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Absence of Novel Translation Products in Relation to Induced Activity of the 93D Puff in *Drosophila melanogaster*

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Abstract. Salivary glands of *Drosophila* larvae were treated in vitro with benzamide or with a homogenate of heat shocked glands to specifically induce high transcriptional activity of the 93D puff. The newly synthesized ^{14}C-amino acids labelled polypeptides in the treated and sister control glands were analysed by polyacrylamide gel electrophoresis, followed by gel autoradiography. The protein synthesis patterns in the treated glands in either case remain the same as in control glands. No novel polypeptide was seen which could be correlated with the high induced transcriptional activity of the 93D puff. This suggests that the 93D transcript/s is/are probably not translated.

Introduction

Temperature induced gene activity in *Drosophila* has been extensively analysed during the last two decades (see review by Ashburner and Bonner, 1979). Among the nine heat shock inducible puffs in polytene chromosomes of *D. melanogaster* larvae, the puff at 93D locus is one of the largest. While the other major heat shock puffs in *D. melanogaster* have been well characterized in recent years with respect to their DNA sequence organization and polypeptide products (Lis et al., 1978; Livak et al., 1978; Schedl et al., 1978; Craig et al., 1979), the functional significance of the 93D puff has remained elusive.

We have earlier shown that a high level of activity at the 93D locus in salivary gland cells of *D. melanogaster* larvae can be specifically induced either by a brief in vitro benzamide (BM) treatment (Lakhotia and Mukherjee, 1980) or by incubation of glands in a homogenate of heat shocked salivary glands (Mukherjee and Lakhotia, 1981). In order to see if the specifically induced activity of the 93D puff is reflected in the appearence of novel protein/s in the treated gland cells, we have now analysed the newly synthesized polypeptides by polyacrylamide gel electrophoresis. The present results, which suggest that the 93D transcripts are probably not translated, complement the unusual properties of the 93D RNA noted by Lengyel et al. (1980).

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Material and Methods

The flies and larvae of the wild type stock (Oregon R^+) of *D. melanogaster* were cultured on standard agar-cornmeal-brown sugar-yeast *Drosophila* food at 24° C ± 0.5° C. The following treatments have been applied to salivary glands from actively migrating healthy late third instar larvae in a salt solution containing only the inorganic salt constituents of Poels' (1972) tissue culture medium.

Temperature Shock (TS) Treatment. Freshly excised salivary glands were heat shocked for 20 min at 37° C, following which they were labelled with ^{14}C-Chlorella protein hydrolysate (Act. 400 μCi/ml; sp. Act. 24 mCi/m atom Carbon, BARC, Trombay) for 30 min at 37° C.

Benzamide (BM) Treatment. Freshly excised sister salivary glands were separated and incubated for 20 min at 24° C either in freshly prepared benzamide (BDH, Poole) medium (1 mg/ml of medium) or in BM free control medium. The treated and sister control glands were labelled as above with ^{14}C-Chlorella protein hydrolysate at 24° C for 30 min. The BM treated glands were labelled in presence of BM while the control glands were labelled in BM-free medium.

Incubation in a Homogenate of Heat Shocked Glands. Freshly excised salivary glands were exposed to 37° C for 1 h and homogenized as described earlier (Mukherjee and Lakhotia, 1981). Fresh salivary glands were incubated in the homogenate of heat shocked glands (HSGH) at 24° C for 45 min, rinsed in fresh medium and were labelled with ^{14}C-Chlorella protein hydrolysate as above for 30 min at 24° C. Freshly excised salivary glands labelled at 24° C with ^{14}C-Chlorella protein hydrolysate served as control for this experiment.

Each of the above treatments was carried out in duplicate and for each sample, glands from six larvae were used.

Electrophoresis. For each treatment, the six labelled salivary glands were briefly washed in radioisotope free medium and dissolved in 6 μl of sample buffer (0.0625 M Tris-HCl; pH-6.8; 1% SDS; 1% 2-marcaptoethanol; 10% glycerol; 20% sucrose; 0.001% Bromophenol blue) and heated at 100° C for 4 min. Electrophoresis was performed in vertical polyacrylamide slab gels (62 mm × 125 mm × 0.45 mm) with 12.5% (W/V) resolving and 3.75% stacking gels. The protein samples (1μl/well) were run at 10 mA constant current using a discontinuous SDS buffer system (Laemmli, 1970). The electrophoresis run was continued till the tracking dye migrated to 125 mm. Samples of heat shocked glands labelled with ^{14}C-Chlorella protein hydrolysate were run parallel to the samples of control and treated glands in each case to compare the mobilities of different labelled polypeptides with those of the heat shock polypeptides (HSPs).

After the run, the gel slabs were fixed and stained with Coomassie Brilliant Blue and processed for fluorography following the procedure of Bonner and Laskey (1974). The dried gels were exposed against pre-flashed (Laskey and Mills, 1975) X-ray film (Sakura, Japan) at −80° C for five days. The exposed films were developed and fixed at 18° C using a Wratten 6 BR safe light filter.

Results

We have shown earlier (Lakhotia and Mukherjee, 1980) that a 10 min or 20 min in vitro treatment with BM at 24° C specifically induces RNA synthesis at the 93D puff while all other chromosomal RNA synthesis is severely repressed (see Fig. 1). Figure 2 shows the patterns of newly synthesized polypeptides in two sets of BM treated and sister control glands. Analysis of labelled polypeptides in BM treated and their sister control glands shows that unlike the effects of BM on RNA synthesis, the pattern of protein synthesis in 20 min BM treated glands is nearly the same as in the control glands (see Fig. 2) since the polypeptides being normally synthesized (control glands) continue to be synthesized at the same rate after the BM treatment while no new labelled bands are

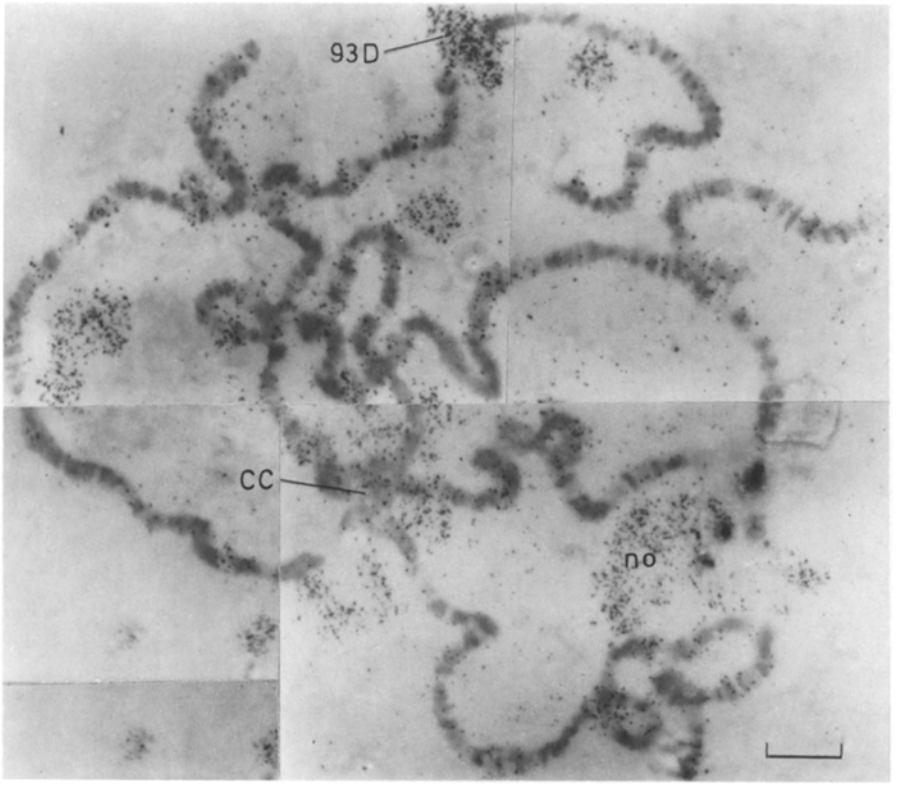

Fig. 1. Autoradiograph of ^{3}H-uridine labelled polytene nucleus after 10 min in vitro benzamide treatment at 24° C (see Lakhotia and Mukherjee, 1980). The 93D puff shows intense labelling while the general chromosomal and chromocentre (*cc*) RNA synthesis is severely inhibited. The nucleolus (seen here in several fragments) shows normal ^{3}H-uridine uptake. Bar represents 10 μm

seen in the treated glands. Small differences occasionally seen in the intensity of the labelling of some bands between control and treated lanes are not reproducible in different gels (Fig. 2) and thus are probably unspecific variations. A comparison of labelled bands in BM treated glands (lanes b and d in Fig. 2) with those in heat shocked glands (lane c in Fig. 2) reveals that synthesis of none of the HSPs is induced by the BM treatment.

We have shown that incubation of intact salivary glands at 24° C in HSGH results in specific induction of the 93D puff, although unlike BM, the HSGH incubation does not inhibit general chromosomal RNA synthesis (Mukherjee and Lakhotia, 1981). In the present study, it is seen that the newly synthesized polypeptides in glands incubated in HSGH for 45 min (lanes c and d in Fig. 3) are similar to those in the freshly excised control glands (lanes a and b in Fig. 3). Like the BM results, no novel labelled polypeptide bands are seen in the HSGH incubated glands. In the HSGH incubated glands the synthesis of the higher molecular weight HSPs is also not induced since the labelling intensity of these HSP bands remains at a low level as in the fresh control

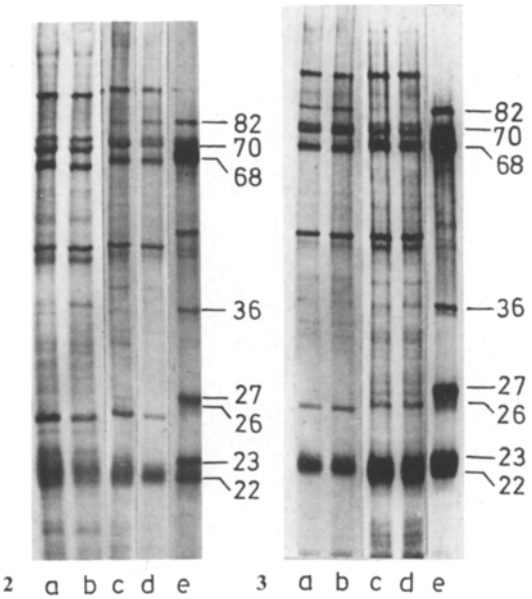

Fig. 2. Patterns of protein synthesis in control (**a, c**) and in corresponding sister glands treated with benzamide at 24° C for 20 min (**b, d**). In lane **e** protein synthesis pattern in heat shocked glands is shown. The molecular weights of heat shock polypeptides are indicated

Fig. 3. Patterns of protein synthesis in freshly excised control glands (**a–b**), in glands incubated in HSGH at 24° C for 45 min (**c–d**) and in heat shocked glands (**e**)

glands. Significantly, however, the 23 and 22 K dalton HSPs are much more heavily labelled in the HSGH incubated glands (Fig. 3).

Discussion

In response to heat shock, the 93D locus forms one of the largest and most active puff (Ashburner, 1970; Murkherjee and Lakhotia, 1979). The nature and fate of the enormous amount of RNA made at the 93D has been analysed earlier (Spradling et al., 1977; Langyel et al., 1980) and it appears that most of the 93D product is devoid of poly-A tails and also the turnover of this RNA is different from other heat shock transcription products. It is significant that as yet it has not been possible to correlate any of the HSPs with the 93D transcription product/s.

In this context, we have analysed the patterns of the newly synthesized polypeptides in salivary gland cells in which the 93D puff has been specifically induced either by BM (Lakhotia and Mukherjee, 1980) or by incubation in HSGH (Mukherjee and Lakhotia, 1981). Since all the known HSPs are translated very rapidly after the heat shock genes are induced, it may be expected that the translation product/s, if any, of the 93D transcripts would also be readilly detected after the specific and strong activation of this puff by BM or HSGH. However, we have not seen any difference in the distribution of the labelled bands in the control and BM or HSGH treated glands. The absence of any novel labelled polypeptide band/s in the BM or HSGH treated glands suggests that the large amount of RNA made at 93D locus has not been translated to detectable levels during the 50 to 75 min (including the 30 min ^{14}C-amino acids labelling period) of activity.

Since the 93D locus is also normally puffed during some stages of larval development (Ashburner, 1970) it is possible that the 93D transcription product is already present in the control glands and therefore, a novel labelled polypeptide band is not seen in the treated glands. However, since the transcriptional activity at the 93D locus is increased several fold in BM or HSGH treated glands (Lakhotia and Mukherjee, 1980; Mukherjee and Lakhotia, 1981), corresponding enhancement in the labelling of the polypeptide band/s would be expected if the induced 93D transcription product/s were translated. This is also not seen. These results thus suggest that the 93D transcripts are not translated, at least not during the first 50 to 75 min period of activity. This is unlike the very rapid translation of the other heat shock puff transcripts (see Ashburner and Bonner, 1979). The possibility that translation of the 93D transcripts is delayed cannot be ruled out from the present results but appears less likely in view of our other observations that the heat shock or BM induced activity of the 93D puff is reverted within minutes after the glands are returned to 24° C or to BM-free medium (Lakhotia and Mukherjee, 1980; and unpublished observations). The rapid reversal of the 93D activity suggests that, whatever function this puff has in the treated glands, it should be of immediate relevance to the cells. The polypeptide product/s should thus be detectable soon after puff activation. The present results, together with this puff's other unique features (Bonner and Pardue, 1976; Ashburner and Bonner, 1979; Mukherjee and Lakhotia, 1979; Lakhotia and Mukherjee, 1980), the absence of poly-A tails on a major fraction of its transcription product/s and the unusual distribution of 93D RNA in nucleus and cytoplasm (Spradling et al., 1977; Lengyel et al., 1980) suggest that the 93D transcript/s may not have a protein coding function.

In our earlier study (Mukherjee and Lakhotia, 1981) a slight increase in RNA synthetic activity of the 67B puff in the HSGH incubated glands was seen. It is now known that the 67B puff codes for 27, 26, 23 and 22 K dalton HSPs (Corces et al., 1980) and therefore, the observed increased labelling of the 23 and 22 K dalton HSPs in HSGH incubated glands may appear to be related to the higher rate of RNA synthesis at the 67B locus. However, the 27 and 26 K dalton HSPs, also coded by the 67B puff (Corces et al., 1980) have not been seen to be synthesized at a higher rate after the HSGH incubation. Thus if the observed higher level of the 23 and 22 K dalton HSPs in HSGH treated glands is related to a higher activity of the 67B puff, the transcription and/or translation of the 27 and 26 K dalton HSPs may appear to be independently regulated. We do not know if the 93D puff activity has any bearing on this aspect.

It is also interesting to note that in spite of the severe inhibition of general chromosomal transcription by BM (Lakhotia and Mukherjee, 1980), the translation process is not at all affected. Thus, unlike the temperature shock which also inhibits general chromosomal RNA synthesis (Ashburner and Bonner, 1979; Mukherjee and Lakhotia, 1979), BM treatment at 24° C does not disrupt the pre-existing polysomes. In this context, it may be noted that we have in other (unpublished) studies seen that BM treatment at 37° C inhibits protein synthesis nearly totally so that even the HSPs are not synthesized to any appreciable

level. Whether this effect at 37° C is an independent action of BM at the higher temperature or is related to the earlier reported (Lakhotia and Mukherjee, 1980) changes in the activity of the heat shock puffs, particularly the repression of the 93D activity caused by BM at 37° C, is not known.

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