Search for a *Drosophila*-93D-like locus in *Chironomus* and *Anopheles*

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Abstract

The results of experiments to explore the possible existence of a heat shock locus in *Chironomus* and *Anopheles* which may be comparable to the 93D heat shock locus of *Drosophila*, are presented. None of the heat shock loci in *C. striatipennis* were inducible by benzamide, colchicine, vitamin B_6, thiamphenicol or a homogenate of heat shocked glands, all of which are known to selectively induce the 93D-like loci in the genus *Drosophila*. Benzamide also failed to induce any locus in *A. stephensi*. The effect of all these treatments on general transcription in *Chironomus* and *Anopheles* polytene nuclei were comparable to those known in polytene cells of *Drosophila*. It thus appears that a heat shock locus homologous to 93D of *D. melanogaster* is absent in *Chironomus* and *Anopheles* so far as inducibility of a puff by specific agents is concerned. The existence of a possible 'functional counterpart' of the 93D locus in *Chironomus* and *Anopheles* genomes cannot be eliminated.

Introduction

Among the major heat shock (TS) loci in *Drosophila melanogaster*, the 93D has generated much curiosity because of its many enigmatic features (for review, see Lakhotia, 1987). The 93D puff can be specifically induced, independent of other heat shock loci, by a number of agents like benzamide, colchicine, homogenate of heat shocked glands, thiamphenicol, *etc* (Lakhotia, 1987). These inducers provide a suitable tool to study the regulation, expression and interaction of this locus.

A number of unusual characteristics has made the 93D locus all the more interesting. Unlike other TS loci, 93D transcripts are associated with large ribonucleoprotein particles with distinct antigenic properties. One of the 93D transcripts undergoes processing and adenylation without any detectable translational product (see reviews by Lakhotia, 1987, 1989). In spite of its apparently non-coding functions and a rapid sequence divergence, the 93D heat shock locus is functionally conserved in the genus *Drosophila* (Lakhotia and Singh, 1982).

In view of this evolutionary conservation, it is important to know if a 93D-like heat shock locus is confined to the genus *Drosophila* or is a component of the heat shock system in other dipterans as well. Since inducibility of this functionally conserved locus by several agents is known in *Drosophila*, the effect of these 93D-specific inducers was studied in two other phylogenetically remote dipteran species, *Chironomus* and *Anopheles*, to search for a *Drosophila*-93D-like locus in these insects.

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Materials and methods

Chironomus striatipennis larvae were raised under controlled (24°C) culture conditions as described by Hägele (1975). Salivary glands from mid-4th instar stage larvae were excised and were subjected to treatments with different agents like benzamide (BM), colchicine, thiamphenicol (TAP), heat shocked glands' homogenate (HSGH) and vitamin B_6 (vit-B_6), in the inorganic salt constituents of Poels' tissue culture medium (Lakhotia and Mukherjee, 1980).

For corresponding controls, isolated sister glands were incubated in medium devoid of these agents at 24°C (Table 1). Thereafter, the treated and control glands were labelled with ^{3}H-uridine (100 μCi/ml; sp. act. 15.2 Ci/mM BARC, Bombay) for 10 min in the presence or absence of BM, colchicine, TAP, HSGH or vit-B_6. After labelling, the control and treated glands were briefly fixed and squashed. Slides were then routinely processed for autoradiography.

Anopheles stephensi stock was kindly provided by Dr S. K. Subbarao, Malaria Research Centre, Delhi, India. The mosquitos were reared as described by Rao (1981). Ovaries from semi-gravid adult females of *A. stephensi* were dissected out during 24—30 h of the post-blood meal period and were incubated in Poels' medium with or without benzamide (1 mg/ml) for 20 min at 29°C following which they were labelled with ^{3}H-uridine in the presence (treated) or absence (control) of BM as in the case of *Chironomus* salivary glands and processed for autoradiography.

Observations

In this study, the effect of the different inducers of 93D locus of *D. melanogaster* on ^{3}H-uridine incorporation in two dipterans was analysed. Mean numbers (± SE) of silver grains on different TS loci (Nath and Lakhotia, 1989a,b) and particular chromosomal segments (chromosome III segment 7A to 8D in *C. striatipennis* and chromosome 2L segment 28A to 28E in *A. stephensi*) were scored to see transcriptional patterns of general chromosomal regions and of the TS loci after the various treatments.

As seen in Table 1 and Figures 1a—d and 2, none of these treatments induced any of the heat shock loci or any other puffs in *C. striatipennis* or in *A. stephensi*. In addition, other concentrations of the chemical inducers were used as follows: BM (0.5 mg/ml, 1.5 mg/ml, 2.5 mg/ml); colchicine (10 μg/ml, 150 μg/ml, 200 μg/ml); TAP (1 mM and 1.5 mM). None of the other concentrations caused induction of any puff either (data not presented).

Both benzamide and colchicine severely inhibited chromosomal transcription in *C. striatipennis* (Table 1) while the nucleolar transcription was not much affected. Treatment with 2 mM TAP caused a slight inhibition of chromosomal transcription, while HSGH incubation had no apparent effect. Vitamin B_6 at 1 mg/ml totally inhibited ^{3}H-uridine incorporation in polytene nuclei (data not presented).

In *A. stephensi* general chromosomal transcription was significantly inhibited by BM. Absence of a coherent nucleolus in nurse cell polytene chromosome squashes (Redfern, 1981) made it difficult to ascertain the effect of BM on nucleolar transcription.

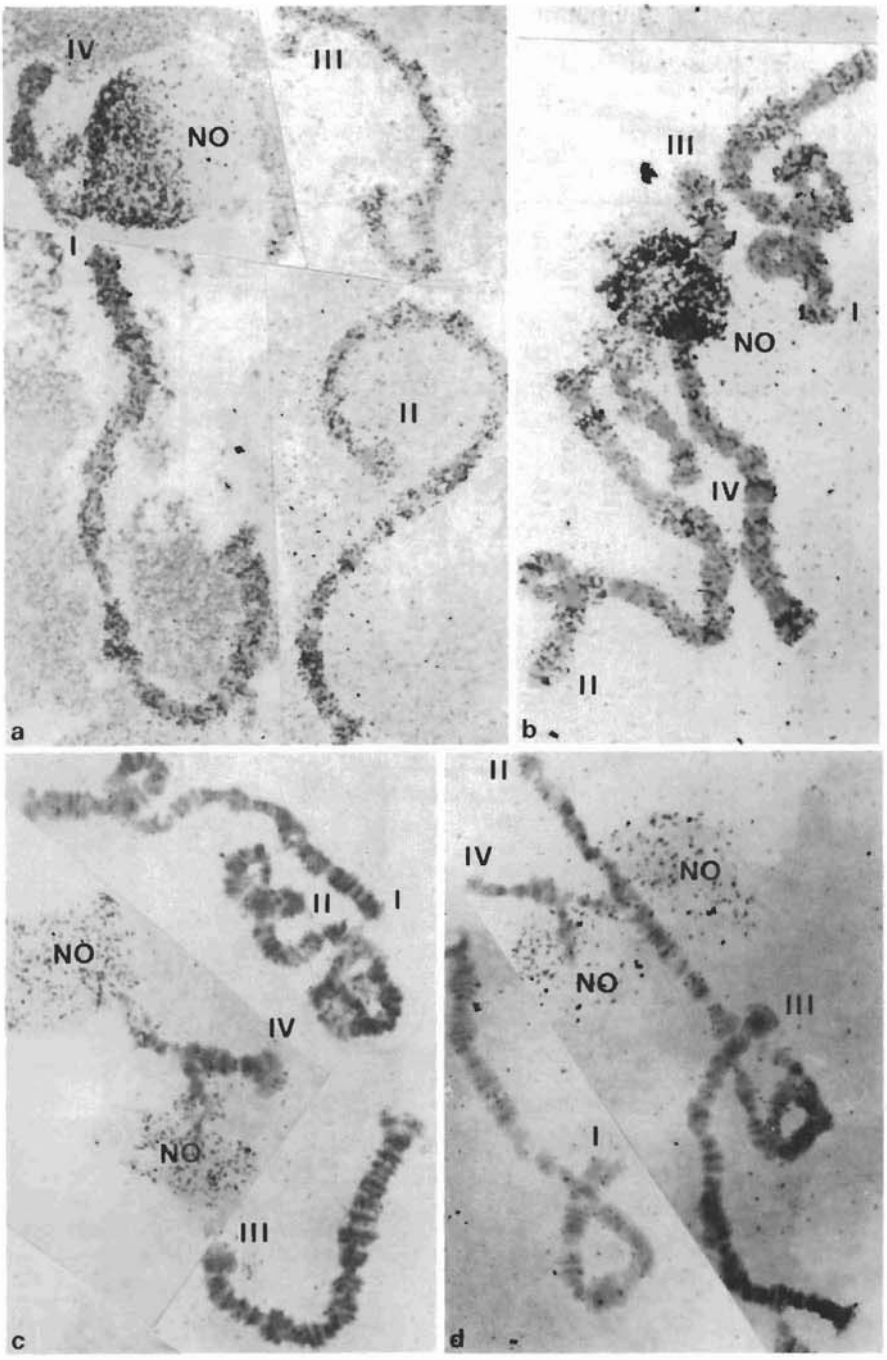

Figure 1 Autoradiograms of polytene chromosomes of *C. striatipennis* showing ^{3}H-uridine labelling after (a) HSGH, (b) thiamphenicol, (c) colchicine, and (d) benzamide treatment. NO, nucleolus. x560.

Table 1a Effect of 93D-inducing agents on activity of heat shock loci of *Chironomus striatipennis*

Treatment		Mean (± SE) grain density on major TS loci in polytene nuclei: I-1D	I-4A	II-3BC	II-10B	II-10D	III-1C	III-2D	III-6C	IV-2D	III-segment*
BM	C	19.0 ± 1.1 (18)	22.9 ± 1.3 (18)	21.8 ± 1.3 (18)	15.0 ± 0.9 (18)	21.2 ± 1.4 (18)	18.9 ± 1.4 (15)	18.3 ± 1.2 (15)	15.9 ± 0.9 (15)	15.2 ± 0.8 (17)	68.7 ± 3.5 (15)
	T	5.0 ± 0.9 (18)	5.9 ± 0.7 (18)	5.3 ± 0.5 (15)	3.2 ± 0.7 (15)	6.1 ± 0.9 (15)	3.5 ± 0.7 (15)	2.8 ± 0.6 (15)	3.3 ± 0.6 (15)	4.1 ± 0.7 (18)	20.0 ± 2.1 (15)
Colchicine	C	16.7 ± 0.1 (16)	18.3 ± 1.3 (16)	20.3 ± 1.4 (18)	14.6 ± 0.7 (18)	17.8 ± 1.3 (18)	18.5 ± 1.1 (16)	13.7 ± 0.6 (16)	14.6 ± 0.5 (16)	16.8 ± 1.2 (16)	53.3 ± 2.3 (16)
	T	8.0 ± 1.0 (16)	6.4 ± 0.6 (16)	5.7 ± 0.5 (15)	5.4 ± 0.3 (15)	7.5 ± 0.5 (15)	7.1 ± 0.6 (16)	5.3 ± 0.4 (16)	5.1 ± 0.5 (16)	5.2 ± 0.4 (18)	15.6 ± 1.4 (18)
TAP	C	35.4 ± 1.3 (17)	33.3 ± 1.3 (17)	33.1 ± 1.2 (16)	28.1 ± 0.7 (16)	33.9 ± 1.2 (16)	32.2 ± 1.7 (16)	25.9 ± 1.1 (16)	33.1 ± 1.3 (16)	26.3 ± 1.6 (16)	112.1 ± 3.5 (16)
	T	33.6 ± 2.2 (16)	30.6 ± 1.3 (16)	31.9 ± 1.2 (15)	28.4 ± 1.9 (15)	32.9 ± 2.0 (15)	27.8 ± 1.2 (15)	22.7 ± 0.9 (15)	27.8 ± 1.2 (15)	25.8 ± 1.3 (16)	91.8 ± 2.8 (16)
HSGH	C	20.1 ± 1.1 (17)	25.7 ± 1.0 (17)	28.9 ± 1.2 (17)	24.9 ± 0.8 (17)	28.7 ± 0.7 (17)	26.7 ± 1.2 (17)	21.2 ± 1.2 (17)	27.2 ± 1.1 (17)	24.6 ± 1.1 (17)	45.8 ± 1.7 (17)
	T	19.8 ± 1.0 (19)	26.4 ± 1.0 (19)	30.3 ± 1.1 (19)	21.8 ± 1.5 (19)	27.7 ± 1.5 (19)	25.7 ± 1.6 (21)	17.9 ± 0.9 (21)	23.8 ± 1.6 (21)	22.5 ± 1.9 (18)	46.2 ± 3.8 (21)

*III-segment, 7A to 8D.
C, control; T, treated. Figures in parentheses indicate the number of nuclei scored in each case. BM, 1 mg/ml for 20 min at 24°C; colchicine, 100 μg/ml for 60 min at 24°C; TAP, 2 mM for 120 min at 24°C; HSGH, homogenate of forty heat shocked glands as described by Mukherjee and Lakhotia (1981).

Table 1b Effect of 93D-inducing agents on activity of heat shock loci of *Anopheles stephensi*

Treatment		Mean (± SE) grain density on major TS loci in polytene nuclei: X-6A	X-2A	2R-19B	2L-21A	3L-38B	3L-40E	2L-seg*
BM	C	14.7 ± 0.9 (25)	9.3 ± 0.6 (25)	12.4 ± 0.6 (25)	11.4 ± 0.4 (25)	20.0 ± 0.8 (25)	12.3 ± 0.6 (25)	20.6 ± 0.7 (25)
	T	6.2 ± 0.5 (25)	3.9 ± 0.3 (25)	5.0 ± 0.3 (25)	3.0 ± 0.3 (25)	5.8 ± 0.5 (25)	5.9 ± 0.5 (25)	9.1 ± 0.4 (25)

*2L-segment, 28A to 28E.
For legend see Table 1a.

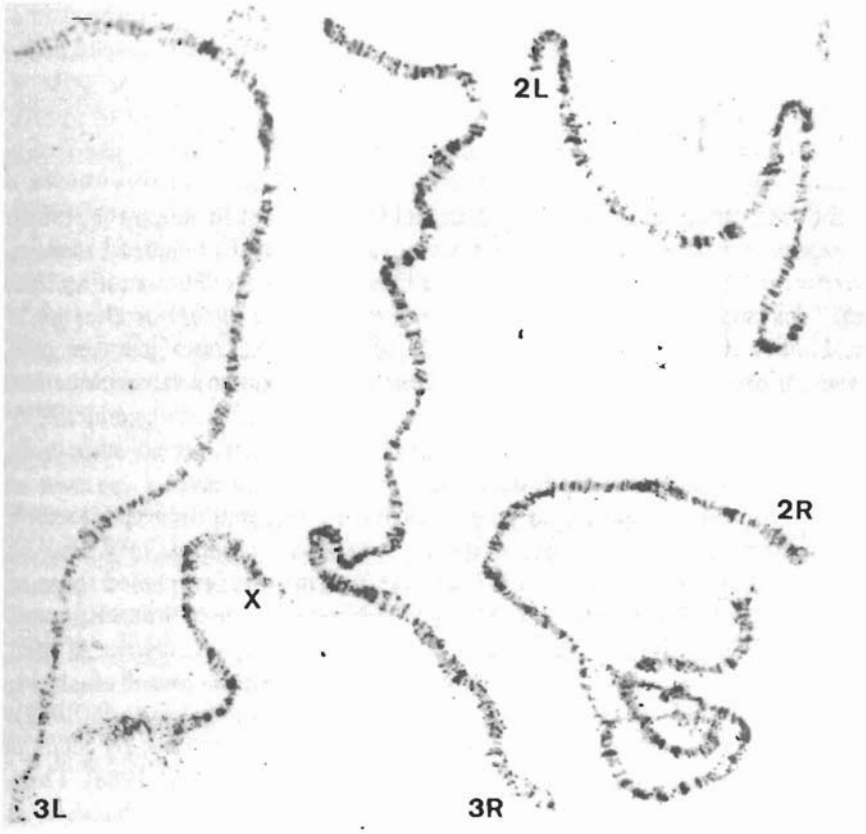

Figure 2 Autoradiogram of a polytene nucleus from ovarian nurse cells of *A. stephensi* showing ^{3}H-uridine labelling after benzamide treatment. x600.

Discussion

In *Drosophila melanogaster*, 93D is one of the major heat inducible loci. The agents used in this study are known to cause specific induction of 93D and its homologous loci in different species of *Drosophila* (see review in Lakhotia, 1987). This unique inducibility was the main criterion for selecting these agents as a tool in the search for 93D-homologues in *C. striatipennis* and *A. stephensi*. However, none of these agents induced any of the heat shock or non-heat shock loci in *C. striatipennis*.

Similarly, benzamide remained ineffective in *A. stephensi* from the induction point of view. Benzamide and colchicine are known to severely inhibit chromosomal RNA synthesis in polytene nuclei of *Smittia* and *Drosophila* without much effect on nucleolar transcription (Sirlin and Jacob, 1964; Lakhotia and Mukherjee, 1980, 1984). Vitamin B_6 also inhibited RNA synthesis in polytene nuclei of *Drosophila* (Leenders *et al.*, 1973; Lakhotia and Singh, 1982).

In *Chironomus* and *Anopheles*, although the 93D inducer/s apparently failed to influence any particular gene locus, their general action was comparable. Several features such as (1) the effect of BM and colchicine in reducing the ongoing chromosomal transcription, (2) persistent nucleolar transcription, and (3) marginal and complete inhibition of transcription by HSGH and vitamin B_6, respectively, mimic the general effects of these 93D-inducers in *Drosophila* polytene cells. This shows that these agents were generally effective on polytene cells of *C. striatipennis* as well as *A. stephensi*. In this context their failure to induce any of the heat shock locus in *Chironomus* and *Anopheles* may suggest that a 93D-like heat shock locus is not present in these dipterans.

Earlier observations in some other dipterans like *Smittia* (Sirlin and Jacob, 1964), *Melanagromyza obtusa* (Singh and Gupta, 1985), and *Chironomus thummi* (Barettino *et al.*, 1988), on the non-inducibility of any heat shock puff by BM *etc* may also suggest that a 93D-like puff is a curiosity of the genus *Drosophila* only. However, before reaching such a conclusion other points must be considered. At present it is not known how these inducers act and cause induction (Lakhotia, 1987), but it would be interesting to see whether the non-inducibility in other dipterans is a reflection of DNA sequence divergence or whether there are any species-specific factor/s operating for the 93D-inducers.

Other recent observations on the properties of the heat shock induced telomeric Balbiani ring (TBR-III) in the temperate midge, *C. thummi*, may also be mentioned. A number of features of TBR-III in *C. thummi* paralleled those of the 93D locus in *Drosophila*, *e.g.* similarity in association of transcriptional products with large RNP particles; internal sequence repetition, absence of open reading frames and a very rapid sequence divergence (Santa Cruz *et al.*, 1984; Carmona *et al.*, 1985; Walldorf *et al.*, 1984; Garbe *et al.*, 1986, Lakhotia, 1987).

In spite of its apparent similarity with 93D-like loci, the TBR-III locus is refractory to specific inducers like BM, HSGH *etc* (Barettino *et al.*, 1988). Thus, although a 93D-like puff is apparently absent in *Chironomus* and *Anopheles* as far as inducibility by specific agents is concerned, the existence of a 'functional homologue' of *Drosophila*-93D in these dipterans cannot be eliminated. Further molecular studies are needed to examine this interesting possibility.

Acknowledgements

We thank Dr S. K. Subbarao of the Malaria Research Centre, Delhi, for providing laboratory facilities for work on mosquitoes. Financial support was obtained from the Council of Scientific and Industrial Research, Delhi, and from the Department of Atomic Energy, Government of India, Bombay.

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Accepted 25 August 1989