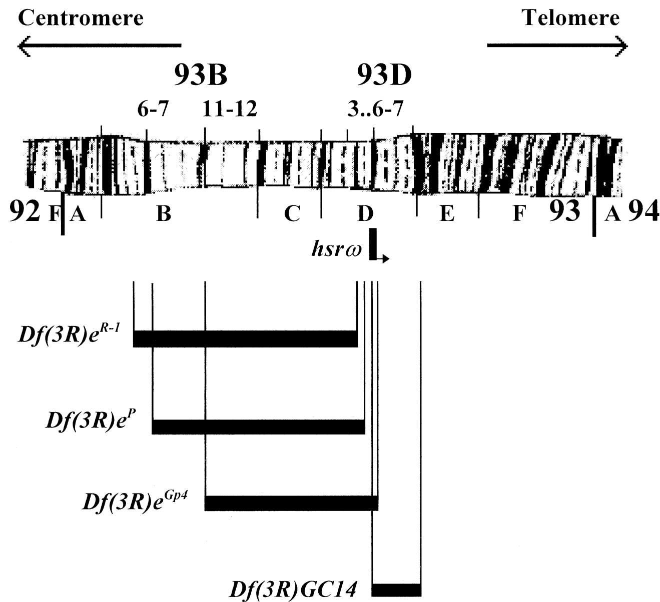


Fig. 1. Diagrammatic representation of the extents of the deleted regions in the four deficiency [*Df(3R)e*$^{R-1}$, *Df(3R)e*^{Gp4}, *Df(3R)e*^{P} and *Df(3R)GC14*] chromosomes used in this study. The polytene chromosome map on top is adapted from Bridges (1935). The *solid vertical bar* with *arrow* below the 93D6–7 region of the map indicates the location and direction of transcription of the *hsr*ω locus

w^{+}*)* transposon inserted at the 93B6–8 region of the right arm of chromosome *3*, were crossed with jump-starter [*P(ry*$^{+}$ Δ2–3)99B Sb/TM6B, Tb e, see Bier et al. 1989] flies to mobilize the P-transposon with a view to obtaining new insert lines that might affect the *hsr*ω locus in the 93D6–7 region. One of the new P-insertion lines showing a recessive *ebony* and *lethal* phenotype proved to be a deletion of region 93B6–8 to 93D3–5 (Figs. 1, 2). This P-transposon mobilization induced deletion spanning the *ebony* locus has been named *Df(3R)e*^{P}. Since *Df(3R)GC14* (deletion of bands 93D6–7 to 93D9–10, Mohler and Pardue 1982) complemented the *Df(3R)e*^{P} chromosome while the *Df(3R)e*^{Gp4} (deletion of bands 93B11–13 to 93D7–9, Mohler and Pardue 1982) and *Df(3R)e*$^{R-1}$ (deletion of bands 93B3–5 to 93D2–4, Eisenberg et al. 1990) deficiencies failed to complement it (detailed data not presented), the extent of deletion in the *Df(3R)e*^{P} chromosome was furhter confirmed to be from 93B6–8 to 93D3–5 (Fig. 1). Normal viability of *Df(3R)e*^{P}/*Df(3R)GC14* heterozygotes indicated that the *hsr*ω locus on the *Df(3R)e*^{P} homolog was not affected by the deletion. This was further confirmed by in situ and Southern hybridization with probes spanning the transcribed region of the *hsr*ω locus (data not presented) and by the results of Southern hybridization with upstream probes presented later.

Treatments and in vitro labeling with [^{3}*H]uridine.* Salivary glands from late third instar larvae of the desired genotypes (see Table 1) were dissected in Poels' salt solution (PSS, 14.7 mM NaCl, 41.98 mM KCl, 7.899 mM $CaCl_2$, 5.64 mM $NaH_2PO_4 \cdot 2H_20$, 1.798 mM $KHCO_3$ and 20.813 mM $MgSO_4$, pH 7.0, Lakhotia and Mukherjee 1980) and treated as follows prior to labeling with [^{3}H]uridine:

a. Control at 21° C in PSS: excised salivary glands were incubated in PSS at 21° C for varying periods of time (10–40 min, corresponding to the following treatments)
b. Heat shock: the salivary glands were heat shocked at 37° C for 30 min in PSS
c. Benzamide, formamide or 3-amino benzamide treatment: the glands were treated for 10 min with one of the amides dissolved at 10 mM concentration in PSS at 21° C
d. Colchicine: colchicine was used at 100 µg/ml in PSS and the glands were incubated at 21° C for 40 min

The control and variously treated salivary glands were labeled with [^{3}H]uridine (activity 250 µCi/ml, sp. act. 18 Ci/mmol, BRIT, India) for 10 min in their respective treatment conditions. Following labeling, the salivary glands were fixed in 1:3 acetic acid: methanol, and squashed in 50% acetic acid. The coverslips were removed following freezing in liquid nitrogen, and the slides were air dried and processed for autoradiography with Kodak NTB-2 emulsion. The autoradiograms were developed after exposure at 4° C for 4–5 days, stained with Giemsa and observed.

Molecular mapping of the distal breakpoint of the Df(3R)e^{P} *deficiency.* Initially the *pUC4.3* and *pUC5.4* clones (corresponding, respectively, to the −1.3 kb to −5.5 and −5.5 to −11 kb upstream regions of the *hsr*ω locus, see Mutsuddi 1995) were used to map the breakpoint in the *Df(3R)e*^{P} chromosome. Since neither of these revealed any differences in restriction patterns (data not presented), a further upstream genomic clone, λ13A (corresponding to the region about −5 kb to about −20.5 kb upstream of the *hsr*λ locus), was isolated by screening a genomic library of *D. melanogaster* with these two clones. A 3.5 kb XhoI-EcoRI fragment from the distal-most upstream end of λ13A was subcloned in *pBluescript* and named *pBS3.5*.

Genomic DNAs were isolated from *Df(3R)e*^{P}*/P(lacZ w*$^{+}$*)93B6/8* and *P(lacZ w*$^{+}$*)93B6/8* male flies and digested with the BamHI,

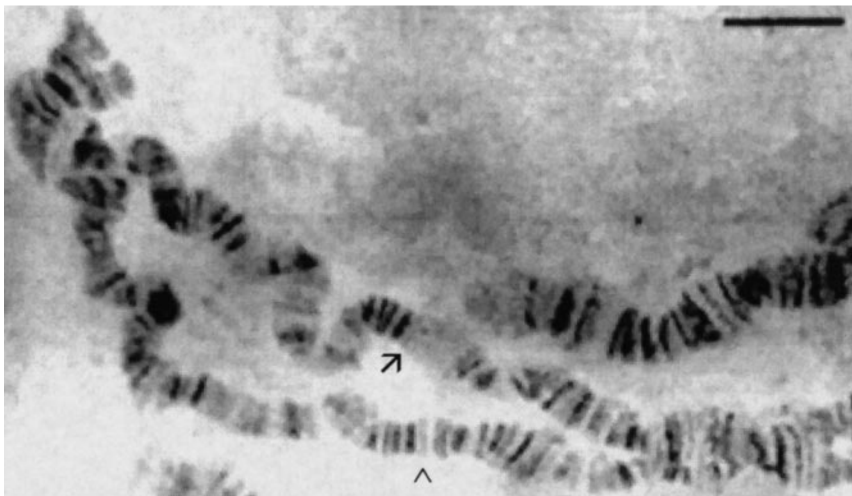


Fig. 2. Cytology of the *Df(3R)e*^{P} chromosome in polytene nuclei from salivary glands of *Df(3R)e*^{P}/+ larvae. The two homologs of the right arm of chromosome *3* are extensively asynapsed. The deletion in the *Df(3R)e*^{P} homolog is marked by an *arrowhead* (>) while the corresponding intact region in the + homolog is marked with an *arrow* (→). The *bar* represents 10 µm

Table 1. Crosses set and genotypes of larvae used for different treatments of late third instar larval salivary glands

Cross	F_1 larvae used (phenotype for selection)
1. +/+×+/+	+/+
2. *P(lacZ+)93B6/8*× *P(lacZ w⁺)93B6/8*	*P(lacZw+)93B6/8P(lacZw+)93B6/8*
3. *Df(3R)e^{R-1}/TM6B*×+/+	*Df(3R)e^{R-1}*/+ (non-tubby larvae)
4. *Df(3R)e^P/TM6B*×+/+	*Df(3R)e^P*/+ (non-tubby larvae)
5. *Df(3R)e^P/TM6B*× *Df(3R)GC14/TM6B*	*Df(3R)e^P/ Df(3R)GC14* (non-tubby larvae)
6. *Df(3R)e^P/TM6B×e/e*	*Df(3R)e^P/e* (non-tubby larvae)

EcoRI, HindIII, PstI and SacI restriction enzymes in appropriate buffers and fractionated on 0.8% agarose gels. The gels were blotted on nylon membranes and the blots hybridized with randomly primed digoxygenin (dig)-dUTP (Boehringer Mannheim) labeled probes (gel-purified DNA inserts from the *pUC4.3, pUC5.4* and *pBS3.5* clones) following the method of Lakhotia et al. (1993). The hybridization signals were detected by colorimetric or chemiluminiscent methods according to the manufacturer's (Boehringer Mannheim) instructions.

Results

The [^{3}H]uridine-labeled autoradiograms were examined for transcription of the 93D puff site after various treatments of the different genotypes (see Table 1). To compare the relative activity of the 93D puff site in different situations, the numbers of silver grains on the 93D puff site and on a chromosomal segment from 99B to 100F of *3R* in a given nucleus were counted and the relative activity of the 93D puff site expressed as the 93D/99B–100F grain ratio.

The 93D locus is induced by heat shock but not by amides on the Df(3R)e^P chromosome

Salivary glands from +/+, *P(lacZ w⁺)93B6/8, Df(3R)e^{R-1}*/+ and *Df(3R)e^P*/+ larvae were examined for inducibility of the 93D puff in response to heat shock or treatment with amides (benzamide, 3-amino-benzamide, colchicine and formamide). Examination of [^{3}H]uridine-labeled autoradiograms revealed that heat shock as well as amide treatment induced a typical puff at the 93D site in +/+, *P(lackZ w⁺)93B6/8* and *Df(3R)e^{R-1}* glands (Fig. 3a–i). However, in the case of *Df(3R)e^P*/+ glands, while heat shock caused the 93D site to be typically puffed and well labeled (Fig. 4a, b), all the amides led to development of an asymmetric puff with labeling being restricted to one side only (Fig. 4c, e, g and i). Nuclei with asynapsis of the 93D region in preparations of amide-treated *Df(3R)e^P*/+ glands clearly showed that while the 93D site on the wild-type homolog was puffed and well labeled, that on the *Df(3R)e^P* homolog was not puffed and showed little or no labeling (Fig. 4d, f, h and j). Compared with the effect of amides, nuclei from heat-shocked *Df(3R)e^P*/+ glands with asynapsis in the 93D region showed equally well developed and labeled 93D puffs on both homolog (Fig. 4b). In the case of *Df(3R)e^{R-1}*/+ glands, the 93D sites on the wild-type and the deficiency homologs were equally puffed and labeled followed heat shock or amide treatment (Fig. 3c, i).

The above observations were confirmed by the grain count data shown in Fig. 5. The mean 93D/3R grain ratios in preparations of heat-shocked glands of the four genotypes [+/+, *P(lacZ w⁺)93B6/8, Df(3R)e^{R-1}*/+ and *Df(3R)e^P*/+] were comparable (Fig. 5a) with no significant difference between them as revealed by Student's *t*-test (data not presented). However, in the case of amide treatment (data for benzamide and colchicine only presented) while the 93D/3R grain ratios in +/+, *P(lacZ w⁺)93B6/8* and *Df(3R)e^{R-1}*/+ glands were similar, those in the *Df(3R)e^P*/+ glands were significantly different, being in fact nearly half of the values in the other three genotypes (Fig. 5a). In the case of *Df(3R)e^P*/+ glands, silver grains were also counted separated on the + and the *Df(3R)e^P* homolog in nuclei where the 93D region displayed asynapsis. This set of data, presented in Fig. 5b, clearly showed that while after heat shock, the 93D puff on the two homologs showed equal labeling, after benzamide or colchicine treatment, the labeling at the 93D site on the wild-type homolog was nearly three times greater than on the *Df(3R)e^P* homolog. With 3-amino-benzamide and formamide also, a similar situation was noted (data not presented but see examples in Fig. 4). It may be noted that the few grains seen on the *Df(3R)e^P* homolog after the amide treatments were generally less than those seen in untreated control preparations (data not presented).

To analyze further the activity of *hsr*ω in the *Df(3R)e^P* homolog after various treatments, we examined salivary glands from *Df(3R)e^P/ Df(3R)GC14* larvae, which have only one copy of the *hsr*ω gene on the *Df(3R)e^P* homolog since the *Df(3R)GC14* chromosome is deficient for the 93D locus (see Materials and methods). Heat shock of salivary glands of larvae of such deficiency *trans*-heterozygotes led to induction of an asymmetric puff at the 93D site on the *Df(3R)e^P* homolog (Fig. 6a). However, benzamide and colchicine failed to induce the 93D puff on the *Df(3R)e^P* homolog in this genotype also (Fig. 6b, c). The *Df(3R)GC14* homolog did not show any detectable increase in [^{3}H]uridine incorporation following any of these treatments (Fig. 6). The 93D/3R grain ratios in control and following heat shock, benzamide or colchicine treatment of *Df(3R)e^P/ Df(3R)GC14* glands shown in Fig. 5a reveal that while in control and heat-shocked

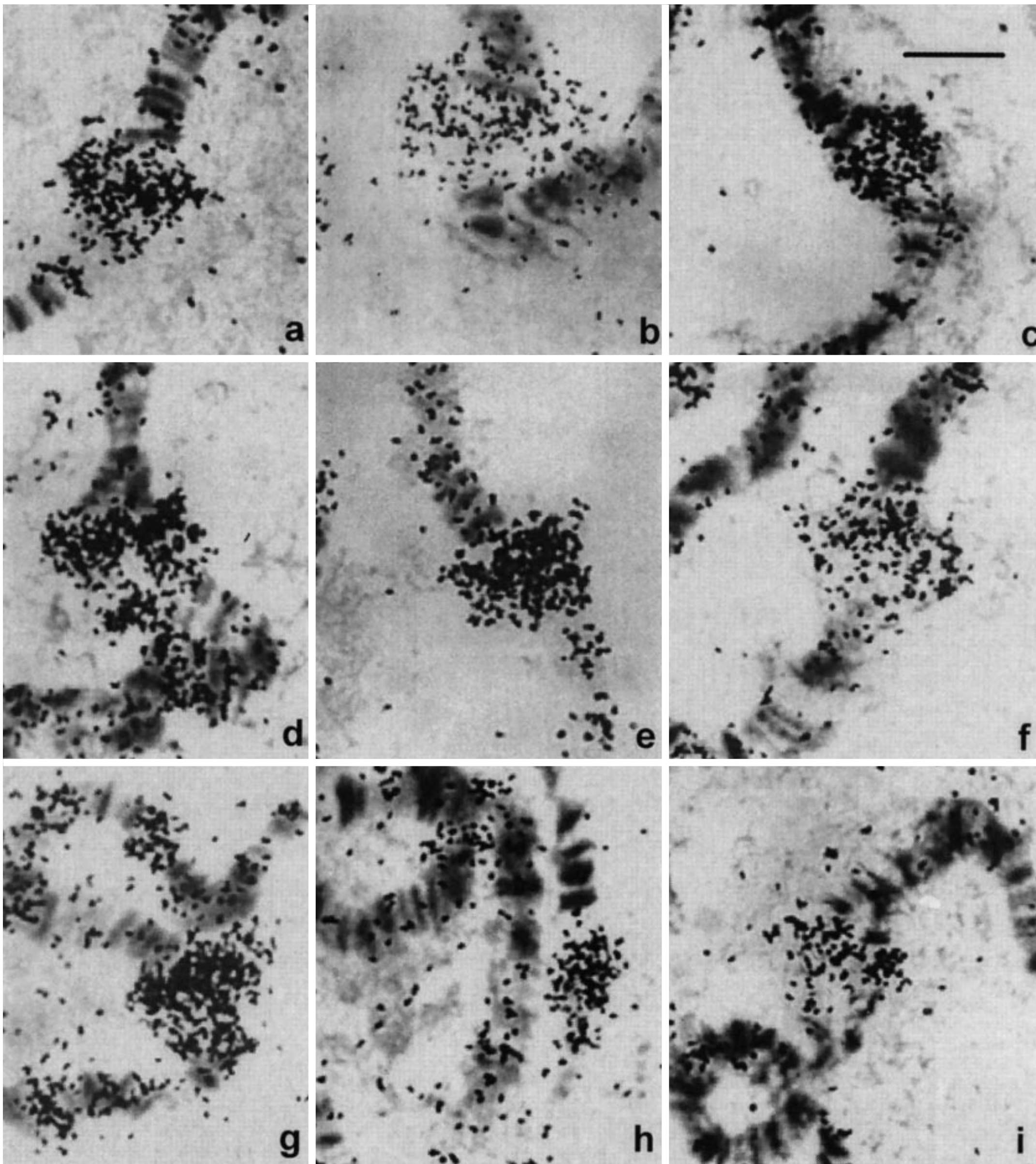


Fig. 3a–i. [^{3}H]uridine-labeled autoradiograms showing the transcriptional activity of the 93D puff site in +/+ (**a, d, g**), *P(lacZ w$^+$)93B6–8/P(lacZ w$^+$)93B6–8* (**b, e, h**) and *Df(3R)e^{R-1}*/+ (**c, f, i**) polytene cells following heat shock (**a, b, c**), colchicine (**d, e, f**) or benzamide (**g, h, i**) treatment. Note the well-labeled symmetric 93D puff in all cases (the two homolog are partly asynapsed in the examples in **c, d** and **i**). The *bar* represents 10 µm

Df(3R)^P/ Df(3R)GC14 glands, the mean ratios were nearly half those in corresponding samples from other genotypes, the ratios in benzamide- or colchicine-treated *Df(3R)e^P/ Df(3R)GC14* glands were only marginally increased over those in the control glands (Fig. 5a). Since the amide treatments cause a drastic inhibition of general chromosome transcription (Lakhotia and Mukherjee 1980; Tapadia and Lakhotia 1997), the apparent increase in the mean 93D/3R grain ratios in the benzamide (0.93±0.07) or colchicine (0.82±0.02) treated *Df(3R)e^P/ Df(3R)GC14* glands over those in corresponding control (0.40±0.03) glands (Fig. 5a) is actually due to the very few grains (usually fewer than 10) on the *3R* reference segment in the treated glands rather than to any real induction of activity of the 93D site by amides in the *Df(3R) Df(3R)e^P* homolog.

These results clearly showed that the deletion in the *Df(3R)e^P* chromosome, while not affecting the heat shock inducibility of the 93D locus, substantially, if not completely, abolished its amide inducibility. On the other hand, neither heat shock nor amide inducibility of the 93D puff was affected by the deletion in the *Df(3R)e^{R-1}* chromosome. The abolition of amide inducibility of the 93D site on the *Df(3R)e^P* chromosome was only a *cis* effect since amide inducibility on the wild-type homolog was not affected, whether the *Df(3R)e^P* and the wild-type homologs were closely synapsed or asynapsed.

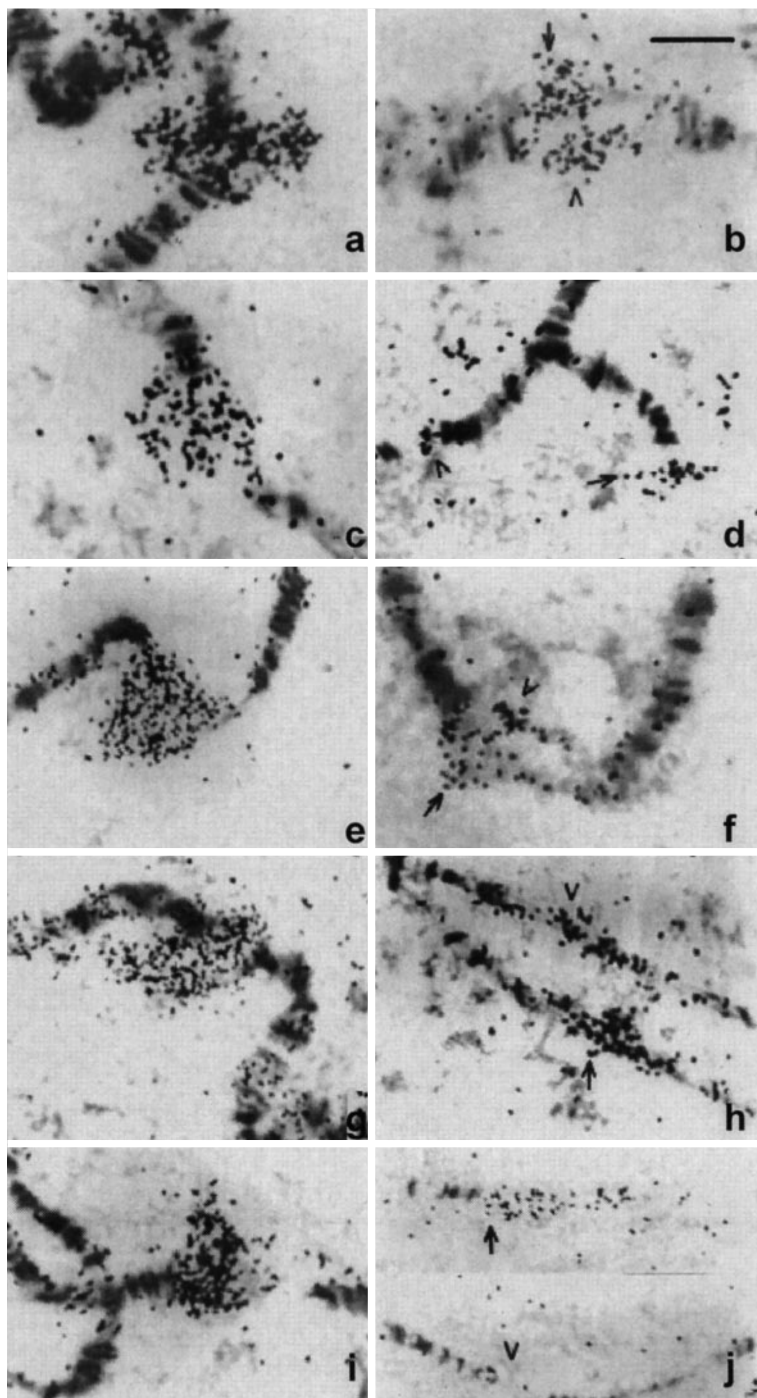
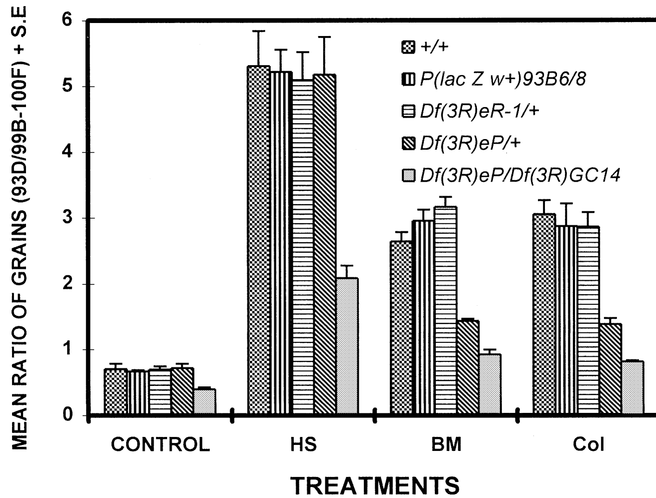


Fig. 4a–j. [^{3}H]uridine-labeled autoradiograms showing transcriptional activity of the 93D puff in *Df(3R)e^P/+* polytene cells following heat shock (**a, b**), benzamide (**c, d**), colchicine (**e, f**), formamide (**g, h**) or 3-amino-benzamide (**i, j**) treatment. The *Df(3R)e^P* and the + homologs are asynapsed in the examples in **b, d, f, h** and **j**. The 93D puff on the + homolog is marked with an *arrow* (→) while the *Df(3R)e^P* homolog is marked with an *arrowhead* (>). Note the symmetric puff in **a** and equal labeling of both homologs in **b** but an asymmetric 93D puff in **c, e, g** and **i** and no labeling on the *Df(3R)e^P* homolog in **d, f, h** and **j**. *Bar* represents 10 μm

Heat shock inducibility of the 93D puff in the Df(3R)e^P chromosome is not affected by the ebony mutation

It has been reported earlier that a mutation at the neighboring *ebony* locus (Lakhotia et al. 1990) or a heterozygous deletion of the 93D locus (Burma and Lakhotia 1986) causes the *hsr*ω locus to be refractory to heat shock induction. Since the *Df(3R)e^{R-1}/+* and *Df(3R)e^P/+* genotypes had only one copy of the *ebony* locus on the wild-type homolog, they were functionally equivalent to *ebony* mutant heterozygotes and, therefore, in view of the earlier report (Lakhotia et al. 1990), the 93D locus was not expected to be heat shock inducible in both these deficiency heterozygotes. However, as noted above, the 93D site showed typical heat shock induction in *Df(3R)e^{R-1}/+* as well as in *Df(3R)e^P/+* genotypes. To examine this further, we generated *Df(3R)e^P/e* genotypes by appropriate crossings (Table 1) and examined heat shock inducibility of the 93D puff in these phenotypically ebony larvae. The data on the size of the 93D puff in wild-type (control and heat-shocked) and in heat-shocked *Df(3R)e^P/e* glands, presented in Table 2, showed that heat shock induced the 93D puff in *Df(3R)e^P/e* glands more or less as strongly as in the wild-type glands.



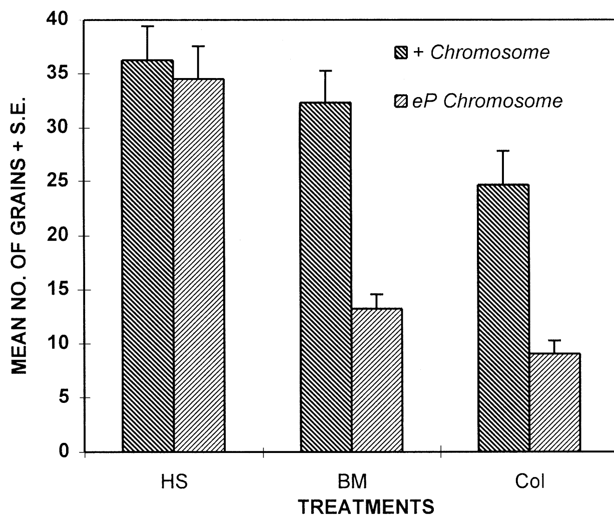


Fig. 5a–b. Histograms showing transcriptional activity of the 93D puff site. **a** Mean ratios of grains (93D/99B–100F) in the various indicated genotypes in controls or after heat shock (*HS*), benzamide (*BM*) or colchicine (*Col*) treatment. **b** Mean numbers of silver grains on the 93D puff site after heat shock (*HS*), benzamide (*BM*) or colchicine (*Col*) treatment in the + and *Df(3R)e*^{P} homolog in *Df(3R)e*^{P}/+ nuclei in which the two homologs were asynapsed

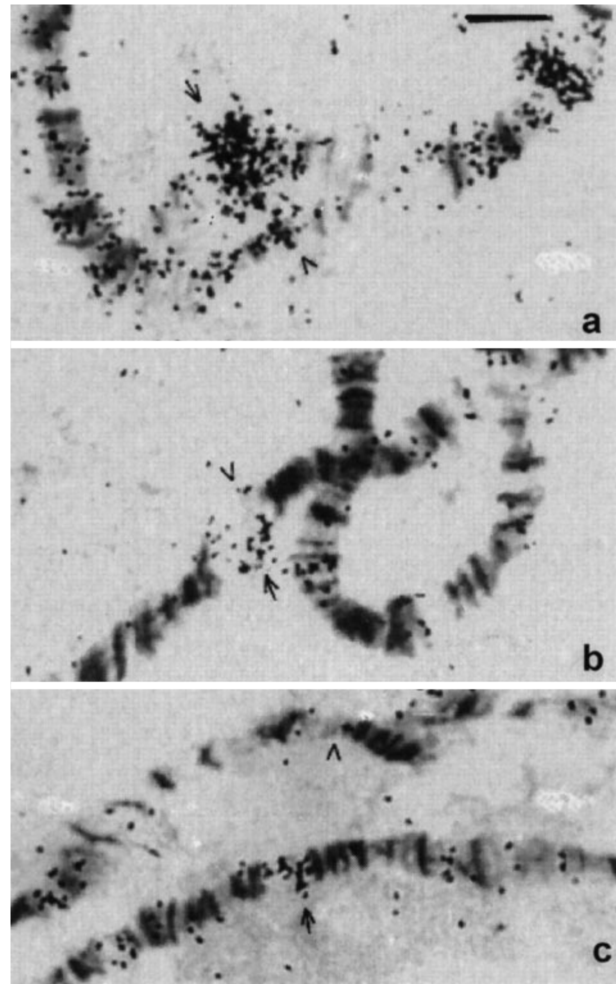


Fig. 6a–c. [^{3}H]uridine-labeled autoradiograms showing transcriptional activity of the 93D puff site in *Df(3R)e*^{P}/ *Df(3R)GC14* polytene nuclei following heat shock (**a**), benzamide (**b**) or colchicine (**c**) treatment. The 93D puff on the *Df(3R)e*^{P} homologs (→) is well labeled after heat shock (**a**) but note its very poor labeling in **b** and **c**; 93D region in the *Df(3R)GC14* homolog (>) remains unlabeled in all three cases. The *bar* represents 10 µm

Table 2. Induction of the 93D puff in wild-type and *Df(3R)e*^{P}*/e* salivary glands

Genotype	+/+		*Df(3R)e*^{P}*/e*
Treatment	Control	Heat shock	Heat shock
Mean puff size±SE[a]	1.04±0.04	2.68±0.09	2.07±0.07
No. of nuclei	14	16	16

[a] Puff size reflects the ratio of maximum width of the 93D puff and that of the 93E1 band

The distal breakpoint of Df(3R)e^P *is about −22 kb upstream of the hsr*ω *transcription unit*

The distal breakpoint of *Df(3R)e*$^{R-1}$ is already known to be about –45 kb upstream of the transcription start point of the *hsr*ω locus (Eisenberg et al. 1990). We have now mapped the distal breakpoint of *Df(3R)e*^{P} using different upstream probes to hybridize with genomic DNAs from flies of different genotypes.

Southern hybridization of restriction enzyme (BamHI, EcoRI, HindIII, PstI or SacI) digested genomic DNA from *P(lacZ w*$^+$*)93B6/8* homozygous and *Df(3R)e*^{P}*/ P(lacZ w*$^+$*)93B6/8* heterozygous flies with dig-labeled inserts from *pUC5.4*, or *pUC4.3* resulted in identical hybridization patterns in the two genotypes (not shown) in all the digests. Likewise, Southern hybridization with the 3.5 probe (Fig. 7) resulted in identical hybridization patterns with HindIII-digested genomic DNA (Fig. 7). However, digestion with the other four enzmes (BamHI, EcoRI, PstI and SacI) resulted in one extra band in the *Df(3R)e*^{P}*/P(lacZ w*$^+$*)93B6/8* lane (Fig. 7) that was obviously due to the *Df(3R)e*^{P} deletion.

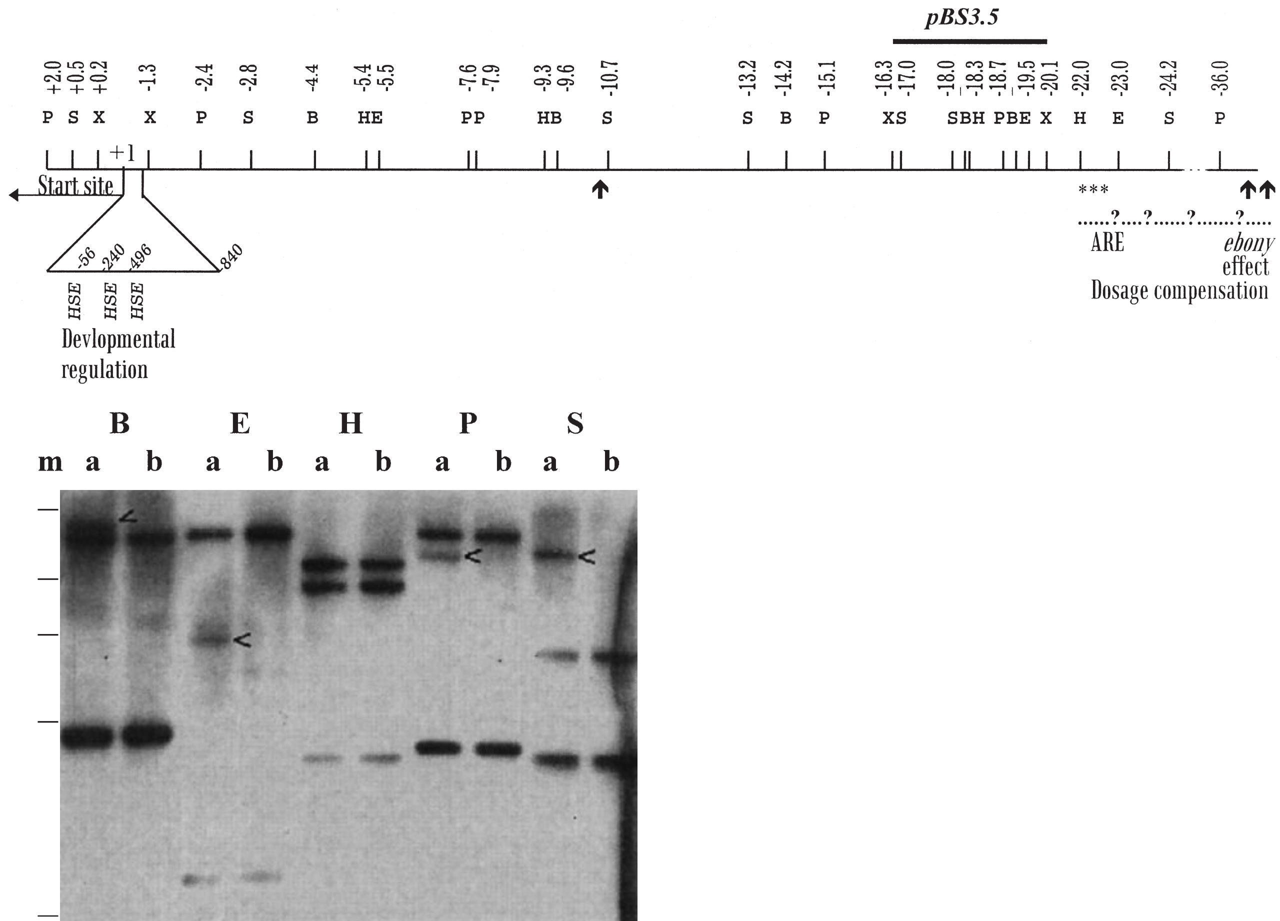


Fig. 7. Southern blot of genomic DNA from *Df(3R)e^P/P(lacZ w^+)93B6–8* (*lanes a*) and *P(lacZ w^+)93B6–8/P(lacZ w^+)93B6–8* (*lanes b*) flies digested with BamHI (*B*), EcoRI (*E*), HindIII (*H*), PstI (*P*) or SacI (*S*) restriction endonuclease and hybridized with digoxygenin-labeled 3.5 kb insert from the *pBS3.5* clone. The left-most panel (*m*) indicates the positions of molecular size markers corresponding to 23, 9.4, 6.5, 4.3, and 2.3 kb, respectively. The bands uniquely seen in BamHI (~18 kb), EcoRI (6.6 kb), PstI (12.5 kb) or SacI (12.6 kb) digested *Df(3R)e^P/P(lacZ w^+)93B6–8* genomic DNA (*lanes a*) after hybridization with the 3.5 probe are marked with *arrowheads* (<). The top panel shows the restriction sites for XhoI (*X*), BamHI (*B*), EcoRI (*E*), PstI (*P*), HindIII (*H*) and SacI (*S*) in the upstream region of the *hsr*ω locus with locations of the sites being marked in kilobases (not to scale). The two bands of ~12.4 and 9 kb, respectively, in the HindIII-digested DNA from *Df(3R)e^P/P(lacZ w^+)93B6–8* (*lane a*) and *P(lacZ w^+)93B6–8/P(lacZ w^+)93B6–8* (*lane b*) flies are due to restriction fragment length polymorphism for HindIII sites on the *P(lacZ w^+)93B6–8* chromosome (map shows locations of the HindIII sites that generate the 9 and 3.7 kb fragments). The transcription start site and the locations of heat shock elements (*HSEs* at –56, –240 and –496) and developmental regulators (Mutsuddi and Lakhotia 1995; Lakhotia and Mutsuddi 1996) are also indicated. The distal (farther from the centromere and thus closer to the *hsr*ω locus, see Fig. 1) breakpoint of the *Df(3R)e^P* deletion, as inferred from the Southern hybridization data, is marked by *three stars* in the interval defined by the HindIII and EcoRI sites at positions −22 and −23 kb, respectively and therefore, the location of a putative amide response element (*ARE*) is suggested to be beyond this region. Putative regulatory sites for dosage compensation and the ''*ebony*'' effect are also indicated to be in this far upstream region. Location of the proximal breakpoint *Df(3R)GC14* at ~−10 kb is shown by a *solid vertical arrow* while that of the distal breakpoint of *Df (3R)e^{R-1}* at ~−45 kb is indicated by *two solid vertical arrows* on the restriction map

On the basis of present hybridization patterns and the previously available genomic maps of the *hsr*ω locus (Walldorf et al. 1984; Eisenberg et al. 1990), a restriction map for the upstream region of the *hsr*ω locus for the BamHI, EcoRI, HindIII, PstI, XhoI and SacI enzymes is shown in the upper part of Fig. 7. Since no difference was observed in the HindIII restriction pattern between the two genotypes, it is concluded that the deletion breakpoint in the *Df(3R)e^P* chromosome is beyond the −22 kb HindIII site. Since the EcoRI site at −23 kb is more proximal than the SacI (−24.2 kb) or the PstI (−36 kb) site but distal to the HindIII at −22 kb, it appears that the distal breakpoint in the *Df(3R)e^P* chromosome is between the HindIII site at −22 kb and the EcoRI site at −23 kb.

Discussion

As noted in the Introduction, the upstream regulatory elements on the *hsr*ω locus that mediate its response to amides are distinct from those that mediate its heat shock induction and are not included in the immediate upstream region up to −840 bp. In the present study we have used

Table 3. Summary of effects of genotypes on dosage compensation and heat shock or amide inducibility of the 93D puff in polytene cells

Genotype	No. of functional gene copies		Relative activity of the 93D puff site[a]			Effect of genotype on 93D activity			References[e]
	ebony	*hsr*ω	Control	Heat shock	Amides	Dosage[b] compensation	Heat shock[c]	Amides[d]	
1. +/+	2	2	++	++++	++++	NA	Yes	Yes	1
2. *e/e*	0	2	++	+	++++	NA	No	Yes	1
3. +/*e*	1	2	++	+	++++	NA	No	Yes	1
4. *Df(3R)GC14*/+	2	1	++	+	++++	Yes	No	Yes	2
5. $Df(3R)e^{Gp4}$/+	1	1	++	+	++++	Yes	No	Yes	2
6. *Df(3R)GC14/e*	1	1	++	++++	++++	Yes	Yes	Yes	3
7. $Df(3R)e^{Gp4}/e$	0	1	++	++++	++++	Yes	Yes	Yes	3
8. $Df(3R)e^{R-1}$/+	1	2	++	++++	++++	NA	Yes	Yes	4
9. $Df(3R)e^{P}$/+	1	2	++	++++	++	No	Yes	No	4
10. $Df(3R)e^{P}$/ *Df(3R)GC14*	1	1	+	++	+	No	Yes	No	4
11. $Df(3R)e^{P}/e$	0	2	++	++++	++	No	Yes	No	4

[a] +, minimal activity; ++, moderate activity; ++++, very high induced activity

[b] NA, not applicable; Yes, the single copy of *hsr*ω is as active as two copies in other genotypes; No, the single inducible copy (on the + or the *e* homolog in the case of amide induction in genotype nos. 9 and 11, resepctively, and on the $Df(3R)e^P$ homolog in genotype no. 10 in control and heat shock conditions) is not dosage compensated

[c] Yes, both copies or the one available copy of *hsr*ω typically induced by heat shock; No, both copies or the one available copy of the locus not induced by heat shock

[d] Yes, both copies or the one available copy of *hsr*ω typically induced by amides; No, the copy on the $Df(3R)e^P$ homolog not induced by amides but that on the + or the *e* homolog in genotype nos. 9 and 11, respectively, induced by amides

[e] 1, Lakhotia et al. (1990); 2, Burna and Lakhotia (1986); 3, Mutsuddi (1995); 4, present study

deficiency mapping to locate the upstream putative AREs (see Tapadia and Lakhotia 1997). We have reported here a new, P-element mobilization generated, deletion mutation, $Df(3R)e^P$, which deletes a short chromosomal segment (93B6–7 to 93D3–5) in the upstream region of its locus.

Our autoradiographic data on transcription of the *hsr*ω locus clearly showed that while heat shock still induced transcriptional activation of the 93D puff on the $Df(3R)e^P$ homolog, its response to different amides was lost as a result of the deletion. This was clear from the fact that in $Df(3R)e^P$/+ cells, while heat shock resulted in typical puffing at the 93D region, amide treatment caused development of an asymmetric puff with the [^{3}H]uridine incorporation being almost half of that in +/+ or $Df(3R)e^{R-1}$/+ or in the parntal line [$P(lacZ\ w^+)93B6–8$] from which the $Df(3R)e^P$ deficiency chromosome was generated. Analysis of [^{3}H]uridine incorporation in $Df(3R)e^P$/+ nuclei with asynapsis of the $Df(3R)e^P$ and the + homolog also revealed that the amide-induced puff with the typically increased [^{3}H]uridine incorporation in the 93D region was present only on the + but not on the $Df(3R)e^P$ homolog. Our results with the $Df(3R)e^P$/ *Df(3R)GC14* genotype further confirmed this. The *hsr*ω locus is included in the deleted region on the *Df(3R)GC14* homolog and, therefore, this homolog does not show any heat shock- or amide-induced puffing at the 93D site (Mohler and Pardue 1982; Burma and Lakhotia 1986); the induction of an asymmetric puff at the 93D region by heat shock but the absence of increased transcriptional activity at this region in amide-treated $Df(3R)e^P$/ *Df(3R)GC14* cells clearly established that the $Df(3R)e^P$ chromosome has lost amide inducibility of the 93D site. The almost complete loss of response to all the amides that were tested in this study on the $Df(3R)e^P$ homolog further illustrates a commonality of the effect of different amides in transcriptional activation of the 93D puff (Tapadia and Lakhotia 1997).

It may further be noted that the effect of the $Df(3R)e^P$ deletion on amide inducibility of the 93D puff is *cis*-limited since the other homolog, carrying a normal *hsr*ω locus, displayed typical induction. This shows that the deleted region includes an upstream *cis*-acting regulatory region that is necessary for the response of the *hsr*ω locus to different amides.

Compared with the $Df(3R)e^P$ deletion, our results showed that response of the *hrs*ω locus on the $Df(3R)e^{R-1}$ deficiency chromosome to heat shock as well as to amides was similar to that in the wild-type chromosome. Therefore, we infer that the interval between the distal breakpoints of the $Df(3R)e^{R-1}$ and $Df(3R)e^P$ deficiencies includes the putative ARE, the loss of which in the $Df(3R)e^P$ chromosome renders it unresponsive to amide treatment. The distal breakpoint in the case of the $Df(3R)e^{R-1}$ chromosome was mapped by Eisenberg et al. (1990) to be about −45 kb upstream of the *hsr*ω transcription unit. Our present mapping of the distal breakpoint of the $Df(3R)e^P$ deficiency places it at more than −22 kb upstream of the *hsr*ω transcription unit. Therefore, we suggest that the AREs are located between −22 kb and −45 kb of the upstream region. The presence of the AREs so far away from the transcription unit adds one more intriguing property to this non-protein-coding *hsr*ω locus. Further studies are required to map more precisely and identify the ARE sequence(s) and their mode of action.

There are two other intriguing aspects of regulation of 93D activity; one concerns dosage compensation at this

locus when in single copy (Burma and Lakhotia 1986) and the other relates to the effect of the *ebony* mutation (or altered levels of β-lamine, Lakhotia et al. 1990) on its heak shock inducibility. The present results reveal further complexities of these regulatory mechanisms (see Table 3 for summary). Burma and Lakhotia (1986) reported that the single copy of the *hsr*ω locus (on the + homolog) in the *Df(3R)e*^{Gp4}/+ or *Df(3R)GC14*/+ genotypes showed as much transcriptional activity (in control and benzamide treatment) as the two copies in a normal diploid genotype while heat shock failed to induce the 93D puff in these genotypes (nos. 4 and 5 in Table 3) but in *Df(3R)e*^{Gp4}/*e* or *Df(3R)GC14*/*e* genotypes (nos. 6 and 7 in Table 3), the single copy of the *hsr*ω locus (on the *e* homolog) is well induced by heat shock and is dosage compensated (see Table 3, Mutsuddi 1995). Compared with these earlier results with the *Df(3R)e*^{Gp4} or *Df(3R)GC14* deficiencies, the present results revealed that in *Df(3R)e*^{P}/ *Df(3R)GC14* cells (genotype no. 10 in Table 3), the single copy of the *hsr*ω locus on the *Df(3R)e*^{P} homolog was not dosage compensated since the [^{3}H]uridine labeling was half that in *Df(3R)e*^{P}/+ cells, both in control and heat-shocked conditions (Fig. 5a, Table 3). It is also interesting to note that in *Df(3R)e*^{P}/+ cells (genotype no. 9 in Table 3) the failure of the 93D site on the *Df(3R)e*^{P} homolog to be induced by amides did not cause the copy on the + homolog to be twice as active as happens after amide treatment in genotype nos. 4–7 or after heat shock in genotype nos. 6 and 7 (see Table 7).

Mutations at the *ebony* and *black* loci or altered levels of β-alanine have complex effects on the heat shock inducibility of the 93D puff (Lakhotia et al. 1990). Homozygosity or heterozygosity for a mutant allele at the *ebony* locus in a normal diploid genetic background causes the 93D locus to be refractory to heat shock induction (genotype nos. 2 and 3 in Table 3), while presence of a mutant allele at the *ebony* locus in a *Df(3R)e*^{Gp4} or *Df(3R)GC14* background allows the 93D locus on the unrearranged homolog to puff normally in response to heat shock (Mutsuddi 1995; compare genotype nos. 4, 5 and 6, 7 in Table 3). In this context, the absence of an effect of an *ebony* mutation (Monosomy or nullosomy for a functional *ebony* gene) on heat shock inducibility of the 93D puff in *Df(3R)e*^{P} as well as *Df(3R)e*$^{R-1}$ backgrounds (nos. 8–11 in Table 3) suggests that the *hsr*ω locus on these deletion chromosomes has lost this sensitivity. These results thus point to the existence of additional regulatory elements that modulate dosage compensation and the β-alanine (*ebony* and *black* mutation) effect (Lakhotia et al. 1990) at the 93D locus. A similar response of the 93D puff in *Df(3R)e*^{P} and *Df(3R)e*$^{R-1}$ chromosomes indicates that these regulatory sites may reside further upstream than the distal breakpoint in the *Df(3R)e*^{P} deletion. Their identification and their mode of action need further studies.

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