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Specific Induction of the 93D Puff in Polytene Nuclei of *Drosophila melanogaster* by Colchicine

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When salivary glands of late 3rd instar larvae of *D. melanogaster* are exposed *in vitro* to colchicine (100 μg/ml) or colcemid (1 to 100 μg/ml) for 40 min at 24 C, ^{3}H-uridine incorporation in polytene chromosomes is severely inhibited while that in nucleolus remains nearly unaffected. At the same time, the 93D puff, which is one of the major heat shock locus, is specifically induced without a concomittant induction of other heat shock loci. These effects of colchicine or colcemid treatment are strikingly similar to those of benzamide [Lakhotia & Mukherjee, *Chromosoma*, **81** (1980) 125]. It appears that the colchicine-induced activity of 93D is not due to the effects of this alkaloid on cellular microtubules. The 93D puff is much less induced when the glands are heat shocked in presence of colchicine. Presence of colchicine during heat shock also causes the 87C puff to be induced to a greater degree (nearly 2.5×) than its duplicate locus at 87A.

Earlier studies from our[1-6] and other laboratories[7-9] have revealed several distinctive features of the 93D locus, a major heat shock gene in *Drosophila melanogaster*: it can be induced independently of the other heat shock loci, its transcription products are perhaps not translated and one of the major heat shock locus in all species of *Drosophila* examined so far, shares functional homology with the 93D of *D. melanogaster*. However, the significance of this locus during heat shock or otherwise is not known. Gubenko and Baricheva[10] have reported that colchicine as well as vitamin B_6 specifically induce one of the heat shock puffs of *D. virilis*. This observation has prompted us to examine the effects of colchicine on the 93D puff of *D. melanogaster* since the vitamin B_6 induced puff in other species of *Drosophila* has been found to be functionally homologous to the 93D[6]. The most well known cellular effect of colchicine is on microtubules[11]. In this context it may be noted that many of the agents, including the heat shock, that induce the heat shock response[9] are also known from other studies to affect the cytoskeletal system in not yet well understood manner[12]. Thus in the hope of understanding the mechanism of induction of 93D and its functional significance, we have initiated a programme to examine the effects of a variety of agents that affect the cytoskeletal system. In this paper, we present our observations on the effects of colchicine or colcemid treatment on the activity of the major heat shock loci in polytene nuclei of *D. melanogaster*.

Material and Methods

Flies and larvae of a wild type strain (Oregon R) of *D. melanogaster* were reared at 24° ± 1°C under standard conditions.

Colchicine or colcemid treatment at 24°C—Colchicine (100 μg/ml) or colcemid (1,10 or 100 μg/ml) was dissolved in Poels' salt solution[1] and salivary glands from late 3rd instar larvae were incubated in the various colchicine or colcemid containing media for 40 min at 24°C. Following the treatment, the glands were labelled with ^{3}H-uridine (500 μCi/ml sp. act. 12.8 Ci/mM, BARC, Trombay) for 10 min in presence of colchicine or colcemid, respectively. Parallel control glands were incubated and labelled with ^{3}H-uridine in colchicine- or colcemid-free medium.

Colchicine treatment at 37°C—Salivary glands from late 3rd instar larvae were incubated in Poels' salt solution containing 100 μg/ml colchicine at 37°C for 30 min and then labelled with ^{3}H-uridine (500 μCi/ml) in colchicine-containing medium for 10 min at 37°C).

Autoradiography—All ^{3}H-uridine labelled glands were immediately fixed and squashed in the usual manner. After the removal of coverslips, the preparations were treated with 5% trichloroacetic acid at 4°-6°C for 10 min, washed in running water, dehydrated through ethanol grades, dried and coated with 1:1.5 diluted Ilford K5 nuclear emulsion. After exposing in dark at 6°-8°C for 4 days, the autoradiograms were developed and fixed as usual. Giemsa stained autoradiograms were scored for labelling (numbers of silver grains) on the major heat shock puff sites (63BC, 87A, 87C, 93D and 95D) and on a segment of 3L (from section 61A to 63A).

Results

Colchicine or colcemid treatment at 24°C—The effects of these two microtubule poisons[11] on RNA synthesis in polytene nuclei are very similar to those of benzamide[4] since as with benzamide, colchicine as well

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as colcemid cause severe inhibition of ^{3}H-uridine incorporation in chromosomes (measured as silver grain counts on the 3L segment, see Table 1) while the 93D puff is induced (Table 1 and Fig. 1). With increasing dose of colcemid, the inhibition of chromosomal RNA synthesis is greater. At higher doses, the labelling on 93D is also found to be reduced so that in glands treated with 100 μg/ml colcemid or colchicine, the absolute numbers of silver grains on the 93D puff are similar to or somewhat lower than those in control glands; however, since in both cases the chromosomal and other puff RNA synthesis is drastically reduced, the lack of a proportionate inhibition on the 93D region indicates its induction (Table 1). Morphologically also, the 93D region appears as a big puff in all colcemid or colchicine treated glands eventhough its rate of ^{3}H-uridine incorporation is reduced at higher dose (Fig. 1c). None of the other heat shock puffs are active, neither morphologically nor in ^{3}H-uridine incorporation in colcemid or colchicine-treated glands. the nucleolar incorporation is partially affected by higher doses of colcemid or colchicine (data not presented).

Colchicine treatment at 37°C—When the glands are heat shocked in presence of colchicine (100 μg/ml), the chromosomal RNA synthesis is nearly completely inhibited but all the heat shock loci are induced (Fig. 1d and Table 1). However, the 87C puff is, on average, 2.5 times more active than the 87A, instead of the two loci being equally active as after a routine heat shock[1]. The 93D locus in these glands forms a smaller puff (see Fig. 1d) and incorporates ^{3}H-uridine less than 87A or even the 63BC puff (Table 1 and Fig. 1d).

Discussion

The specificity with which colchicine or colcemid induce only one of the heat shock locus, the 93D puff in *D. melanogaster*, is remarkable. In species like *D. hydei*, *D. nasuta* also the 93D-like puff[16] has been found (A.K. Singh, unpublished data) to specifically respond to colchicine. Thus the response of the 93D locus to colchicine is specific. These alkaloids are most well known for their binding to tubulins and the consequent disruption of cellular microtubules[11]. In view of this and the possible involvement of the cytoskeleton in heat shock response[12], we suggested in a preliminary study[13] that the specific induction of 93D by colchicine may be related to its effects on microtubules.

The effects of colchicine on polytene nuclei of *Drosophila* are strikingly similar to those of benzamide[4] since both inhibit the general chromosomal but not nucleolar RNA synthesis and specifically induce the 93D locus. However, unlike colchicine, benzamide is not known to bind to tubulins. Eventhough, benzamide has certain effects on mitosis in mammalian cells[14] and on motility in plasmodia[15], it does not block mitotic cells at metaphase as colchicine does (unpublished observations). Thus while benzamide may affect the

Table 1 – ^{3}H-Uridine Incorporation in Major Heat Shock Puff Loci and a Chromosome (3L) Segment in *D. melanogaster* Polytene Nuclei Treated with Colcemid or Colchicine

Treatment	Mean ± SE numbers of silver grains on					
	Major heat shock puff sites					3L segment (61A to 63A)
	63BC	87A	87C	93D	95D	
Control 24°C 40 min	12.4 ± 0.9 (25)	5.3 ± 0.8 (17)	5.1 ± 0.7 (17)	26.0 ± 1.0 (26)	15.4 ± 0.9 (25)	71.2 ± 3.4 (27)
Colcemid 24°C 1 μg/ml 40 min	(27)*	(25)*	(25)*	44.4 ± 2.9 (35)	(32)*	30.9 ± 2.3 (27)
Colcemid 24°C 10 μg/ml 40 min	(40)*	(36)*	(36)*	41.8 ± 2.1 (54)	(49)*	16.1 ± 1.3 (40)
Colcemid 24°C 100 μg/ml 40 min	(37)*	(28)*	(28)*	30.0 ± 0.5 (28)	(31)*	11.5 ± 1.1 (20)
Colchicine 24°C 100 μg/ml 40 min	(26)*	(20)*	(20)*	21.1 ± 6.9 (30)	(28)*	16.1 ± 1.3 (24)
Colchicine 37°C 100 μg/ml 30 min	28.1 ± 1.2 (27)	32.1 ± 0.2 (27)	79.9 ± 6.7 (25)	25.8 ± 2.6 (26)	9.4 ± 0.7 (22)	(22)*

*Indicates that the mean grain count was less than 3. Figures in parentheses indicate the number of nuclei examined for each case.

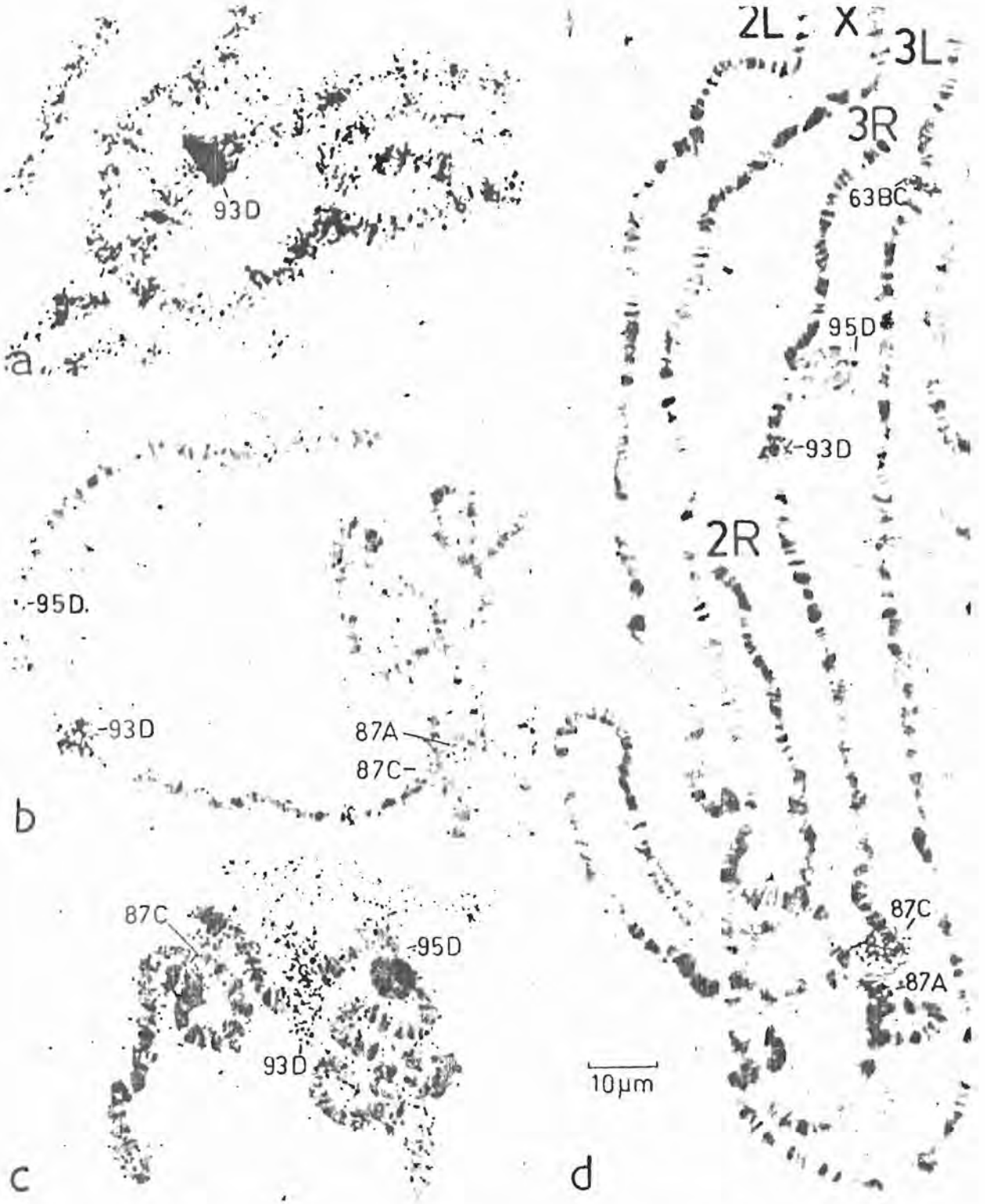

Fig. 1—Autoradiographs of polytene nuclei labelled with ^{3}H-uridine after treatment with 1 µg/ml (a), 10 µg/ml (b) or 100 µg/ml (c) colcemid at 24°C for 40 min or with colchicine (100 µg/ml) at 37°C (d) for 30 min. [Note the greater inhibition of ^{3}H-uridine labelling of chromosomes in b and c and the large puff at 93D, especially in c. In d, only the major heat shock puffs show appreciable labelling; the 87C puff is larger and significantly more labelled than 87A; the 93D puff is smaller and much less labelled.]

cytoskeletal components in some ways, it perhaps does not act upon microtubules in a manner comparable to that of colchicine. These considerations, therefore, raise the possibility that the colchicine induced 93D activity may not be due to this alkaloid's effect on microtubules *per se*, as was suggested earlier[13]. In another study in our laboratory (A.K. Singh and Lakhotia; in preparation) a number of other microtubule poisons like vinblastin, pdophyllotoxin, nocodazole and several others are being examined for their 93D-inducing potentiality. Results of this study indicate that unlike colchicine, none of them specifically induce the 93D puff. These observations also thus suggest that the colchicine-induced 93D activity in the present study is perhaps not directly due to its effect on microtubules but is more likely to be due to some other actions of colchicine. In plant as well as animal meiotic cells, colchicine, but not vinblastin etc, is known to inhibit homologus pairing and chiasma formation[16,17] and these effects have been ascribed to binding of colchicine to a nuclear associated protein distinct from the cytoplasmic tubulins[16]. In view of these reports, it appears tempting to speculate that in polytene cells also, a colchicine-binding protein, distinct from cytoplasmic microtubules, may exist and this may mediate the specific induction of the 93D locus by colchicine or benzamide. This needs further study.

It is to be noted here that unlike in polytene cells, colchicine as well as benzamide do not have any apparent effect on ^{3}H-uridine incorporation in non-polytene cells of *Drosophila*[13]. Thus the inhibition of chromosomal RNA synthesis by benzamide or colchicine may be a phenomenon specially associated with polyteny. At present we do not know if in the non-polytene cells of *Drosophila*, these chemicals induce the 93D locus as in polytene cells. This aspect is being examined.

It is interesting that when colchicine is present during heat shock at 37°C, the 93D puff is much less induced than after either of the treatments alone. This result is in agreement with our earlier observations[2-4] that when two 93D inducing treatments are applied together or in sequence, the 93D is much less induced or even not induced. The reasons for this behaviour of the 93D locus are not clear. It may, however, be noted that when colchicine and benzamide are given together at 24 °C (data not presented here), the activity of the 93D puff is not inhibited although it is also not additively induced to a greater degree.

Colchicine treatment at 37°C also leads to a striking difference in the activity levels of the duplicated gene loci at 87A and 87C[18], with the later being more than twice as active as the former. Benzamide treatment, which also selectively induces the 93D locus at 24°C, preceding or following the heat shock too was found to cause a differential activity of the 87A and 87C loci[4]. However, two important differences exist between the effects of colchicine and benzamide on the heat shock induced activity of 87A and 87C loci :(i) benzamide when present during the heat shock does not differentially affect the 87A and 87C activity while colchicine does, and (ii) in the case of benzamide treatment preceding or following the heat shock, the 87A locus is much more active than 87C[4] while in the present case, the 87C locus is found to be more active. The basis and the significance of these differences in the effects of colchicine and benzamide on the heat shock induced activity of 87A and 87C loci are not known but it seems very interesting that both the 93D inducing agents also affect the activity of 87A and 87C heat shock loci. These effects may reflect some unknown functional relationship between these loci. Whether this effect on the 87A and 87C loci is due to the activity of the 93D locus itself or is due to direct action of colchicine and benzamide on these loci at 37°C also remains to be known.

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