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Cytological Identity of 93D-like and 87C-like Heat Shock Loci in *Drosophila pseudoobscura*

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The 2-58C heat shock locus of *D. pseudoobscura* is specifically inducible by benzamide and colchicine. The present results, together with an earlier report, show that this heat shock locus is homologus to 93D as well as to 87C of *D. melanogaster*.

The heat shock locus 93D of *Drosophila melanogaster* has been shown to be specifically induced by benzamide[1,2] and colchicine[3]. Interestingly, in all species of *Drosophila* so far examined, one of the major heat shock puffs is also specifically induced by benzamide, vit-B_6 and by colchicine[3,4]. In view of their common inducibility and other properties, these puffs have been designated 93D-like puffs[4]. The conservation of a 93D-like puff in even distantly related species of *Drosophila* reflects fundamental importance of this locus.

Heat shock has been reported[5] to result in the induction of four major heat shock puffs in larval salivary gland chromosomes of *D. pseudoobscura*. Two of them, the 53 and 58 loci, are present on the second chromosome[5] which evolutionarily corresponds to the 3R of *D. melanogaster*[6] and therefore, on the basis of earlier evidence[4], it may also be expected to contain a 93D-like heat shock locus. However, on the basis of *in situ* hybridization of clones of DNA sequences complementary to the 87A/87C mRNA of *D. melanogaster* to *D. pseudoobscura* chromosomes, Pierce and Lucchesi[5] concluded that the two major heat shock loci, the 53 and 58 puffs on the chromosome 2 of *D. pseudoobscura*, are both homologus to the duplicate gene loci 87A and 87C of *D. melanogaster*[7]. Since there is no other major heat shock locus on chromosome 2 of *D. pseudoobscura*[5], it becomes interesting to search for a 93D-like locus in this species.

In the present study we have looked for 93D-like puff in *D. pseudoobscura* chromosomes by using benzamide and colchicine as specific probes. Our observations reveal that in addition to its DNA sequence homology with 87A/87C loci[5], the 2-58 heat shock locus of *D. pseudoobscura* behaves also as a 93D-like puff.

Materials and Methods

Salivary glands of late third instar larvae of a wild strain (ST-322) of *D. pseudoobscura* grown on standard agar-cornmeal-brownsugar-yeast food at 20° ± 1°C have been used for these studies. The medium used for incubation was a modified Poels' medium as described by Mukherjee and Lakhotia[8].

Heat Shock (HS)—The sister lobes of freshly excised salivary glands were separated and incubated at 37°C or at 24°C for 10 or 40 min. Treated (37°C) and control (24°C) glands were fixed, stained and squashed in the usual manner and observed for puff induction using phase-contrast microscope.

Benzamide (BM) treatment—Salivary glands were incubated in medium freshly mixed with benzamide (1 mg/ml) for 10 min at 24°C. Sister lobes of the glands were incubated in BM-free medium for the same time at 24°C (control). The treated and control glands were either directly fixed, stained and squashed for observation under phase contrast microscope or some of them were labelled with ^{3}H-uridine (activity, 300 μCi/ml; sp. activity, 13.8 Ci/mM, BARC, (Bombay) for 10 min in presence (treated) or absence (control) of BM.

Colchicine treatment—Treated glands were incubated in medium containing 100 μg/ml of colchicine for 45 min at 24°C while the sister glands were incubated in medium for the same time at the same temperature in absence of colchicine (control). The treated and control sets were labelled with ^{3}H-uridine for 10 min as above either in absence (control) or presence (treated) of colchicine.

After ^{3}H-uridine labelling the control and treated glands of different experiments were fixed, stained, squashed and processed for autoradiography with Ilford L4 nuclear emulsion. After 4-5 days' exposure in dark at 4° to 6°C the autoradiograms were developed, fixed, washed, stained with Giemsa and mounted for examination.

Results

Using the photomaps of polytene chromosomes of *D. pseudoobscura* prepared by Stocker and Kastritsis[9], Pierce and Lucchesi[5] designated the four major heat

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shock loci as 53 and 58 loci on chromosome 2, and 23 and 39-40 on the XR. Our observations on heat shocked preparations confirm that only these four major puffs are induced by heat shock (data not presented). BM and colchicine have been found to induce a prominent puff in the 58 region of chromosome 2 (Figs 1&2). The 58 region of chromosome 2 in the photomap prepared by Stocker and Kastritsis[9], however, is a large segment and thus to localise the HS, BM or colchicine induced puffs in this region with greater precision, it was necessary to prepare a more detailed map of the distal segment of the chromosome 2, which is presented in Fig. 1a. A cytological comparison of the puff induced by HS, BM or by colchicine shows that each of these treatments causes the same lightly stained region in the 58C to develop into a puff (Fig.1).

When the salivary glands are heat shocked for a brief period (10 min), this puff is already very large (Fig. 1b) while the other heat shock loci remain nearly uninduced (not shown). As may be seen in the examples in Figs. 1b-d and 2, the morphology of the puff induced in the 2-58C region by HS (10 & 40 min), BM or colchicine is remarkably similar so that the differently induced puffs cannot be cytologically distinguished. Thus we believe that the same cytological region is involved in puffing after these different treatments and we designate this puff as the 2-58C puff.

Examination of ^{3}H-uridine labelled autoradiograms reveals that as in other species of *Drosophila*[2-4], in polytene nuclei of *D. pseudoobscura* also, BM and colchicine inhibit chromosomal RNA synthesis and at the same time considerably enhance the incorporation

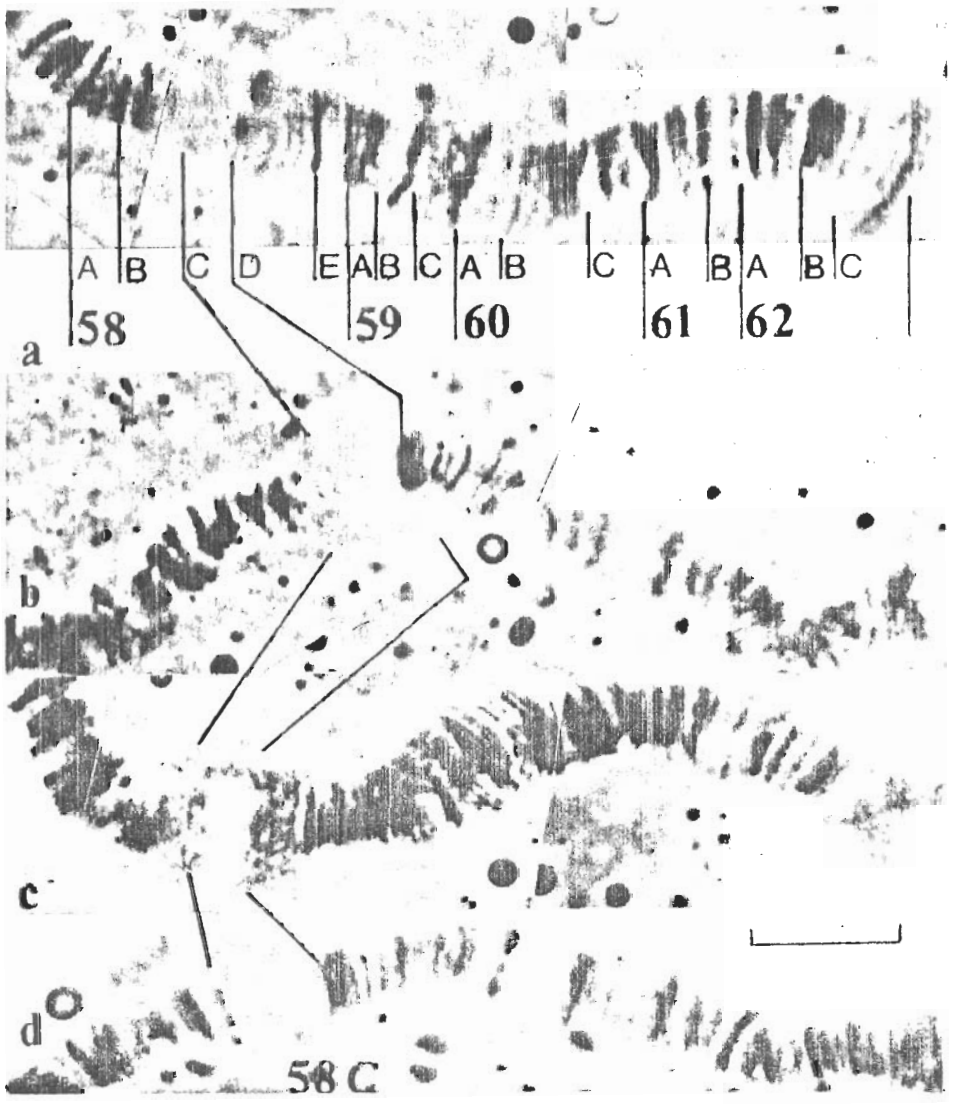

Fig. 1(a-d) Phase contrast photomicrographs of a segment (58-62) of polytene chromosome 2 of *D. pseudoobscura*: (a) control: showing our cytological map of this segment (the major cytological divisions correspond to the photomap prepared by Stocker and Kastritsis[9]); (b) heat shock for 10 min; (c) heat shock for 40 min and (d) 10 min BM treatment. Bar in figure represents 10 μm

of ^{3}H-uridine at the 2-58C heat shock locus (Figs 2&3). RNA synthesis at the other heat shock loci is not stimulated by either of these treatments, rather as exemplified by the labelling of the heat shock locus at 2-53 (Figs 2&3). ^{3}H-uridine incorporation in them is greatly inhibited by BM as well as by colchicine. ^{3}H-uridine incorporation in nucleolus is affected neither by BM nor by colchicine treatment.

Discussion

The present results show that the 2-58C heat shock locus of *D. pseudoobscura* is functionally homologus to the 93D of *D. melanogaster* in several respects: (i) like the 93D of *D. melanogaster*, the 2-58C puff of *D. pseudoobscura* is a developmental puff[9] and is one of the largest and the first to be induced after heat shock as shown by Pierce and Lucchesi[5] and by our present observations; (ii) is located on the chromosome arm homologus to 3R of *D. melanogaster* and most importantly; and (iii) is specifically induced by benzamide and colchicine. Therefore, in view of the earlier observations in other species of *Drosophila*[3,4], we consider the 2-58C of *D. pseudoobscura* to be a 93D-like heat shock locus.

Pierce and Lucchesi[5] found the 2-53 and 2-58C heat shock loci of *D. pseudoobscura* to specifically hybridise *in situ* with DNA clone complementary to the mRNA of the 87A and 87C heat shock loci of *D. melanogaster* and therefore, concluded that these two heat shock loci in *D. pseudoobscura* are counterparts of the duplicate gene loci[7] at 87A and 87C of *D. melanogaster*. Furthermore, the 2-58C heat shock locus was found to show twice as much hybridization with 87A/C clone than the 2-53 locus[5]. Thus the 2-58C heat shock locus of *D. pseudoobscura* seems to be homologus to 87C[5] as well as to 93D (present study) of *D. melanogaster*.

In *D. melanogaster*, the 87A/87C loci code for the 70KD heat shock polypeptide[7-10] while the 93D

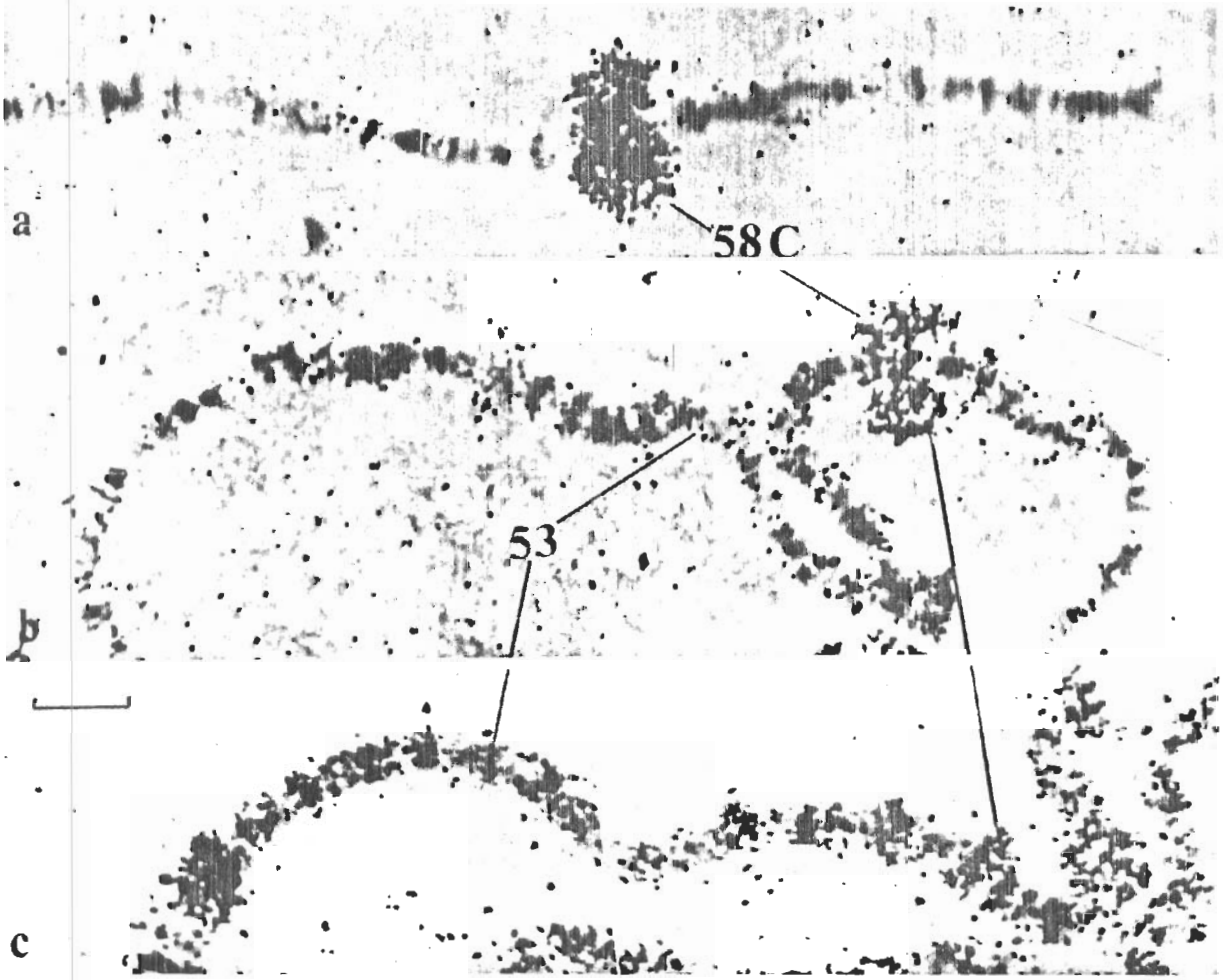

Fig. 2 (a-c)—^{3}H-uridine labelled autoradiograms of a segment of chromosome 2 from (a) benzamide (1 mg/ml); (b) colchicine (100 μg/ml)treated and (c) control salivary glands of *D. pseudoobscura*. Note the high level of ^{3}H-uridine incorporation at the 2-58C puff in (a) and (b). Bar in figure represents 10 μm

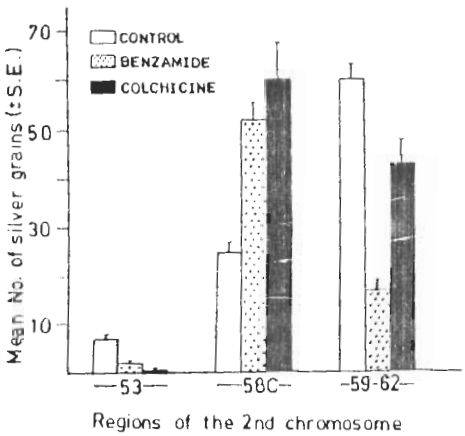

Fig. 3—Histograms showing pattern of ^{3}H-uridine incorporation on two HS puffs, 53 and 58C, and on a 2nd chromosomal segment (59-62) in variously treated salivary glands of *D. pseudoobscura*. Each data-point is mean of grain counts scored in 20-25 polytene nuclei

apparently does not code for any heat shock polypeptide[11]. As in *D. melanogaster*, in other species like *D. hydei*, *D. virilis* etc. also, the heat shock loci equivalent to 87A/C and 93D of *D. melanogaster* are cytologically distinct[12,13]. In this light, the cytological identity (or very close linkage) of these two distinct heat shock loci in *D. pseudoobscura* is intriguing from the evolutionary as well as functional points of view. In this context, earlier observations in our laboratory[2,3,14] on a strong influence of 93D on the heat shock induced activity of the 87A and 87C loci appear relevant: it has been seen that if during heat shock, the induction of 93D is prevented for some reasons, the 87A and 87C loci are induced to very unequal levels instead of being nearly equally active. These observations suggest that in *D. melanogaster* also, a functional relationship between 87A/C and the 93D heat shock loci exists.

The *obscura* species group, to which *D. pseudoobscura* belongs, is closely related to the *melanogaster* species group and is presumed to have originated from a protomelanogaster lineage[15]. Thus in view of this evolutionary relationship between *D. pseudoobscura* and *D. melanogaster* and in view of the above noted functional interaction between the 93D and the 87A/87C loci in *D. melanogaster* it is tempting to speculate that the cytological identity of the 93D-like and 87C-like heat shock loci in *D. pseudoobscura* may reflect an ancestral situation. During evolutionary divergence of species, these two heat shock loci would have cytologically become distinct while retaining some of the ancestral relationship. This could explain the influence of 93D activity on the 87A and 87C heat shock loci in *D. melanogaster*[2,3,14]. Further search for 93D-like and 87A/C-like loci in other species of the *obscura* and *melanogaster* species group and their molecular characterisation are necessary to understand the evolutionary and functional significance of the unique duality of the 2-58C locus of *D. pseudoobscura*.

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