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Differential induction of chromosome puffs in two cell types of *Melanagromyza obtusa*

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Abstract. The patterns of puffing activity in polytene nuclei of salivary gland (SG) and midgut (MG) tissues of *Melanagromyza obtusa* have been studied after heat shock (HS), 2-4-dinitrophenol (DNP) or benzamide treatment. This study has revealed that HS and DNP treatments induced the same set of puffs but in a tissue-specific pattern. Benzamide treatment was found ineffective in inducing puffing activity. Some HS genes were also found to be more or less active during normal development, indicating some function in the normal metabolism of the cells.

Introduction

The interest in experimentally induced puffing patterns goes back to the early studies of Beermann (1952). He observed peculiarities in the behaviour of some puffs after cold treatment. It was Kroeger (1960) who first made a detailed study of experimentally changed puffing patterns in the chromosomes of isolated salivary gland (SG) nuclei. Since the discovery of the induction of unique set of puffs by HS (Ritossa 1962) in salivary chromosomes of *Drosophila*, much information has been accumulated on these loci (see Ashburner and Bonner 1979; Bonner 1982; Alahiotis 1983). Our knowledge of puffing phenomena in different cell types as a result of experimental induction is, however, still scanty. Because of the low degree of polyteny in cells other than SG such studies are obviously more difficult.

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Melanagromyza obtusa, a dipteran pest of an important pulse crop in the oriental regions (*Cajanus indicus*), possesses polytene chromosomes that are extremely suitable for study in cells other than SG (Singh and Gupta 1981; Gupta and Singh 1983). This prompted us to study the patterns of puffing activity in SG and midgut (MG) epithelial cells of the third instar larvae after exogenous treatment with several agents.

Materials and methods

The larvae of *M. obtusa* were collected from field-grown plants of *Cajanus indicus*. Early larvae, present in the infected seeds, were allowed to complete their development in the laboratory. Well-developed third instar larvae of similar age were chosen for the present investigations. To avoid any possibility of discrepancy in the puffing due to differences within the tissues, the distal lobes of SG and the proximal portion of MG were used throughout the present study.

Heat shock (HS). The distal lobes of sister SG from well-developed third instar larvae were excised and incubated separately for 30 min at 39° C and 24° C. The latter served as controls. Also freshly excised proximal portions of MG from third instar larvae were incubated separately at 39° C and 24° C for 30 min. The medium used for dissection and incubation of the tissues for treatments included only the inorganic salt constituents of Poels' (1972) tissue culture medium.

To determine possibly different timing of puff induction at different loci the material from both tissues was alloted to eight different groups and incubated at 39° C for various periods of time. At time intervals scaled up every 5 min the material from each group was fixed and then processed for chromosome preparations.

DNP treatment. During this treatment both SG and MG tissues were incubated at 24° C in medium containing 10^{-3} M 2-4-dinitrophenol for 30 min. Control material was incubated in DNP-free medium.

Benzamide treatment. Freshly excised SG and MG tissues were incubated in medium containing benzamide (1 mg/ml) for 10 min, the control being in benzamide-free medium.

After the treatment the tissues were fixed in acetomethanol (1/3) and stained in lacto-orcein for 5 min before squashing. The activity of each puff was estimated by taking the ratio between the maximum diameter of the puffed region and the diameter of an adjacent band not involved in puffing activity (Ashburner 1972).

Results

Three major puffs were induced in SG nuclei and five puffs in MG epithelial nuclei of third instar larvae after HS treatment. The loci are identified as 2C on the X chromosome and 31E, 33A/B, 35A, 37B on the D chromosome. Among them, three loci, i.e. 2C, 31E and 37B, were found in both, SG and MG nuclei, whereas activity of the two loci 33A/B and 35A was noticed exclusively in the MG nuclei (Figs. 1–3). Analysing the kinetics of puff induction we found remarkable differences with respect to puff initiation. For instance, the loci 2C, 33A/B, 35A and 37B become active between 5 and 10 min of treatment. On the other hand,

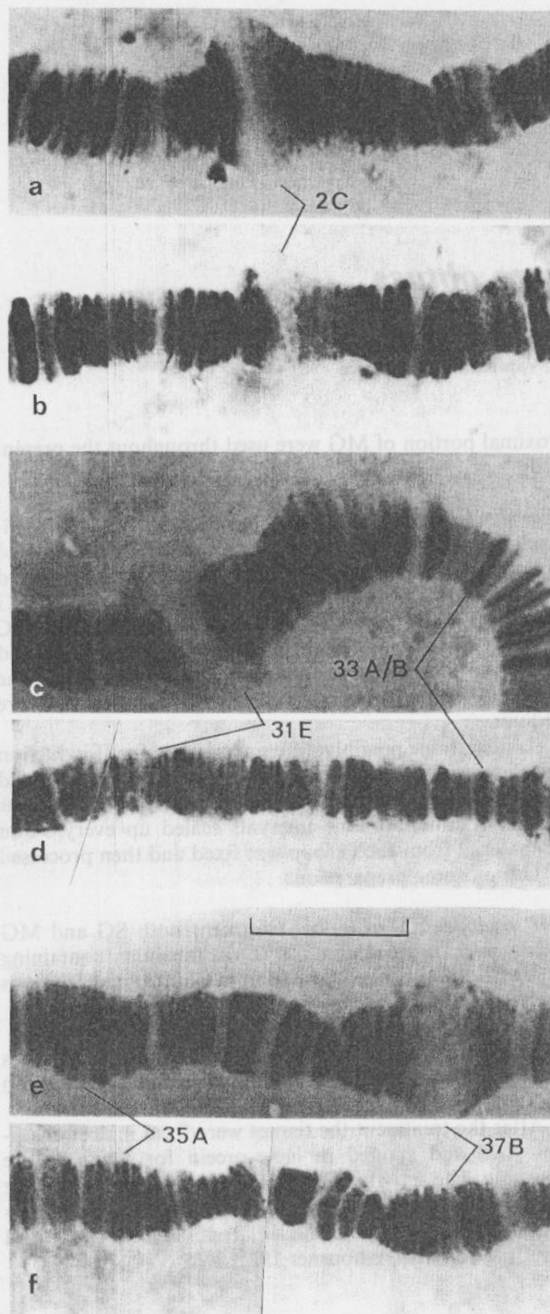

Fig. 1. Photomicrographs of polytene chromosome segments from HS treated (a, c, e) and control (b, d, f) salivary glands showing major induced puffs (loci 33 A/B and 35 A did not show any activity after HS treatment). The bars in Figures 1 and 2 represent 10 μm

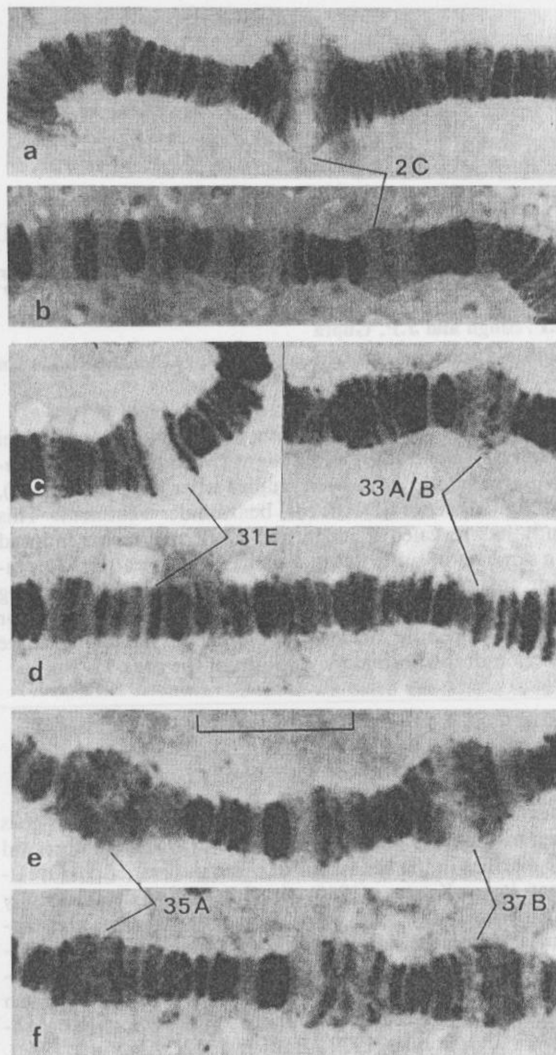

Fig. 2. Photomicrographs of polytene chromosome segments from HS treated (a, c, e) and control (b, d, f) midgut showing the major induced puffs

the puffing activity at 31 E locus in both tissues starts only after 20 min of treatment (Fig. 4).

Treatment with DNP showed in both tissues the same loci activated that had responded to HS treatment. Slight activity of loci 2C in both SG and MG, and of 37 B in MG could also be seen in the controls, but a much stronger increase in the puff-diameter was always noticed after treatment either with HS or DNP (Figs. 1–3). Apart from the induction of puffing, a marked inhibition of normal activity was observed also at several loci after experimental treatments. When both tissues were exposed to benzamide no puff could be induced and the activity of all normal puffs was inhibited.

Discussion

Heat shock and treatment with DNP have long been known to induce certain loci to puff in polytene chromosomes of *Drosophila*. These studies have also revealed that both treatments induce a more or less identical puffing pattern in polytene chromosomes (Ritossa 1962). It has further been reported that the synthesis of heat shock polypeptides after HS is not tissue-specific (Tissieres et al. 1974; Lewis et al. 1975). Hybridisation studies between mRNAs extracted

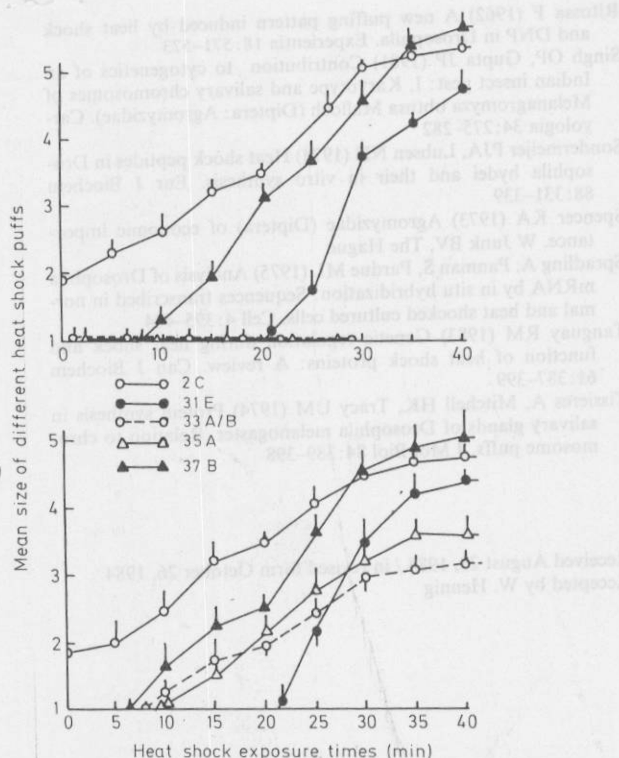

Fig. 3. Histogram showing the mean size of puffs in heat-shocked and control tissues (SG and MG) of *M. obtusa*

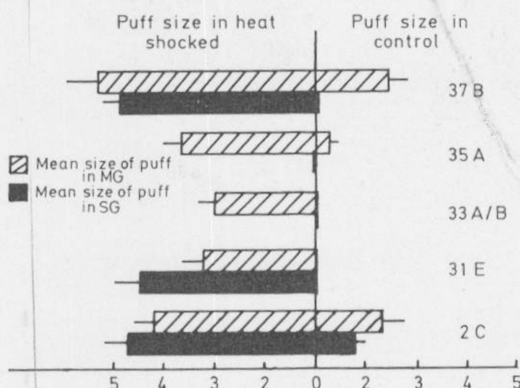

Fig. 4. Diagrams showing the timing of initiation of puffing activity (mean size of puffs calculated from ten different larvae) at different loci in SG and MG tissues of *M. obtusa* after HS treatment

from heat-shocked, non-polytene cells and the HS puffed loci suggested that different cell types of *Drosophila* respond to HS in a similar fashion (Spradling et al. 1975). However, Sondermeijer and Lubsen (1978) detected some tissue-specific differences in heat shock polypeptides. Our present investigation on puffing activity in polytene chromosomes of two different cell types from third instar larvae of *M. obtusa* revealed that a total of three puffs in SG and five puffs in MG could be induced by subjecting the tissues to either HS or DNP treatment (Fig. 1, 2). This clearly indicates that SG and MG nuclei respond in different ways. SG and MG tissues are quite different in their physiological activities and different responses of their genomes to the exogenous agents is therefore, not unexpected. Moreover, the responses of the loci differ. While other loci respond within 5–10 min to HS treatment, 31 E is activated only after 20 min of treatment (Fig. 4).

Three different categories of HS puff can be recognised. For instance, some HS puffs (i.e. 31 E in SG and 31 E and 33 A/B in MG) appear de novo, since these loci are never puffed during normal development. A second category includes those HS puffs (37 B in SG and 35 A in MG) that normally do not form puffs at the third instar larval stage but become active during the pre-pupal stage of development (Gupta and Singh 1983). The third category of puffs (2 C in both SG and MG and 37 B in MG) comprises already active loci, which after HS treatment display a substantially enhanced activity.

Obviously, some HS genes are active during normal development. This supports the hypothesis further that the products of HS genes might protect cells against damage caused by various kinds of stress (Li and Werb 1982; Minton et al. 1982; Cosgrove and Brown 1983; Tanguay 1983; Marx 1983). Loci 2 C and 37 B might belong to this category because they remain more or less active during normal development.

Lakhotia and Mukherjee (1970) reported that benzamide selectively induces only 93 D to puff out of nine HS loci of *D. melanogaster*. Recently, Lakhotia and Singh (1982) showed that the 93 D locus of *D. melanogaster* is fairly conserved among the members of the family Drosophilidae. However, the present study has shown that benzamide treatment fails to induce any puffing either in SG or MG nuclei. This shows the absence of a locus comparable to 93 D in *M. obtusa*. Since Agromyzidae, to which *M. obtusa* belongs, are considered to be a primitive dipteran family (Spencer 1973) the function of 93 D-like loci has probably been introduced much later in evolution.

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