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Heat Shock Puff Activity in Salivary Glands of *Drosophila melanogaster* Larvae during Recovery from Anoxia at Two Different Temperatures

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All heat shock puff sites are induced in salivary gland polytene nuclei of *D. melanogaster* during recovery from anoxia at 24°C. ^{3}H-uridine autoradiographic analysis reveals that the puff at 93D locus is most active in glands recovering from anoxia at 24°C but in glands recovering at 37°C this puff is completely repressed. During recovery from anoxia at 24°C as well as 37°C, the 87C puff is nearly twice as active as its duplicated locus at 87A.

When *Drosophila* or their excised tissues are briefly heat shocked at 37°C, a rapid change take place in the patterns of transcription and translation[1]. The same set of genes are activated when larvae or their excised tissues recover from anoxia[1]. In our earlier studies[2,3] we have reported on certain unusual features of heat shock induced transcriptional activity of the 93D locus in polytene nuclei of *D. melanogaster*. In the present communication we show that during recovery from anoxia, the response of the 93D puff is again distinctive from the other members of the heat shock loci. It has also been observed that the 87A and 87C puffs, which are duplicated gene loci[4], are induced to very different levels during recovery from anoxia, although after routine heat shock these two gene loci show equal levels of activity.

Materials and Methods

Eggs of *Drosophila melanogaster* (Oregon R$^+$) were collected in petridishes at hourly intervals and grown under standard conditions at 24° ± 1°C. Healthy late third inster larvae were transferred to a small injection vial with a moist filter paper. The air in the vial was replaced by dry nitrogen. The larvae were kept in the anaerobic condition for 3 hr at 24°C. After the anaerobic period, salivary glands were dissected out from the larvae within a very short period (3-5 min) and the glands from one set of larvae were labelled with ^{3}H-uridine (activity 500 μCi/ml; sp. act. 10.9 mCi/mM, BARC, Trombay) at 24°C for 10 min. Salivary glands from another set of larvae were dissected out and allowed to recover from anoxia at 24°C for 20 min while glands from a third set of larvae were immediately transferred to 37°C for 20 min. Following the 20 min period of recovery either at 24°C or at 37°C, the glands were labelled at 24°C with ^{3}H-uridine as above for 10 min. For dissection and incubation of glands a modified Poels'[5] medium was used[3]. After labelling the glands were fixed in acetomethanol (1:3), stained in acetocarmine and squashed. Squash preparations were processed for autoradiography as described earlier[2].

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Results

The data on ^{3}H-uridine incorporation on the major TS puffs in glands pulse labelled immediately (within 3-5 min) after 3 hr anoxia or after 20 min recovery from anoxia at 24°C or at 37°C are presented in Table 1. Data on relative labelling of different temperature shock (TS) puff sites, expressed relative to the labelling on 87A puff, are presented in Table 2. In both the tables, data on activity of these puffs after a routine heat shock[3] are also presented for comparison. It has been observed that after anoxia, all the heat shock puffs appear rapidly during the period of 10 min labelling with ^{3}H-uridine although the labelling density on all the major TS puffs is much less than what is seen after a routine heat shock or after 20 min recovery from anoxia at 24°C (Fig. 1). When salivary glands recover from anoxia at 37°C the activity of different TS puffs is less than in glands recovering at 24°C since the mean grain counts on all TS puffs in 37°C recovering glands are considerably lower than on respective puffs in glands recovering from anoxia at 24°C.

As revealed by data (Table 2) on the relative labelling of different puffs, the 87C puff is nearly two times more active than 87A puff in glands recovering from anoxia (Fig. 1). The data on Table 2 also show that, except in the case of 93D puff, the relative activity of different TS puffs is closely similar whether the 20 min recovery from anoxia occurs at 24° or 37°C. The 93D puff, however, is seen to be completely regressed when glands recover from anoxia at 37°C although recovery at 24°C causes a high induction of this puff as seen after routine heat shock condition (Fig. 1 and Tables 1-2).

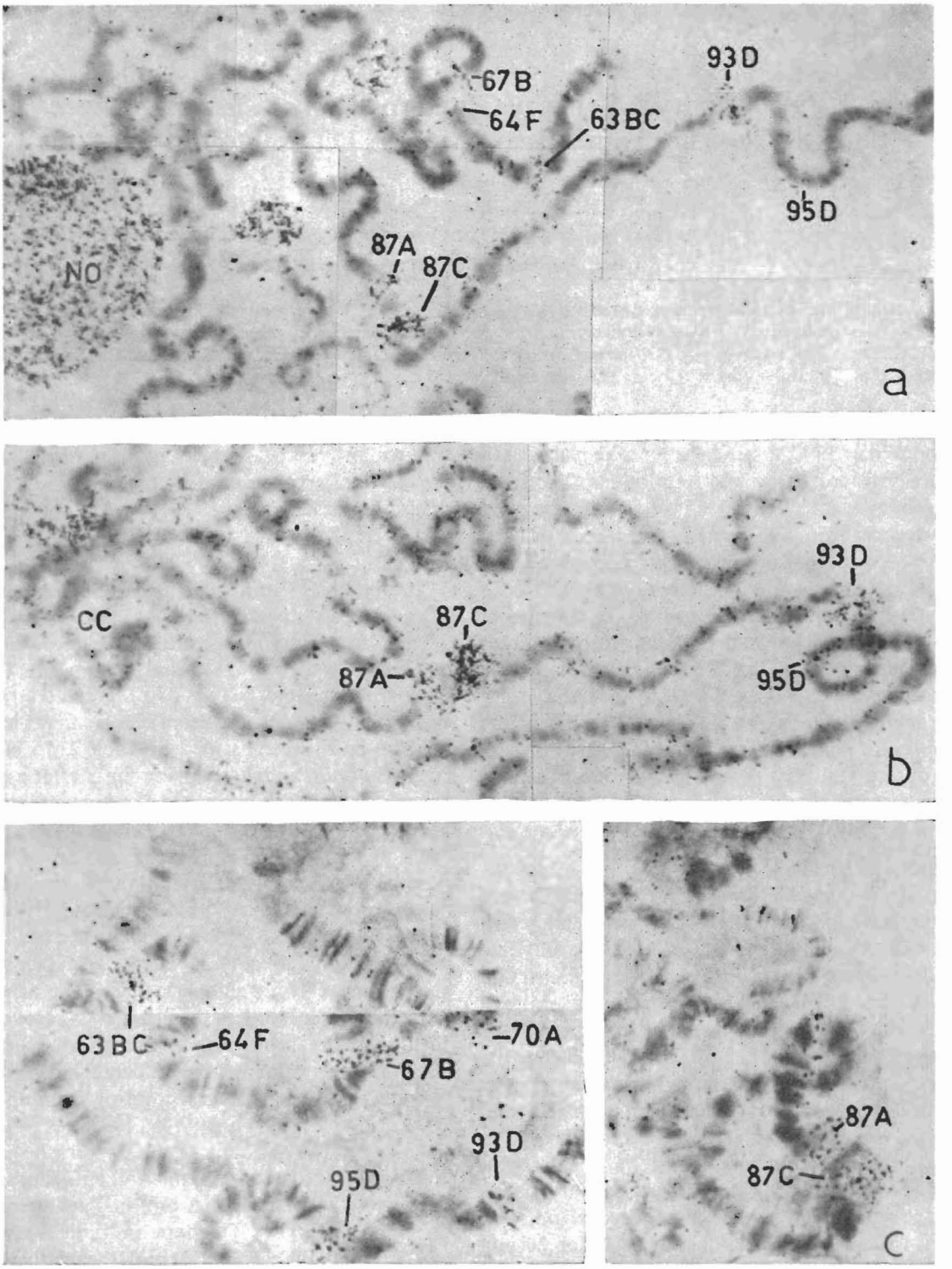

Fig. 1 - - Autoradiographs of ^{3}H-uridine labelled nuclei from glands labelled immediately after 3 hr anoxia (a), labelled after 20 min recovery at 24°C(b) and labelled after 20 min recovery from anoxia at 37°C(c). In c, the 87A and 87C puff regions are from a different nucleus than the rest. Note the lack of activity at 93D region in glands recovering from anoxia at 37°C(c). Also note that the 87C site in all cases is more active than 87A, × 1200

Table 1—Mean Autoradiographic Grain Densities on Major Heat Shock Puffs after Different Treatments

Experiments	Mean (±SE) No. of silver grains on major TS puffs					
	63BC	67B	87A	87C	93D	95D
Heat shock*	40.09±1.6 (41)	54.08±2.1 (40)	65.32±2.5 (38)	64.98±3.1 (42)	76.12±2.5 (42)	22.72±3.8 (39)
Anoxia	18.79±0.7 (48)	12.59±0.6 (44)	19.9±0.9 (50)	45.24±1.5 (50)	28.4±1.0 (51)	7.9±0.4 (47)
Recovery from anoxia at 24°C, 20 min	25.8±1.0 (38)	27.52±2.1 (33)	32.9±2.8 (39)	54.7±2.8 (39)	47.8±1.9 (40)	10.2±0.4 (32)
Recovery from anoxia at 37°C, 20 min	15.9±0.8 (45)	15.6±0.8 (39)	18.9±0.9 (45)	38.5±2.1 (45)	6.11±2.1 (44)	5.8±0.5 (34)

*Data taken from Lakhotia and Mukherjee[3].
(Figures in parentheses indicate the number of nuclei examined in each case).

Table 2—Relative ^{3}H-Uridine Incorporation on the Major TS Puff Sites after Different Treatments

Experiments	Ratios of mean grain densities over major TS puffs				
	63BC/87A	67B/87A	87C/87A	93D/87A	95D/87A
Heat shock*	0.61	0.82	0.99	1.16	0.35
Anoxia	0.95	0.63	2.27	1.43	0.40
Recovery from anoxia at 24°C, 20 min	0.78	0.84	1.66	1.45	0.31
Recovery from anoxia at 37°C, 20 min	0.84	0.82	2.03	0.32	0.31

*Data from Lakhotia and Mukherjee[3].

Discussion

The present results confirm the earlier cytological observations[6] that recovery from anoxia causes activation of the heat shock puff sites. All the heat shock puffs are seen to be active in transcription and their activity increase with the recovery time.

The response of the 93D puff glands recovering from anoxia at 37°C is remarkable. In glands recovering from anoxia at 24°C, the 93D puff appears to be highly active. As mentioned earlier[2] a 20 min heat shock by itself also induces the 93D puff maximally. In this context, it is interesting that in glands recovering from anoxia at 37°C, the 93D puff is seen to be completely repressed although other TS puff sites in glands recovering from anoxia at 37°C are induced in a characteristic manner. In our earlier studies[3,7] on the effects of other combinations of the 93D inducing agents (like benzamide and heat shock or incubation in homogenate of heat shocked glands and heat shock) also we had found that the effects of two inducing agents on 93D activity are not additive but somewhat repressive. In parallel with these results, in the present study also the two agents (recovery from anoxia and heat shock) together have been found to totally inhibit the activity of 93D but not of other TS puff loci. Thus the response of the 93D locus to anaerobiosis is also unique as to heat shock[1,2].

The 87A and 87C puffs are now known to be duplicated gene loci[4] but unlike the nearly equal levels of activity of the two sites induced by heat shock[2], during recovery from anoxia the 87C puff has been found to be much more active. These observations provide further evidence for independent regulation of these two duplicated loci[3]. The difference in the response of 87A and 87C puffs to heat shock and anoxia also suggests that these two puff inducing treatments do not act via a common pathway. The biological significance of the greater activity of the 87C puff during recovery from anoxia remains to be analysed.

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