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^{3}H-Uridine Incorporation in the Puff 93D and in Chromocentric Heterochromatin of Heat Shocked Salivary Glands of *Drosophila melanogaster*

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Abstract. The autoradiographic patterns of ^{3}H-uridine labelling of the major temperature shock puff sites and the chromocentric (β-)heterochromatin in heat shocked (37° C) salivary glands of *Drosophila melanogaster* have been studied. It is seen that in response to the heat shock treatment, four of the major temperature shock puffs (63BC, 87A, 87C and 95D) show a correlated level of ^{3}H-uridine incorporation in a given nucleus. However, although the mean grain density on 93D puff is maximum in the heat shocked preparations, in individual nuclei this puff is labelled to a widely varying level and this variation in its labelling is statistically not correlated to the labelling of the other four temperature shock puffs in a nucleus. The chromocentric β-heterochromatin, which has been shown in several earlier studies to hybridize with temperature shock RNA fractions, is seen to be totally inactive in transcription after the heat shock.

Introduction

In recent years, the transcription and translation patterns in heat shocked cells of *Drosophila* have evoked considerable interest. As is now well known, a set of puffs is induced by temperature shock in *Drosophila* polytene nuclei and the same set of puffs is also inducible by other agents that disturb the oxidative metabolism of cells (Ritossa, 1964; Ashburner, 1970; Schoon and Rensing, 1973). The correlated inducibility suggests that these puff sites are under common regulation and may form a "battery" of genes. However, in *D. melanogaster* the puff at 93D, one of the major temperature shock puff, is distinctive in that it can be induced to a high level of RNA synthesis activity independent of the other temperature shock puff loci (Lakhotia and Mukherjee, 1970; Bonner and Pardue, 1976). Therefore, it would be interesting to know whether after a normal heat shock to salivary glands, the response of 93D is correlated with other temperature shock puff sites or is independent. Another interesting aspect of RNA metabolism in heat shocked cells is that a significant amount

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of heat shock induced RNA shows in situ hybridization with chromocentric heterochromatin in polytene nuclei (Spradling et al., 1975, 1977; McKenzie et al., 1975). In the present study, therefore, we have autoradiographically analysed the transcription patterns on 93D puff in relation to other temperature shock puffs and the transcription of chromocentric heterochromatin after exposure of larval salivary glands of *Drosophila melanogaster* to 37° C for 20 min. These observations were preliminarily reported earlier (Mukherjee, 1979).

Material and Methods

Eggs of wild type (Oregon R) flies of *Drosophila melanogaster* were collected at 1 h intervals and the larvae were grown at 24° ± 1° C on standard food. Actively migrating late third instar larvae from healthy cultures were dissected in *Drosophila* Ringer at 24° C. The glands were immediately transferred to fresh Ringer, pre-warmed to 37° C, and were incubated at 37° C for 20 min for temperature shock (TS). In some cases, the sister glands were kept at 24° C for 20 min as controls. After 20 min, the heat-shocked and control glands were transferred to fresh Ringer at 24° C and were labelled with ^{3}H-uridine (activity – 250 μCi/ml; sp. act. – 3.6 Ci/mM, obtained from BARC, Trombay) for 10 min. After labelling, the glands were fixed, squashed and processed for autoradiography with Ilford L4 emulsion (Lakhotia and Roy, 1979). The autoradiographic exposure was for 9 days.

Observations

^{3}*H-uridine Labelling of TS Puff Loci in Heat Shocked Glands*

As reported by other workers, in our preparations of heat shocked salivary glands, the TS puff sites are very well labelled with ^{3}H-uridine (Fig. 1). Five of these TS puffs (63BC, 87A, 87C, 93D and 95D) are more prominent than others (Ashburner, 1970) and show intense ^{3}H-uridine incorporation. These five puffs in a nucleus are not labelled to the same extent and also in different nuclei, the degree of TS-induced ^{3}H-uridine labelling varies considerably. A casual examination of autoradiograms suggested that while the 63BC, 87A, 87C and 95D puffs show a co-ordinated variability in their labelling, the puff at 93D was in many nuclei more or less heavily labelled than other puffs (see Fig. 1). In order to analyse this apparent variability in ^{3}H-uridine incorporation by the different TS puff sites, silver grains over each of these five puff sites in well spread nuclei from six heat-shocked salivary gland preparations

Fig. 1a–d. ^{3}H-uridine labelled autoradiograms of polytene chromosomes of *D. melanogaster* from heat shocked (**a–c**) or Control (**d**) salivary glands. In **a–c**, parts of three labelled nuclei are shown to demonstrate the independently variable grain density on 93D puff after TS. Locations of the other major TS puffs are indicated. **a**: The 93D puff is very heavily labelled while the other TS puffs are moderately labelled; **b** the 93D puff shows nearly as heavy labelling as in **a** but the other TS puffs (only 87A and 87C are seen here) are more heavily labelled than in the previous example; **c** the 93D puff is very low labelled whereas the other TS puffs show heavier labelling. The lack of ^{3}H-uridine incorporation in chromocentre (*cc*) in heat-shocked glands may is also seen in b and c. d The chromocentre region from a control nucleus showing heavy ^{3}H-uridine labelling. *NO* nucleolus. Magnification × 1,200

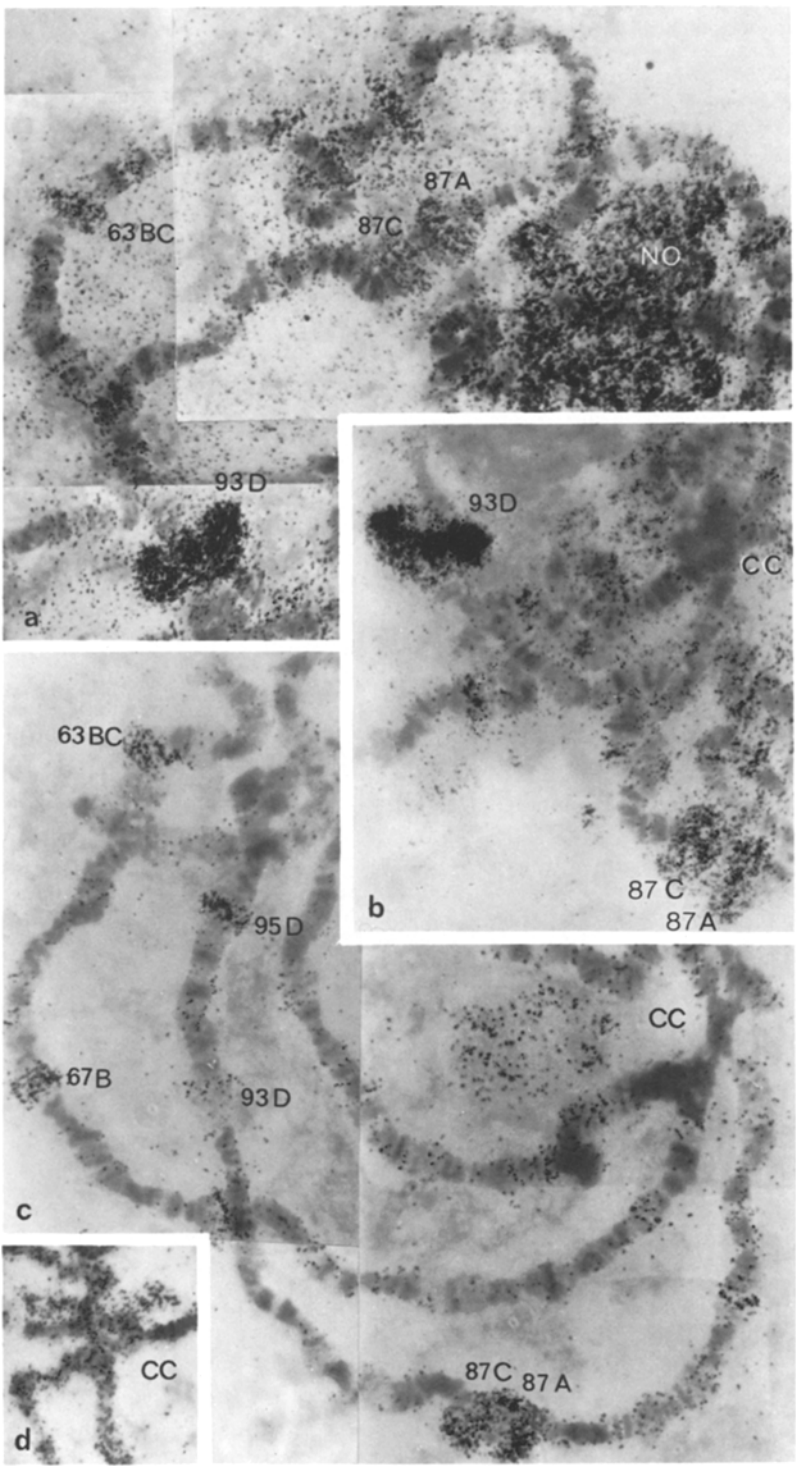
87A
63BC
87C
NO
93D
a
93D
CC
b
63BC
95D
67B
93D
c
CC
87C
87A
CC
d

Table 1. Mean autoradiographic grain densities on a normal (58DE) and five major temperature shock puff sites in control and heat-shocked salivary glands labelled with ^{3}H-uridine

	Mean number of grains (±S.E.) on different puff sites						
	58DE	63BC	87A	87C	93D	95D	N
Control	32.26 ±1.63	14.18 ±1.31	11.83 ±1.8	10.61 ±1.01	25.77 ±2.74	9.18 ±0.82	27
Heat-shocked	7.11 ±1.03	90.97 ±6.76	132.18 ±9.41	109.18 ±7.31	242.4 ±33.43	58.02 ±4.93	44

have been counted. In addition, a normally active puff site (58DE on 2R) has also been included in this analysis. The data on the mean grain counts on these puff sites in control and heat shocked glands are presented in Table 1.

In control preparations, with the exception of 93D, the other TS puff loci show a very low ^{3}H-uridine incorporation. The 93D puff site is known to be active in normal development (Ashburner, 1970) and, accordingly, shows a moderate labelling in control nuclei. Heat-shocked polytene nuclei show greatly reduced incorporation in most chromosomal regions (as exemplified by 58DE labelling density). All the five TS puffs are very active in ^{3}H-uridine labelling and on the basis of the mean grain count, the 93D puff may be considered to be the most active site in heat shocked glands. However, a more detailed analysis of the relative labelling of different TS puffs in each of the 44 nuclei reveals that the 93D puff is not always the most heavily labelled puff after heat shock. In fact, in certain nuclei, this puff is much less labelled than the other puffs. To examine this aspect, the 44 nuclei examined from six heat shocked glands have been sub-grouped into six categories on the basis of the grain density on the 93D puff. The means and the range of the silver grains on the other four TS puffs in nuclei grouped on the basis of the 93D labelling are given in Table 2. It is seen that 93D puff shows a very wide range of grain counts (from 16 to 700) in the 44 nuclei which have been studied, while the other puffs in these nuclei show a relatively limited range of grain counts. It is also seen from the data in Table 2 that in those nuclei with low labelling of the TS puffs (groups 1 and 2), 93D is generally less heavily labelled than the other puffs. On the other hand, in nuclei with a heavy labelling of 93D (Groups 3 to 6), the other TS puffs show lower mean grain counts than 93D. Furthermore, it is very interesting to note that while the mean grain density over 93D increases severalfold between nuclei of Groups 2 and 4, the mean grain density on other TS puffs in these nuclei shows only a marginal, if any, increase. This means that the transcriptional activity of the 93D puff in response to heat shock is not directly correlated with that of other TS puff loci as substantiated by a statistical analysis of correlation between the labelling on these five puff sites (see Table 3). In this analysis, we have measured the linear correlation co-efficient between grain density on 87A and the other four puff sites, respectively. When all the 44 nuclei are considered, it is seen that the grain densitites on 87C, 93D, 95D and 63BC, respectively, show a positive

Table 2. Distribution of autoradiographic grain densities on the five TS puffs in the 44 heat shock nuclei grouped on the basis of 93D grain density

Group	Means and ranges of silver grains on different TS puff sites										N
	93D		95D		87A		87C		63BC		
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
1	16– 25	18.5 ±1.1	21– 30	32.0 ±4.3	34– 88	60.5 ±9.7	32– 67	44.7 ±7.2	16– 70	45.5 ±10.5	4
2	26– 50	37.4 ±2.4	11–101	52.8 ±10.7	31–212	104.6 ±18.9	46–121	86.7 ±13.1	42–101	78.7 ±6.8	10
3	51–150	119.6 ±11.2	32– 48	41.0 ±3.5	52–162	98.5 ±11.3	61–157	93.5 ±11.3	32– 81	60.1 ±8.2	8
4	151–250	202.0 ±15.5	35– 48	37.7 ±3.4	83–155	118.8 ±11.8	78–125	103.4 ±7.5	36– 98	69.8 ±8.3	6
5	251–350	296.7 ±25.3	40– 63	51.5 ±5.0	130–164	148.5 ±8.1	107–134	123.0 ±6.0	74–121	100.7 ±9.9	4
6	351–700	559.2 ±33.4	34–140	98.2 ±7.7	115–280	187.7 ±17.3	101–238	151.3 ±14.0	48–200	141.6 ±14.7	12

Table 3. Analysis of linear correlation between ^{3}H-uridine labelling on different TS puff sites in heat shocked glands

Sample of nuclei	Co-efficient of correlation between grain density on 87A and on other TS puffs			
	$r_{87A\text{-}63BC}$	$r_{87A\text{-}87C}$	$r_{87A\text{-}93D}$	$r_{87A\text{-}95D}$
A All the 44 nuclei taken together	+0.86	+0.89	+0.5	+0.76
B Nuclei (21) with 26 to 200 silver grains on 93D	+0.55	+0.83	−0.2[a]	+0.7

[a] No linear correlation between 87A and 93D labelling ('t'=0.896; $P>0.3$). In all other cases, a high positive correlation is seen with 87A grain density

correlation with the grain density on 87A, although the correlation co-efficient between 87A and 93D labelling is much less than between 87A and other TS puffs (Table 3). However, when only those nuclei are considered for correlation analysis in which the grain density on 93D varies between 26 to 200, the ^{3}H-uridine labelling of 93D and 87A does not show any linear correlation. Nevertheless, the grain densities on 63BC, 87C and 95D puffs in these nuclei still show a high positive correlation with the grain density on 87A. These data, thus, show that in most of the moderately labelled nuclei, the level of ^{3}H-uridine incorporation on 93D varies independent of the other TS loci. Furthermore, even in very heavily labelled nuclei (Group 6 in Table 3), the increase in grain density over the 93D puff is severalfold higher than on other puffs.

^{3}H-uridine Labelling on the Chromocentre in Heat Shocked Glands

As shown earlier by Lakhotia and Jacob (1974), the chromocentric β-heterochromatin actively incorporates ^{3}H-uridine in control polytene nuclei (Fig. 1 d). The mean grain count on the chromocentre in control nuclei examined in the present analysis was 34.2 ± 1.79 and in nearly 68% of the control nuclei examined, the grain density over the chromocentre was between 26 and 80; only one nucleus had a low grain count of 14 in the chromocentre (Table 4). TS drastically inhibits ^{3}H-uridine incorporation in the chromocentre (Fig. 1 a, c), i.e., in the heat shocked preparations, in more than 50% of nuclei, the labelling over the chromocentre was negligible with less than 5 silver grains. In none of the heat shocked nuclei examined, the chromocentre grain density was more than 25 grains over its area (Table 4).

Table 4. ^{3}H-uridine labelling of chromocentre region in control and heat shocked glands

	% nuclei with different ranges of grain counts on chromocentre						
	0–5	6–15	16–25	26–35	36–50	51–80	N
Control	0.0	3.4	27.5	37.8	13.8	17.2	29
Heat-shocked	52.5	33.4	14.2	0.0	0.0	0.0	42

Discussion

The behaviour of the 93D puff site in *D. melanogaster* is known to be distinctive under certain experimental conditions. This puff can be specifically activated to transcribe very heavily either by in vitro benzamide treatment (Lakhotia and Mukherjee, 1970, and other unpublished data) or by heat shock in aged Grace's medium (Bonner and Pardue, 1976) without concomittant activation of other TS puff loci. On the other hand, Compton and McCarthy (1978) found that in their in vitro system of heat shock puff induction, the 93D locus fails to respond while the other TS puffs do. These studies show that under certain altered physiological conditions, the activity of the 93D locus can be dissociated from the other members of the TS puff set.

Analysis of ^{3}H-uridine labelled autoradiograms of heat shocked glands reveals that the overall level of induced activity in different nuclei varies. The reasons for such quantitatively variable induction of RNA synthesis in different nuclei of same gland are not clear. However, it is very significant that within a given nucleus, the relative incorporation on all TS puff sites, other than 93D, remains co-ordinatedly low or high. The level of heat shock induced ^{3}H-uridine incorporation by 93D puff, on the other hand, varies independent of the other TS loci as evidenced by the grain count data. It may, therefore, be surmised that while the other TS puffs appear to be under common co-ordinated regulation, the 93D puff responds independently to the altered

cellular physiology. Probably, the specific response of 93D puff to benzamide and other treatments as referred to above, and the independently variable level of ^{3}H-uridine incorporation after normal TS, are related to each other and may reflect a common aspect of the organization of this locus.

In a detailed study, Spradling et al. (1977) found that none of the specific poly-A containing mRNAs extracted from heat shocked cells of *Drosophila melanogaster* hybridize in situ with the 93D locus. However, total cellular RNA extracted from heat shocked cells does show in situ hybridization with 93D (Bonner and Pardue, 1976). These observations suggest that the large amount of RNA synthesized at 93D puff may not be of same quality as the RNA synthesized on other TS puffs. The significance of the independent response of the 93D puff to TS and the apparent lack of poly-A sequences in the RNA synthesized at 93D (Spradling et al., 1977) is not clear at present. When benzamide (which inhibits most of the chromosomal RNA synthesis but specifically activates a higher rate of RNA synthesis at 93D puff, Lakhotia and Mukherjee, 1970) and TS treatments are given simultaneously or one follows the other, RNA synthesis on all TS puffs, including 93D, is almost completely inhibited (Lakhotia and Mukherjee, 1978; detailed manuscript is under preparation). It seems possible that modulation of 93D activity by benzamide treatment may influence the entire TS response. Further studies on this aspect are in progress.

A high rate of transcription in the β-heterochromatic chromocentre region of polytene nuclei of *D. melanogaster* under normal conditions was shown earlier by Lakhotia and Jacob (1974). In the present study, we show that in heat shocked glands, ^{3}H-uridine uptake in the chromocentre heterochromatin is almost totally inhibited. In this context, the involvement of chromocentre DNA sequences in heat shock RNA metabolism in *Drosophila* cells is interesting. Spradling et al. (1977) found that nearly all the heat shock induced mRNA fractions show in situ hybridization with or very close to the chromocentre heterochromatin in *D. melanogaster*. It is also known that some specific DNA sequences which are transcribed after heat shock, are shared by the 87C puff and the chromocentre region (Ish-Horowicz et al., 1977; Livak et al., 1978). Thus it seems that after a heat shock to salivary glands, a selected set of DNA sequences (in heat shock puff sites) are activated to transcribe, but similar sequences present at the chromocentre region are not transcribing under the same conditions. This differential response of similar DNA sequences located in different parts of the genome raises interesting possibilities about their transcriptional control.

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