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Further Observations on Inducibility of 93D Puff of *Drosophila melanogaster* by Homogenate of Heat Shocked Cells

AJIT KUMAR SINGH & S C LAKHOTIA*
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005

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The 93D-inducing activity of the homogenate of heat shocked *D. melanogaster* larvae remains unaffected even if the homogenate is heated to 80°C (30 min) or to 100°C (10 min). In contrast, a homogenate of heat shocked *D. hydei* larvae loses its 2-48C (a 93D-like puff) inducing activity if heated to 80° or 100°C. Thus it appears that the 93D-inducing factor is different and perhaps is non-proteinaceous in nature. Cytoplasmic fraction of heat shocked brain cells of *D. melanogaster* larvae has been found to have 93D-inducing activity.

Following the initial report by Compton and McCarthy[1] that the cytoplasm from heat shocked cells can induce all, except the 93D, heat shock puffs in isolated polytene nuclei of *Drosophila melanogaster*, it was reported from our laboratory[2,3] that incubation of intact salivary glands of late third instar larvae in a homogenate of heat shocked larval salivary glands (HSGH) results in specific induction of 93D puff or the 93D-like puff in other species. Our 93D inducing homogenate system provides an opportunity to examine the nature of the factor/s responsible for the 93D activity. As a first step in this direction, in the present study, we have sought to know if the factor/s in the homogenate responsible for the 93D puff induction, is/are heat-labile and they are compartmentalized to nucleus or to the cytoplasm of the heat shocked cells. Our results show that unlike the heat-labile factor/s, which induce TS puffs in *D. hydei*[4], the 93D inducing activity is not heat-labile and is present in cytoplasmic fraction. We have also examined the heat-lability of the 2-48C inducing activity of heat shocked homogenate of *D. hydei*[3]. It is very interesting that in spite of functional homology between the 93D and the 2-48C, the 2-48C inducing activity in *D. hydei* homogenate is heat—labile and thus different from the 93D-inducing activity of *D. melanogaster* homogenate.

Material and Methods

All studies have been done with healthy and actively migrating late third instar larvae of wild type strains of *D. melanogaster* or *D. hydei*, reared under standard laboratory conditions at 24°±1°C. All incubations were made in Poels' salt solution[5].

Incubation of salivary glands in larval homogenates—Late third instar larvae were homogenized either after a 2 hr heat shock at 37°C (heat shocked larval homogenate or HSLH) or without any treatment (control larval homogenate or CLH). The larvae were homogenized in Poels' salt solution as described earlier[2]. The paired salivary glands from fresh late third instar larvae were excised, the sister lobes were separated and incubated at 24°C for 45 min either in the control or heat shocked larval homogenates, respectively. Following the incubation period, the glands were washed with fresh medium, briefly fixed in acetomethanol, stained with acetoorcein and squashed in 50% acetic acid. The chromosome squashes were examined under phase-contrast optics for activity of the major heat shock puff loci.

In another experiment, the *D. melanogaster* CLH was kept at 24°C or at 37°C for 60 min or at 80°C for 30 min, cooled (in case of heated CLH) to 24°C. Fresh *D. melanogaster* salivary glands were incubated in it for 45 min. After the incubation, the salivary glands were squashed as above. In a third set, the *D. melanogaster* or *D. hydei* HSLH was kept at 80°C for 30 min or at 100°C for 10 min, allowed to cool to 24°C (5-10 min) and then used for incubation of freshly excised salivary glands from *D. melanogaster* or *D. hydei* larvae, respectively, for 60 min. Squash preparation of the treated and control glands were examined for TS puff activity.

Incubation of salivary glands in cytoplasmic fraction of heat shocked larval brain cells—Cytoplasmic fraction was prepared from heat shocked (incubation *in vitro* for 60 min at 37°C) or control (60 min at 24°C) larval brain ganglia of *D. melanogaster* following the method of Compton and McCarthy[1]: the heat shocked or control ganglia cells were disrupted with mild strokes in a glass homogeniser containing chilled homogenizing buffer (20 mM Tris, 80 mM KCl, 20 mM NaCl and 4 mM $MgCl_2$, *p*H-7). The homogenate was centrifuged at 800 *g* for 14 min at 4°C and the resulting supernatent was recentrifuged at 8000 *g*

*Addressee for correspondence

for 5 min at 4°C. The final supernatent was collected as the cytoplasmic fraction[1]. Paired salivary glands, freshly excised from *D. melanogaster* larvae, were separated and the sister lobes were incubated for 45 min in the freshly prepared cytoplasmic fractions of control or heat shocked brain ganglia, following which the glands were squashed as earlier.

Results

Squash preparations of glands incubated in different homogenates or cell fractions have been examined for activity of the major TS puffs (activity indices, measured as ratio of maximum diameter of the puff size to a given reference non-puffed band) and the observed activities compared with those in glands incubated in freshly prepared CLH. In glands incubated in fresh CLH, the activities of major TS puff sites remain as in freshly dissected glands (Figs. 1-4). Thus as noted earlier[2] for control salivary gland homogenate, the CLH incubation also has no effect on TS puffs. However, in glands incubated in autologous HSLH, the 93D puff of *D. melanogaster* (Figs. 1 & 2) and 2-48C puff of *D. hydei* (Figs. 3 & 4) become distinctly larger while other TS puff sites do not show any effect (data on puff size of other TS puffs of *D. melanogaster* are not presented, but see Fig. 1). Interestingly, CLH which has been kept at 24°C or at 37°C for 60 min or at 80°C for 30 min has been found to significantly increase the 93D puff activity in *D. melanogaster* (Figs. 1 & 2). Also HSLH heated to 80°C for 30 min or to 100°C for 10 min, retains its 93D inducing property (Figs. 1 & 2). However, HSLH of *D.*

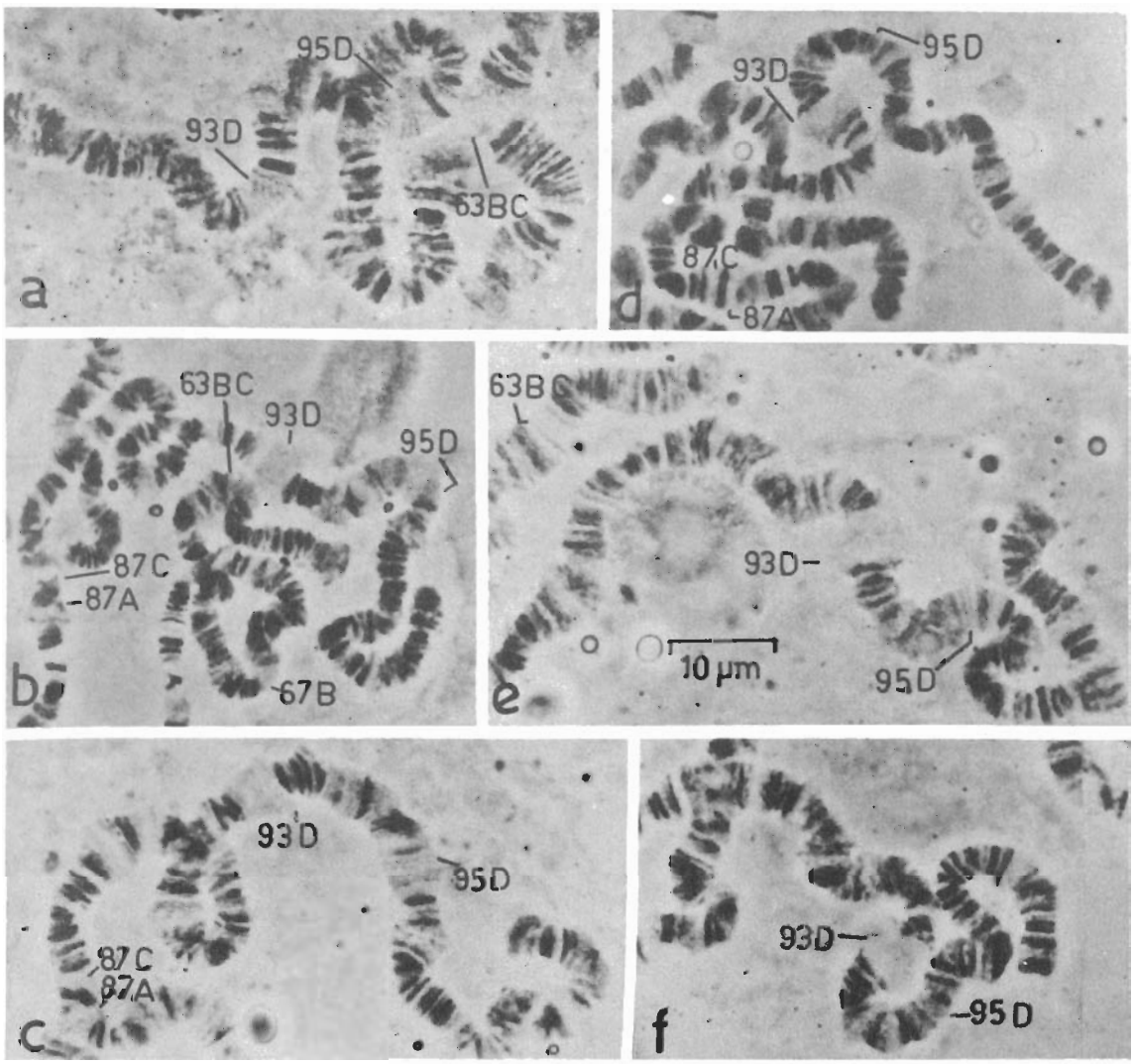

Fig. 1—Phase-contrast photomicrographs of parts of polytene nuclei of *D. melanogaster* salivary glands incubated for 45 min in different homogenates of control (CLH) or heat shocked (HSLH) *D. melanogaster* larvae to show the activity of 93D and some other (63BC, 67B, 87A, 87C, 95D) TS loci. **a**. fresh CLH; **b**. fresh HSLH; **c**. HSLH preheated to 80°C for 30 min; **d-e**. CLH stored at 24°C or 37°C, respectively, for 1 h prior to gland incubation; **f**. CLH stored at 80°C for 30 min prior to gland incubation. Note the enlarged size of the 93D puff in all except in **a**; other TS loci are not affected.

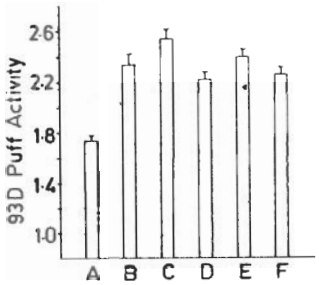

Fig. 2—Puff activity indices of the 93D locus in salivary glands incubated for 45 min in fresh CLH (**A**), CLH kept at 24°C (**B**) or at 37°C (**C**) for 1 hr, CLH preheated at 80°C for 30 min (**D**), fresh HSLH (**E**) or in HSLH preheated at 80°C for 30 min (**F**). Each data point in this and in Figs. 4 and 5 represents mean ±SE of puff activity indices in 25-30 polytene nuclei from 5-6 larvae.

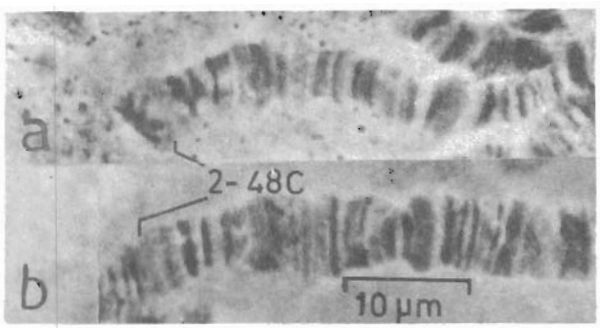

Fig. 3—Phase-contrast photomicrographs of the tip of polytene chromosome 2 of *D. hydei* from glands incubated for 45 min in fresh HSLH (a) or in HSLH preheated at 100°C for 10 min (b). Note lack of induction of 2-48C in b.

hydei heated to 80°C for 30 min or to 100°C for 10 min, fails to induce the 2-48C puff (Figs. 3 & 4).

When salivary glands are incubated in cytoplasmic fraction of control brain ganglia cells, all the major TS puff sites (including the 93D puff) show their normal level of activity, i.e. the puffs at 63BC, 87A, 87C and 93D show either their normal developmental activity (63BC and 93D) or remain uninduced (87A and 87C, see Fig. 5). However, incubation of glands in the cytoplasmic fraction of heat shocked brain ganglia cells results in a greater activity of the 93D puff while other TS puff loci remain unaffected (Fig. 5).

Discussion

The heat shocked larval homogenate has been found to be as effective in specifically inducing the activity of the 93D puff as the homogenate of the heat shocked larval salivary glands[2]. It may be noted here that we have also seen (data not presented) that homogenate of heat shocked larvae, whose salivary glands have been removed, is equally effective in inducing 93D activity. These observations further show that response to heat shock is generally alike in polytene and non-polytene cells[6]. As with the heat shocked salivary glands' homogenate[2], the HSLH or the cytoplasmic fraction of heat shocked larval brain cells also fails to induce other TS puffs in intact salivary glands. As suggested earlier[2] the non-inducibility of other TS puffs in our system appears to be related to the presence of intact polytene cell organization which perhaps does not permit the proteinaceous inducing factors[7] to act upon the other heat shock loci in the nucleus.

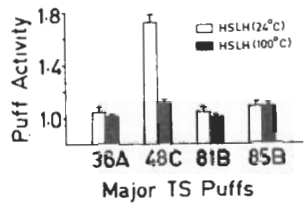

Fig. 4—Mean (±SE) puff activity indices of the major TS loci in *D. hydei* salivary glands incubated in *D. hydei* fresh HSLH (□) or in 100°C preheated HSLH (■).

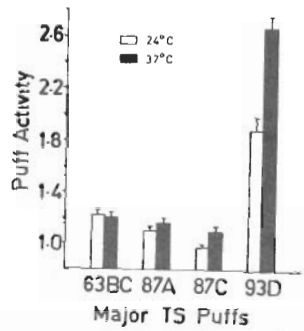

Fig. 5—Mean (±SE) puff activity indices of the major TS loci in *D. melanogaster* salivary glands incubated in cytoplasmic fraction of control (24°C) or heat shocked (37°C) brain ganglia of *D. melanogaster* larvae.

It is interesting that storage of the *D. melanogaster* CLH at 24°C or even at higher temperatures generates 93D-inducing factor/s. In this context it may be noted that in their *in vitro* assay system, Compton and McCarthy[1] also observed induction of TS puffs in isolated polytene nuclei incubated for longer than one hour in control cell cytoplasm. The nature of the inducing factors generated during storage is not known. It is also not known whether these factors in the stored homogenate are the same as those generated *in vivo* after heat shock or whether the puff inducing activity of the stored control homogenate is due to the accumulation of some degradation products in the homogenate.

Our present result in *D. hydei* on the loss of 2-48C inducing activity in the 'heated' HSLH is in agreement with an earlier report on the heat-lability of TS puffs inducing (including the 2-48C) factor/s of *D. hydei*[4]. However, the 93D inducing activity is not lost even after heating the HSLH at 100°C. Thus although the 93D and 2-48C puffs are homologous with respect to

many of their inducible properties[3], the factors that are responsible for their activation are obviously distinct. This perhaps also explains our earlier observation[3] that heterologous HSGH fails to induce 93D or the 93D-like puffs in other species. The present results on differences in the heat sensitivity of the inducing factor/s for 93D and 2-48C add to complexity of the situation with regard to their homology and differences[3].

In view of the differences in the experimental procedures used by us and by Compton and McCarthy[1] and in view of the differences in the response of the 93D puff in polytene nuclei in the two systems, it was suggested earlier[2] that the 93D inducing activity is intranuclearly located. Our present results, however, show that the inducing activity is also present in cytoplasmic fraction since we have found that cytoplasmic fraction of heat shocked larval brain cells is significantly effective (see Fig. 5) in inducing the 93D puff activity. The nature of the cytoplasmic factor remains to be ascertained. However, in view of its heat stability we may tentatively suggest it to be non-proteinaceous and thus different from the proteinaceous factors[7] which induce the other TS loci.

Acknowledgement

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