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Specific induction of the *hsrω* locus of *Drosophila melanogaster* by amides

M. G. Tapadia & S. C. Lakhotia

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We report here that 3-aminobenzamide and other amides, such as formamide, acetamide and nicotinamide, specifically induce a high rate of transcription at the 93D puff (the *hsrω* heat shock gene) in polytene chromosomes of *Drosophila melanogaster*. Other chemicals, such as benzamide, colchicine, thiamphenicol and paracetamol, that are already known to specifically induce transcription at the *hsrω* locus are also identified as amides. In view of the specific induction of the 93D puff by different amides and other data that demonstrate *hsrω* transcription in response to benzamide and colchicine etc. to be independent of its heat shock induction, it appears likely that amides induce this locus through distinct regulatory elements that we propose to designate amide response elements (AREs).

Key words: amides, *Drosophila*, heat shock, *93D* locus

Introduction

The *93D* or the *hsr-omega* (*hsrω*) locus of *Drosophila melanogaster* is a very interesting gene in view of its many unusual properties, which include its ready inducibility with a variety of agents, its wide developmental expression and its not coding for any protein (reviewed in Lakhotia & Sharma 1996). This locus was distinguished from the other heat shock loci because it is an unusual member of the heat shock family of genes (Mukherjee & Lakhotia 1979) and by its specific induction in larval salivary glands by benzamide (BM) (Lakhotia & Mukherjee 1980). Subsequent studies have shown that, besides BM, the *93D* or *hsrω* locus is also selectively induced, independent of any other heat shock gene, by chemicals such as colchicine/colcemid (Lakhotia & Mukherjee 1984), thiamphenicol (Behnel 1982) and paracetamol (Srivastava & Bangia 1985). An intriguing aspect of the selective induction of the 93D puff by all these chemicals is that these chemicals are known to have very diverse effects on cells. Colchicine and colcemid are well-known inhibitors of microtubule polymerization (Dustin 1978). However, other microtubule poisons, such as vinblastine, nocodazole, chloral hydrate and podophylotoxin, fail to induce this locus (Singh & Lakhotia 1984). In contrast, BM and its derivatives are often used as potent inhibitors of poly-ADP-ribose polymerase (PARP) (Sims *et al*. 1983) and are also reported to have some effect on the depolymerization of contractile fibrils in *Physarum polycephalum* (Korohoda & Wohlfarth-Bottermann 1976) but with no known effect on microtubules. Paracetamol is an analgesic and antipyretic drug which, like salicylates, is an uncoupler of oxidative phosphorylation; however, unlike salicylates, which induce all the heat shock genes, paracetamol induces only the 93D heat shock puff (Srivastava & Bangia 1985). Finally, thiamphenicol, another specific inducer of the 93D puff, is an analogue of chloramphenicol and interferes with the energy-conserving metabolism in the mitochondria (Behnel 1982). Thus, in the absence of an apparently common target, the basis for specific induction of the 93D puff in salivary glands of late third instar larvae of *D. melanogaster* briefly treated with any of these chemicals has remained enigmatic.

An examination of chemical structures of the four above-mentioned selective inducers of the 93D puff reveals all of them to be amides. The IUPAC name for colchicine is (S)-*N*-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl) acetamide, for thiamphenicol it is D-threo-2,2,-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-(mehysulphonyl) phenethyl] acetamide and for paracetamol the IUPAC name is *N*-(4-hydroxyphenyl) acetamide. Therefore, to examine if the specific inducibility of the 93D puff in response to all these chemicals is because they are amides, we have now examined transcriptional activity of the 93D puff after treatment of larval salivary glands with a number of other amides, namely formamide (FM), acetamide (AM), nicotinamide (NM) and 3-aminobenzamide (3-AB). Out results showed that, like BM, colchicine, thiamphenicol and paracetamol, all the amides tested in the present study, caused a selective activation of the 93D puff.

Materials and methods

Flies and culture conditions

The wild-type Oregon R^+ strain of *Drosophila melanogaster* was used. Flies were maintained at 21 ± 1°C on standard corn meal,

M. G. Tapadia and S. C. Lakhotia (corresponding author) are at the Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221005, India. Tel: (+91) 542 316145; Fax: (+91) 542 317457, 317074; Email: lakhotia@banaras.ernet.in.

agar and yeast food. For cytological preparations, staged larvae were grown in Petri dishes and periodically supplemented with yeast. Salivary glands from actively migrating late third instar larvae were dissected in Poels' salt solution (PSS, Lakhotia & Mukherjee 1980) and subjected to the following treatments:

- control: Incubated in PSS at 21°C for 10 min and then labelled with [^{3}H]uridine for 10 min (activity 250 μCi/ml, sp. act. 18 Ci/ml, Brit, Bombay, India) at 21°C.
- Amides: different amides (formamide, acetamide, nicotinamide and 3-aminobenzamide) were used at a final concentration of 10 mM in PSS. The excised salivary glands were incubated in PSS containing the given amide at 21°C for 10 min before labelling with [^{3}H]uridine as above for 10 min at 21°C in the continued presence of the amide.

After the treatment described above, salivary glands were fixed in 1:3 acetomethanol for 30 s and then squashed in 50% acetic acid. The slides were frozen in liquid nitrogen and the coverslips were flipped off with a razor blade. The slides were kept in absolute ethanol for a few minutes and then air dried. They were coated with Kodak NTB-2 emulsion and exposed for 4–5 days before the development of the autoradiograms. The slides were finally stained with 3% Giemsa for 2–3 min, mounted in DPX and examined.

Results and discussion

Examination of autoradiograms of [^{3}H]uridine-labelled polytene chromosomes revealed that, as after BM or colchicine treatment (Lakhotia & Mukherjee 1980, 1984), the general chromosomal transcription was considerably reduced (Figure 1), the nucleolar uptake of [^{3}H]uridine was not much affected, but the transcription at the 93D site was markedly increased after treatment with all the amides (Figure 1). None of the other heat shock loci showed any increase in [^{3}H]uridine incorporation (data not presented but see Figure 1), indicating a specific activation of the 93D puff. The numbers of silver grains at the 93D site and a segment of the 3R (99B to 100F) were counted and the ratios of 93D/3R segment in control and the various amide-treated glands are presented in Table 1. Student's *t*-test revealed that [^{3}H]uridine incorporation at the *hsrω* locus in the amide-treated salivary glands was significantly greater than in control cells ($P < 0.01$ in all cases, Table 1).

It has been reported previously (Lakhotia 1987) that 3-aminobenzamide, at a concentration ranging from 0.1 mg/ml to 1 mg/ml is unable to induce the *hsrω* locus. However, in the present study, 3-aminobenzamide was found typically to induce the *hsrω* locus when used at a higher concentration of 1.5 mg/ml (10 mM). This suggests a dose-dependent effect of amides on induction of the *hsrω* locus.

The present results clearly showed that all four amides that were tested induced the *93D* heat shock locus in the same manner as the previously reported induction with BM, colchicine, thiamphenicol or paracetamol (Lakhotia & Mukherjee 1980, 1984, Behnel 1982, Srivastava and Bangia 1985) and, further, like the BM or colchicine, all four amides caused inhibition of general chromosomal transcription without affecting the nucleolar activity. BM, colchicine, paracetamol and thiamphenicol, besides sharing an amide group, also have a benzene ring in common. Therefore, amides with (nicotinamide and 3-aminobenzamide) or without (formamide and acetamide) a benzene ring were used in the present study to determine their effect on the *hsrω* locus. As all of them induced the 93D puff similarly, we believe that it is the amide group rather than the benzene ring that is responsible for the 93D puff induction.

Several previous studies in our laboratory have suggested that the HS induction of the *93D* locus occurs via a pathway different from the BM and colchicine pathways as only certain conditions affect the heat- or the chemical-induced activation of this locus differently, the profile of its multiple transcripts in response to heat shock and chemicals is also different (Lakhotia & Sharma 1996). Furthermore, Lakhotia & Mutsuddi (1996) demonstrated that a region up to −846 bp upstream of the *hsrω* locus conferred typical HS inducibility on the *lacZ* reporter gene, but not BM or colchicine inducibility. HSP83 specifically accumulates at the 93D puff site after heat shock but not after BM induction (Morcillo *et al*. 1993). A deletion map of the

Table 1. Relative transcriptional activity of the 93D puff site in control and amide treated salivary glands

	Mean (± SE) 93D/3R grain ratios				
	Control	Formamide	Acetamide	Nicotinamide	3-Amino benzamide
	2.59 ± 0.24	3.95 ± 0.15	3.94 ± 0.27	4.01 ± 0.28	3.98 ± 0.24
Number of nuclei	15	19	11	13	13
t		5.02	3.6	5.2	3.99
P		< 0.001	< 0.001	< 0.001	< 0.001

The *t*-values in each case were obtained after comparison of the treated sample with the control.

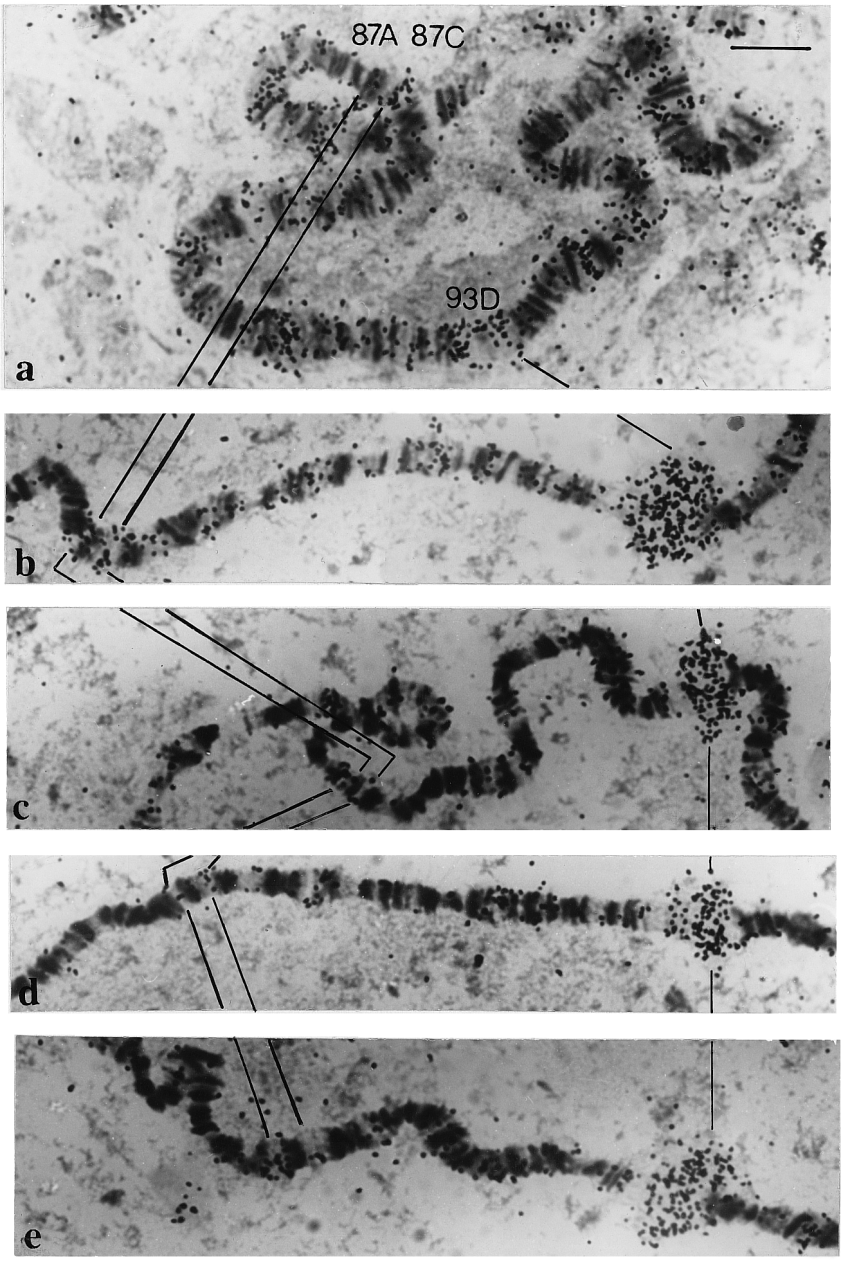

Figure 1. Autoradiograms showing [^{3}H]uridine incoporation at the 93D site in control (**a**) or amide-treated (**b–e**) salivary glands of wild type larvae (**b** formamide, **c** acetamide, **d** nicotinamide and **e** 3-amino benzamide). The 93D puff site is very strongly labelled in each of the treated examples. Note the absence of sliver grains on 87A and 87C sites. Bar = 10 μm.

amide inducibility of the 93D puff has revealed that an upstream region between approximately −21 kb and −45 kb of the *hsrω* locus is necessary for response of the 93D puff to any amide, but the absence of this region does not affect its HS inducibility (Lakhotia & Tapadia 1997). In view of these considerations and the present data that all amides in general induce the *93D* locus in a similar fashion, it appears likely that the amides function through a common pathway not involving the HSEs located within the −846 bp upstream region (Lakhotia & Mutsuddi 1996). We propose that this hypothetical regulatory site in the far upstream region (approximately −21 kb to −45 kb) of the *hsrω* gene be named as the amide response element (ARE; see also Lakhotia & Tapadia 1997).

The existence of multiple regulatory elements for the

hsrω is not unique. Several other heat shock genes are known to have multiple regulatory elements. The small hsp genes in *Drosophila* have ecdysone response elements and are expressed in a developmentally regulated manner (Marin *et al*. 1993). For the *hsrω* locus also, two sequence motifs bearing close homology to the ecdysone receptor binding sites have been identified in the upstream promoter region (Mutsuddi & Lakhotia 1995). The human *hsp70* genes, in addition to the heat shock elements also have cAMP response elements (Choi *et al*. 1991) and serum response elements (Visvader *et al*. 1988).

A comparable activation of a specific gene or a set of genes by amides in other eukaryotes is not known, although in prokaryotes such as *Pseudomonas aeruginosa* propionamide and acetamide etc. are known to activate the amidase gene (Wilson *et al*. 1993). However, activation of the *hsrω* locus by amides does not appear to be analogous as this locus is known not to code for any protein (Lakhotia & Sharma 1996). Whether its transcripts are involved in the activity of amidase or any other protein involved in amide metabolism remains to be studied.

Amides such as NM, BM and 3-aminobenzamide are well-known inhibitors of PARP (Sims *et al*. 1983). Therefore, it may appear that activation of the *93D* locus by amides is related to the inhibition of PARP. However, inhibition of PARP by amides may not be a direct cause for *hsrω* activation as amides can inhibit PARP at as low a concentration as 3 mM (Shall *et al*. 1984), but at this concentration *hsrω* is not induced by any amides. Nevertheless, this aspect needs further study.

We believe that the identification of chemical basis (the amides) for the specific induction of the 93D puff by agents, with otherwise diverse effects on cells, will pave the way for a better understanding of the functional significance of this enigmatic locus.

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