Chromosoma (Berl.) 84, 207–219 (1981)

CHROMOSOMA

Cytogenetic Analysis of the 2B3-4–2B11 Region of the X Chromosome of *Drosophila melanogaster*

II. Changes in 20-OH Ecdysone Puffing Caused by Genetic Defects of Puff 2B5

E.S. Belyaeva[1,5], I.E. Vlassova[1], Z.M. Biyasheva[2], V.T. Kakpakov[3], G. Richards[4], and I.F. Zhimulev[1]

[1] Institute of Cytology and Genetics, Siberian Division of the USSR Academy of Science, Novosibirsk, 630090, U.S.S.R.; [2] Kasakh State University, Alma-Ata, U.S.S.R.; [3] Institute of General Genetics of the USSR Academy of Science, Moscow, U.S.S.R.; [4] C.N.R.S. Laboratoire de Genetique Moleculaire des Eucaryotes, 67085 Strasbourg Cedex, France; [5] address for reprint requests

Abstract. Larvae homozygous or hemizygous for the *l(1)t435* mutation located within the early ecdysteroid puff 2B5, or carrying a deletion of the 2B5 band, die at the end of the third larval instar. In the salivary gland chromosomes of these larvae only intermoult puffs are detected. If these salivary glands are incubated in vitro with 20-OH ecdysone for 6 h the intermoult puff 68C remains large, some early puffs (74EF and 75B) are induced to 30–40% of their normal size, other early (63F) and all late puffs (62E, 78D, 82F and 63E) are not induced at all. Puff 2B5 reaches its normal size but does not regress after 6 h incubation with 20-OH ecdysone, as it does in normal stocks. The data obtained in this study show the existence of a locus (or loci) in the band (puff) 2B5 which is necessary for the normal response of the salivary gland chromosomes to the hormone 20-OH ecdysone.

Introduction

We have previously described the cytogenetic analysis of the X chromosomal region 2B in *D. melanogaster* that allowed us to locate a series of mutations at the site of the early ecdysteroid puff 2B5. The complex overlapping complementation map of these mutations (Belyaeva et al., 1980) as well as preliminary data on the considerable recombinant distances between them (Aizenzon and Belyaeva, 1981) indicate the complex informational organisation of this puff. We have called this locus *o.c.c.* (overlapping complementation complex). It consists of the complementation groups *rbp, br, l(1)pp1* and *l(1)pp2* partially or completely overlapped by a series of non-complementing mutations.

By morphological and autoradiographical criteria the puff 2B5 is already active at PS1. However its size and activity dramatically increase by PS2 in vivo as well as within the first minutes of salivary gland incubation with 20-OH ecdysone in vitro (Ashburner, 1972). On these latter criteria this puff is considered an early ecdysteroid puff. The puff completely regresses by PS9–10 and again occurs at

0009-5915/81/0084/0207/$02.60

PS18–20. The morphology of the puff has been described in detail in our previous paper (Belyaeva et al., 1980).

There are reasons for believing that the activity of late ecdysteroid puffs depends not only on 20-OH ecdysone, but also on the functioning of early puffs (Ashburner et al., 1974). In connection with this suggestion, we decided to investigate the effect both of mutations at this locus, and of the complete removal of this puff by deletion, on the ecdysteroid dependant puffing activity in other chromosome regions. Our data indicate that normal puffing activity at 2B5 is one of the first events necessary for the development of a normal ecdysteroid induced programme in salivary gland cells.

Materials and Methods

Stocks and Culture Conditions. All the mutant stocks have been described in detail (Belyaeva et al. 1980, 1981). Here we give only essential additional information.

Larvae homozygous (♀♀) or hemizygous (♂♂) for *l(1)t435*, EMS-induced in a *yellow* (*y*) stock were used. This mutation is a "long" allele overlapping all mutation in the locus *o.c.c.* (Table 5 in Belyaeva et al., 1980). It is one of the developmentally earliest mutations of this complex: the mutants cease development and subsequently die at the end of the third larval instar. Males deficient for the 2B5 puff were produced according to the scheme presented in Figure 1. Males, carrying *Df(1)S39* which removes 1E3–4—2B5 and the locus *o.c.c.*, and also carrying the duplication *Dp(1)y²Y67g* covering bands 1A—2B18, were mated to females carrying *Dp(1)YSz280* and an attached X chromosome. *Dp(1)YSz280* is a translocation of 1A–2C1–2 with an internal deletion which includes bands 2B5, 2B6 and perhaps the margins of 2B3–4 and 2B7–8. Genetically this duplication covers all the loci to the left of *o.c.c.* and loci *l(1)HM40, l(1)HM38* and *l(1)HM32* to the right of *swi*. This duplication was synthesised by Kiss and Koczka (see Belyaeva et al., 1981). Male progeny of the cross carry an X chromosome with *Df(1)S39* and a Y chromosome with *Dp(1)YSz280*, i.e. a deletion of band 2B5 and perhaps a small part of the right hand margin of band 2B3–4, and duplications of 1A1–1E2 and 2B6–2C1.2. These we term "puffless" males.

Homozygotes or hemizygotes for *y l(1)t435*, "puffless" males and control *y* larvae were reared on standard medium at 25 °C., about 50–60 animals in a 2.5 cm diameter tube. Larvae were synchronised by taking those which hatched within 1 h. Such larvae pupate within a twelve hour period (Zhimulev and Kolesnikov 1975a).

Organ Culture and Cytology. Salivary glands were incubated in vitro using Ashburner's technique (Ashburner, 1972) at 25° ± 1° C. *Drosophila* tissue culture medium (Poluektova et al., 1980) and 20-OH ecdysone (Serva) were used.

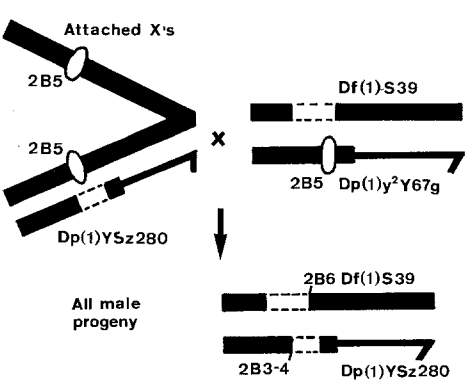

Fig. 1. Mating scheme for obtaining male larvae with a deficiency of the 2B5 band

Aceto-orcein slides were prepared as previously described (Zhimulev and Belyaeva, 1975). Autoradiographs were prepared from salivary glands incubated for 5 min with ^{3}H-uridine (200 μCi/ml, 43 Ci/mm, Amersham). The number of silver grains above puff 2B5 in both the asynapsed homologues of one nucleus was counted (21 nuclei from 5 animals at PS2–7).

The chromosome response to 20-OH ecdysone was estimated by the determination, with a filar optical micrometer, of puff ratios (Ashburner, 1972) in twenty nuclei (four from each of five animals) for each experimental point. The mean puff ratio and the standard error of the mean were calculated for each puff. The diameter of puff 2B5 was referred to the diameter of the reference band 4A1.2. The same puffs and reference bands studied by Ashburner (1972) were analysed so that the results would be comparable. Where measurements were impractical (see Results) puff size was evaluated visually in 20–30 nuclei from 5–10 animals.

Results

Development of the Mutants

Males lacking the puff 2B5 and homozygotes (or hemizygotes) for *y l(1)t435* are very similar in development. Their growth and development are delayed by one to two days compared with control larvae, even under optimal growth conditions. In unfavourable conditions (overcrowding, dense medium etc.) "puffless" males die when they reach sizes normal for the end of the third larval instar. Homozygotes (or hemizygotes) for *y l(1)t435* and "puffless" males at 120 h have a smaller body size, salivary glands and chromosomes than larvae of normal stocks. These mutants reach normal size only by 130–140 h. Their salivary glands are full of secretion by this time and the secretion is also found in the lumen. The larvae live two-three days longer and die as larvae.

Puffing in Mutants in vivo

The salivary gland chromosomes in the mutants have a distinct banding pattern except for the X chromosome in *y l(1)t435* males and "puffless" males, which has a diffused appearance. Larvae at the age of 120–130 h and younger have intermoult puffs of normal sizes (Fig. 4a). Thereafter puffs 3C and 25B (=25AC) regress and at the same time the chromosomes shorten. However 68C does not regress and remains large at the time of death. The chromosomes most suitable for analysis are those of 120–130 h *y l(1)t435* females, 130–140 h *y l(1)t435* males and those of "puffless" males.

We could not detect any sign of ecdysteroid induced puffs in mutant larvae throughout the delayed third instar. Perhaps the only exception is 2B5 in *y l(1)t435*. However, it is very difficult to estimate its size as the male X cannot be analysed, as mentioned above, and the female X also has a diffused appearance in the 2B region which obscures the banding pattern of this region. This diffuse structure of 2B has been observed only in homozygous females. In heterozygous females, *y l(1)t435/FM6*, both homologues have a normal morphology and the puff 2B5 develops normally in the *y l(1)t435* homologue as well as showing ^{3}H-uridine incorporation (Table 1).

Table 1. Incorporation of ^{3}H-uridine in the 2B5 puff of *y l(1)t435* and FM6 asynapsed chromosomes of the same nucleus

Number of larva and Puff Stage (PS)	Number of silver grains under puff: 2B5 in *y l(1)t435 /FM6* homologue for each nucleus scored in the larva					
1. PS7	6/7					
2. PS7	8/9					
3. PS5	14/12 15/18	14/13 8/7	12/10 15/16	10/11	12/13	11/9
4. PS5	12/11	13/14	20/22	17/14		
5. PS2	15/12	24/27	39/35	32/24	19/25	34/30

Puff Induction in the y l(1) t435 Mutant and "puffless" Males in vitro

To estimate induced puffing quantitatively we used salivary glands from 96 h larvae of the control stock *y*, 120–130 h females homozygous for *y l(1)t435*, 130–140 h males *y l(1)t435* and from "puffless" males.

An optimal 20-OH ecdysone concentration was determined in trial experiments. Glands from homozygotes *y l(1)t435* and from the *y* stock were incubated for 2 h in medium with various 20-OH ecdysone concentrations. As can be seen in Figure 2, the puffs 2B5, 74EF and 75B are maximally induced in the *y* stock at the concentration 10^{-6} M. As for chromosomes in *y l(1)t435* homozygotes, the maximal response of puffs 74EF and 75B (2B5 was not measured) is observed at the same concentration, but the induction is weaker than in the control stock, since the puff sizes do not reach even half that of the controls. A further increase of concentration does not produce any effect and at the highest concentration tested (10^{-2} M), the puffs are somewhat smaller. Similar data have been reported by Ashburner (1973). To study the time course of puff induction, the salivary glands were incubated for 0–6 h with or without 20-OH ecdysone (10^{-5} M). In glands from the *y* stock the sequence of 20-OH ecdysone induced puffing progresses to PS11 during the 6 h incubation (Fig. 3), as is observed in wild-type stocks (Ashburner, 1972) cultured in other incubation media. At the same time intermoult puffs regress. The addition of hormone in vitro induces responses in chromosomes of homozygous (or hemizygous) *y l(1)t435* and "puffless" males, but in both cases it cannot induce the normal puffing process observed in the controls. By the end of the 6 h incubation with 20-OH ecdysone the intermoult puffs 25B and 3C completely disappear, but the 68C puff remains active (Figs. 3a and 4). Its behaviour does not depend on the presence of 20-OH ecdysone in the medium. The unusual behaviour of this puff stimulated us to repeat the experiments taking sister salivary glands. 10 pairs of salivary glands from 120–130 h female *y l(1)t435* larvae were divided into two lobes. One lobe was fixed immediately, while the sister lobe was incubated for 3 to 6 h with 20-OH ecdysone. In this experiment the size of the 68C puff did not change during a 6 h incubation.

Early 20-OH ecdysone puffs are only partly induced in mutant larvae. The

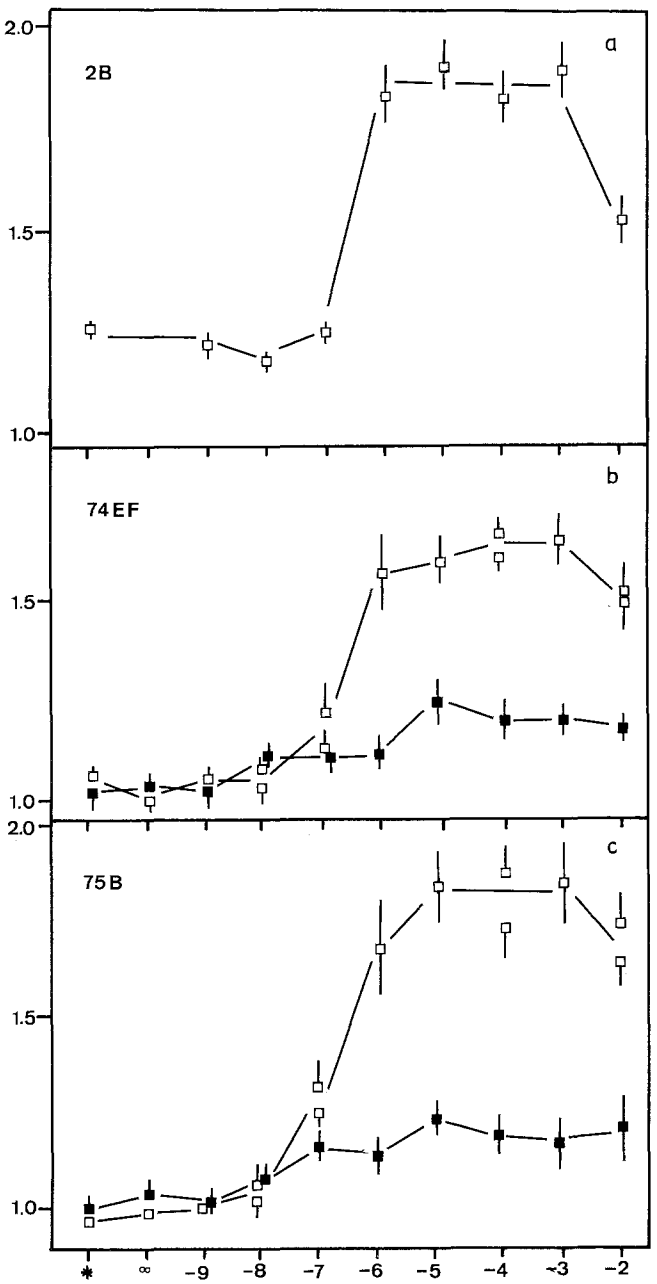

Fig. 2a–c. Response of **a**. 2B5, **b** 74EF, **c** 75B as a function of $\log_{10}$ 20-OH ecdysone concentration. Ordinate: Puff size after 2 h culture. Abscissa: Log_{10} of molar 20-OH ecdysone concentration. ■——■ = *yl(1)t435*; □——□ = *y*

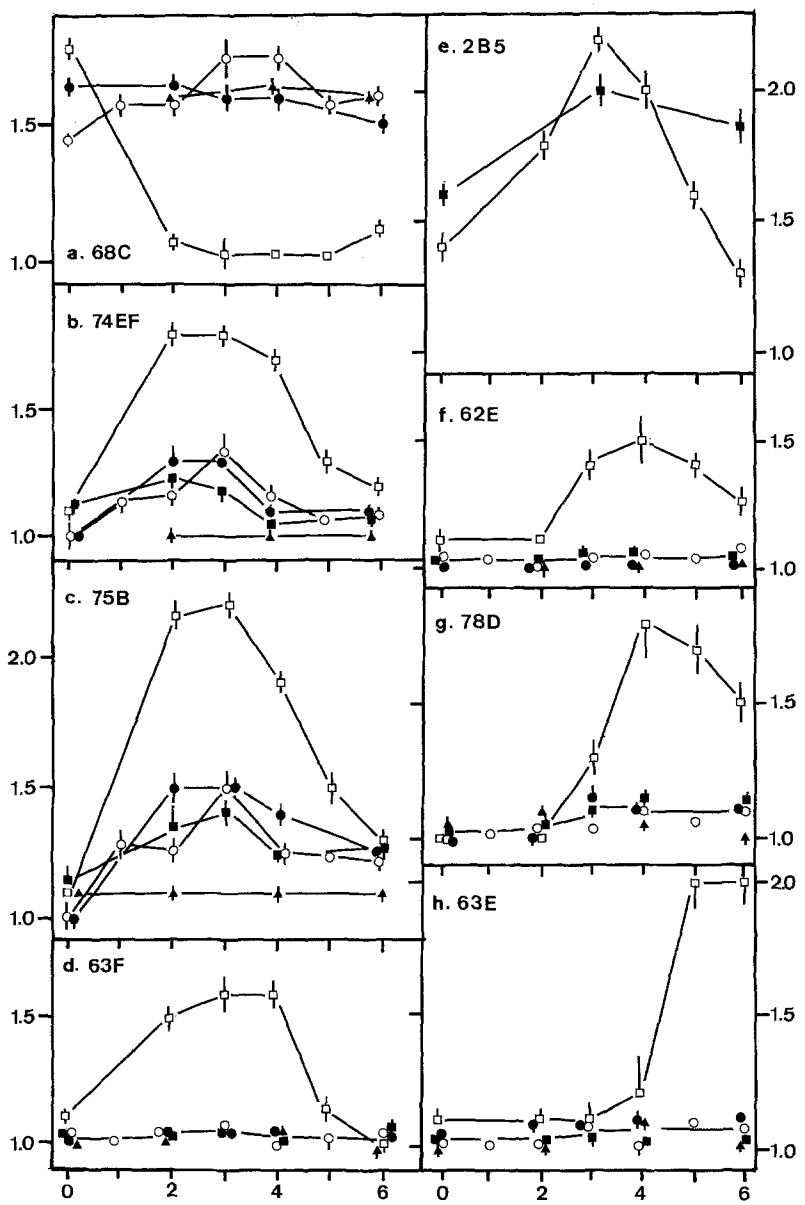

Fig. 3a–h. Activity of **a** 68C, **b** 74EF, **c** 75B, **d** 63F, **e** 2B5, **f** 62E, **h** 78D, **d** 63E during culture in vitro with 20-OH ecdysone (10^{-5} M). Ordinate: Puff size. Abscissa: Time of incubation in hours. □——□ = *y*; ○——○ = *y l(1)t435* males; ■——■ = *y l(1)t435* females; ●——● = males, deficient for 2B5, incubation with 20-OH ecdysone; ▲——▲ = males deficient for 2B5, incubation without 20-OH ecdysone

clearest induction is observed in puffs 74EF and 75B – they develop to 30–40% of the control size (Fig. 3b, c). The maximum is reached after 2 to 3 h, as in the control, then the puffs regress and after 6 h these regions in the mutants do not appear different from the controls. Puff 75B, however, regresses slower than 74EF (Fig. 5).

In some chromosomes one can observe the initial stages of puff development at

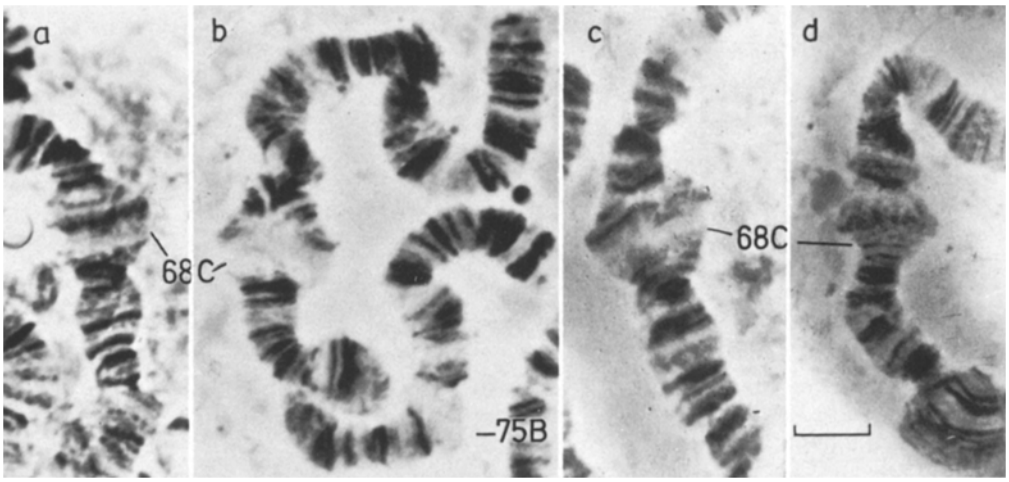

Fig. 4a–d.[1] Intermoult puff 68 C in *y l(1)t435* (**a, b**) and *y* (**c, d**) larvae, before (**a, c**) and after (**b, d**) 6 h incubation in vitro with 20-OH ecdysone

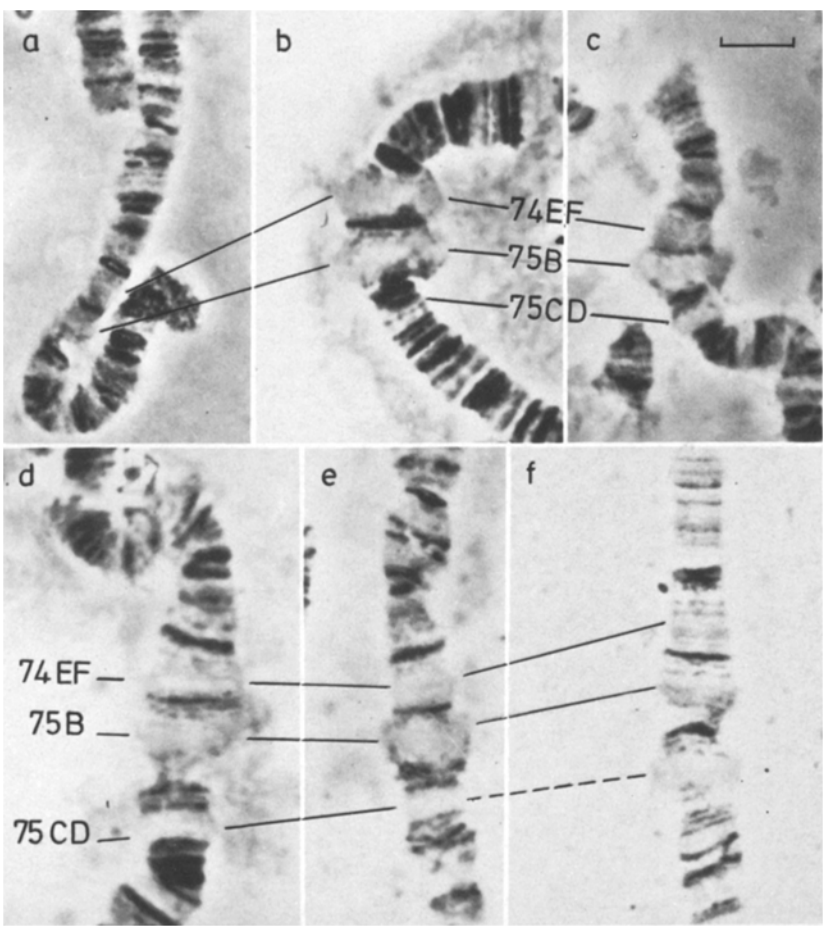

Fig. 5a–f. Early 20-OH ecdysone puffs 74EF and 75B in *y l(1)t435* (**a, c, d, e**) and *y* (**b**) larvae, before (**a**) and after (**b–e**) 2 h incubation with 20-OH ecdysone. **f** Puff 75CD in 10 h prepupa, Canton-S. **a, b, c, f** = females, **d, e** = males

1 Figures 4–7: bar = 5 μm

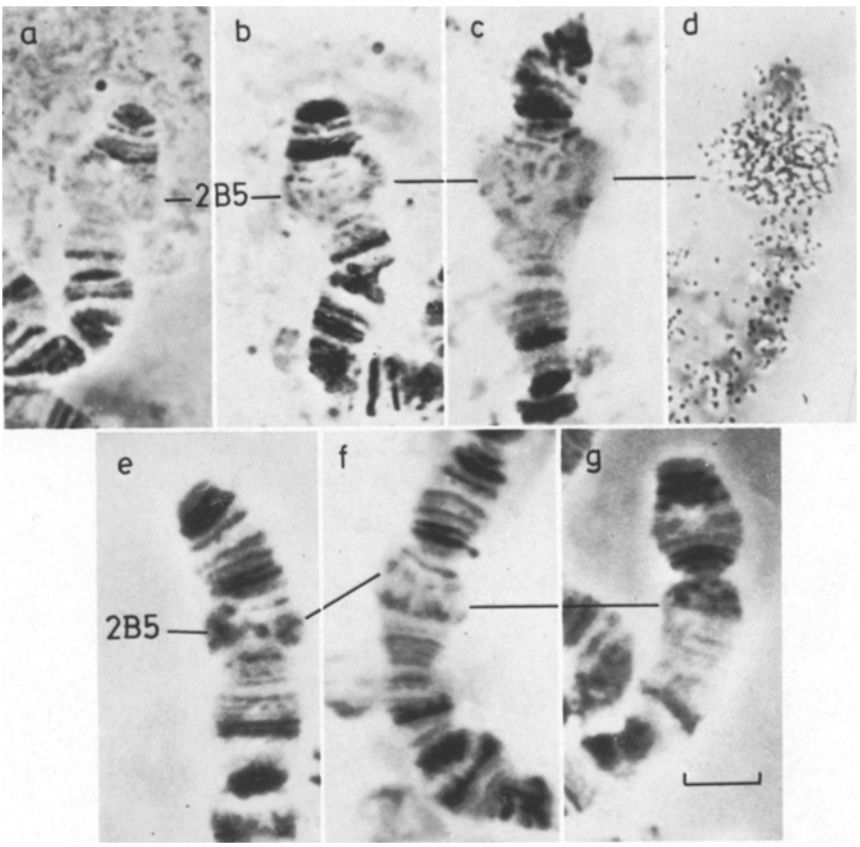

Fig. 6a–g. 2B5 puff in *y l(1)t435* (**a–d**) and *y* (**e–g**) larvae, before (**a, e**) and after 2 h (**b, f**) and 5 h (**c, d, g**) incubation with 20-OH ecdysone

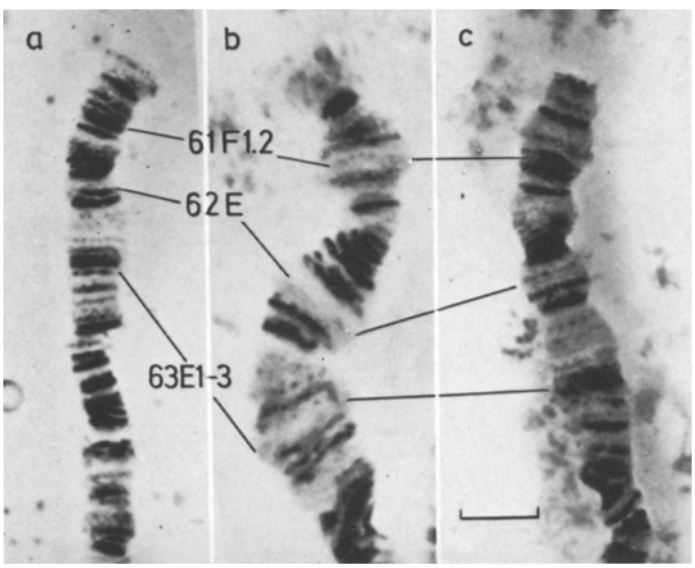

Fig. 7. Tip of 3L chromosome in *y l(1)t435* (**a, c**) and *y* (**b**) larvae, before (**a**) and after 5 h (**b, c**) incubation with 20-OH ecdysone

the loci 23E and 63F, but this slight activity is not detectable on the criteria of measuring chromosome puff ratios (Fig. 3d).

The interesting exception in the reaction of the early puffs to 20-OH ecdysone is the behaviour of 2B5 (Figs. 3e and 6). In the controls the puff rapidly increases in size, reaches a maximum after 3 h and regresses by 6 h, but in the chromosomes of *y l(1)t435* females this puff is also induced after 3 h but does not regress sharply on further incubation (Figs. 3e and 6). In this case also, we have carried out the additional analysis of puff behaviour in sister glands, to exclude the effect of variability of the initial puffing state in this region in different larvae. It should be noted that the estimation of activity in this region by puff ratio is difficult since it is not known how the diffuse structure of the region in the mutant affects the puff ratio at decondensation. Nevertheless the active ^{3}H-uridine incorporation in the puff area with pulse label after 5 h incubation of *y l(1)t435* glands with 20-OH ecdysone demonstrates its transcriptional activity (Fig. 6d).

None of the late puffs, including early-late (62E and 78D) and late-late (63E) ones show any sign of induction in vitro in the mutants (Figs. 3f–h, 7). On incubation of mutant salivary glands with 20-OH ecdysone, a puff is induced in the region 75CD (Fig. 5c–e). There is a puff active in this region in normal development only in 6–10 h prepupae (Fig. 5f), whose activity is inhibited even low concentrations of 20-OH ecdysone (Richards, 1976a).

We considered whether or not the absence of normal ecdysteroid puff induction in mutants was the result of developmental changes in the old (130–140 h) larvae. For this reason we incubated glands from younger larvae (110–115 h, males and females) with hormone. As mentioned above, the chromosomes from these larvae have a lower polyteny, and are difficult to analyse quantitatively, but by inspection we did not find any differences in the chromosome response to 20-OH ecdysone in the larvae of different ages. Puff induction per se is not affected as we were able to induce the complete set of "heat shock" puffs (Ashburner and Bonner 1979 for review) in both "young" and "old" larvae.

Discussion

The similarity of the mutant phenotype of the *l(1)t435* mutation, located within the 2B5 puff interval, and a deficiency of this puff is most interesting. The larvae having either this mutation, or a deficiency, stop their development at the end of the third larval instar at PS1 without any sign of the 20-OH ecdysone puffs in the salivary gland chromosomes. At the same time the processes of synthesis and secretion of the salivary gland glue appear to proceed normally.

Culture of mutant salivary glands with 20-OH ecdysone in vitro results in the partial development of some early 20-OH ecdysone puffs. Late 20-OH ecdysone puffs do not appear, and one of the intermoult puffs, 68C, does not regress even after a 6 h incubation. Moreover 68C remains active during the prolonged larval life of the mutant in vivo. Finally a puff is induced by 20-OH ecdysone in the region 75CD.

It is perhaps worth repeating the limits of such cytological analyses in the absence of extensive genetic data as are available in the case of 2B5. We cannot exclude the possibility that the puff we see at 68C is the transcriptional activity of a locus other than that for a glue component. Similarly the unexpected activity at

75CD, although morphologically similar to that seen later in normal development, may represent an entirely different gene activity. What is perhaps striking is that this "new" 20-OH ecdysone induced puff appears in a region known to be 20-OH ecdysone sensitive.

In this discussion we will consider results obtained both with *l(1)t435,* and also *l(1)npr-1* and *l(1)npr-2* two other "long" *o.c.c.* alleles that overlap all four *o.c.c.* complementation subgroups (Belyaeva et al., 1980). The mutation *l(1)t435* itself affects neither the developmental puff 2B5, nor ^{3}H-uridine incorporation in this region. Together with the result that heterozygotes of *l(1)t435* [or *l(1)npr-1*] with other alleles of *o.c.c.* show partial complementation (i.e., die later in development, Kiss et al., 1980; Aizenon et al., 1981), this suggests that it is not a microdeletion and that the mutant effect seems to be realised at the post-transcriptional level.

"Puffless" males lack the region between the left margin of the deletion in *Dp(1)YSz280* and the right one of *Df(1)S39*. According to cytogenetic data the left margin of this region is situated between the loci *sta* and *o.c.c.* The recombination distance between these loci is 0.021 m.u. The right margin of this region is between *o.c.c.* and *dor* where the recombination distance is 0.03 m.u. So "puffless" males may lack a region up to 0.05 m.u. On the cytological map this region is occupied by the band 2B5 and perhaps by the right margin of the band 2B3–4. In this interval are situated *o.c.c.* and the puff 2B5. We suggest that it is the *o.c.c.* activity that is responsible for the development of this puff.

This conclusion derives from the following facts:

1. The phenotypical effect of either removing the puff by deletion (and thus also *o.c.c.*) or the mutation in *o.c.c.* is the same, hence in this region there are no other essential genes expressed before the end of the third larval instar.

2. The time of death of defective *o.c.c.* carriers coincides that of the increase in puff activity (Aizenzon et al., 1981).

3. According to preliminary data the size of the *o.c.c.* locus is of the same order of magnitude as the region removed in "puffless" males – three of the four complementation groups of *o.c.c.* occupy about 0.02–0.03 m.u. Together with *dor* and *swi*, the genetic length of the six complementation groups of the 2B5.6 region (Table 5, Belyaeva et al., 1980) can be much greater.

Thus, the presence of the defective product in mutants *l(1)t435*, or the complete absence of this product in "puffless" males, have the same consequences. These consequences are very different from those described for other mutations affecting development and 20-OH ecdysone dependant puffing. So far four mutations with effects on 20-OH ecdysone puffing have been studied, *l(2)gl, DTS3, l(3)tl* and *dor*^{lt187} (Richards, 1976b; Holden and Ashburner, 1978, Zhimulev et al. 1976, Biyasheva et al., 1981). All these mutations disrupt development at the larval/prepupal transition when the processes of 20-OH ecdysone induction occur. Homozygous mutant larvae of these stocks, just as the larvae examined in our experiments, have chromosomes which do not develop normal 20-OH ecdysone puffs in vivo. Intermoult puffs are observed in "young" larvae and in a number of cases the salivary gland secretion is clearly produced. In these mutants an apparently normal induction of 20-OH ecdysone puffs can be produced by exogenous hormone. Hence, these mutations would act at the level of synthesis and release of ecdysone by the ring gland, or its transport to the target cells, without

altering the cellular mechanism responsible for the hormone entering into the salivary gland cells and binding with specific chromosomal loci. Our in vitro experiments have shown that the distortions caused by defects in the puff 2B5 occur at another level compared with these mutants. The genetical defects in the puff 2B5 change the cellular response to 20-OH ecdysone in such a way that neither the late, nor some of the early 20-OH ecdysone puffs are inducible. In addition an intermoult puff, 68 C, does not regress either in vivo or in vitro.

There are some other facts that support the conclusion that the defect lies in the cellular response. For example it has been demonstrated that when ring glands from *l(1)npr-1* or *l(1)npr-2* are transplanted into larvae from another mutant stock which have a low ecdysteroid level, puparium formation is induced in the latter (Kiss et al. 1980). It is perhaps surprising that during the whole delayed third instar in vivo we have never detected 20-OH ecdysone puffs as such mutant ring glands can apparently produce ecdysteroids, and in vitro, several puffs (74EF, 75B and 2B5) are induced. It can not be excluded that a 20-OH ecdysone excess in vitro is capable of compensating partly the defects of mutant cells.

The exact consequences of *o.c.c.* mutations and puff removal are still unknown. If we accept that 20-OH ecdysone is not rapidly metabolised in the salivary gland (Ashburner et al., 1974), it is possible to suggest that defects occur between the hormone entering the cell and its binding with specific chromosome sites. In the *l(1)t435* mutants and "puffless" males, "glue" is secreted from cells into the gland lumen. This process in normal conditions is an ecdysteroid induced function (Poels, 1972; Zhimulev and Kolesnikov, 1975; Boyd and Ashburner, 1977), and this is apparently not disturbed in animals having puff 2B5 defects. Hence the regulatory system of induction of certain gene loci by 20-OH ecdysone appears to be damaged. Furthermore, in genetic mosaics, portions of tissues homozygous for *l(1)npr-1* or *l(1)npr-2* are not able to pupariate although exposed to hemolymph with normal amounts of 20-OH ecdysone (Kiss et al., 1976). Similarly many tissues in *l(1)npr-1* fail to develop normally during prolonged culture (up to three weeks) in culture medium containing 20-OH ecdysone (Kiss and Molnar, 1980).

It is to be kept in mind that the critical developmental period in the animals with genetic defects of the puff 2B5 is at the beginning of puparium formation when the ecdysteroid concentration is high (Richards, 1981 for review). These mutants have successfully undergone the preceding larval moults. So in these mutants only the mechanism responsible for the third instar 20-OH ecdysone puff induction is damaged.

Thus, the data presented in this paper demonstrate the importance of the puff 2B5, which is apparently active both in salivary glands and other tissues (unpublished observations), in the regulation of 20-OH ecdysone gene activation. They demonstrate also the remarkable properties of this puff whose behaviour distinguishes it from the other early 20-OH ecdysone puffs: unlike other puffs it may be present in mutant cells, its activity is increased in vitro by exogenous 20-OH ecdysone, but it does not dramatically decrease during subsequent incubation with hormone. In vivo this could be evidence that the locus has a higher sensitivity to low ecdysteroid concentrations, but the dose response curve (Fig. 2a) does not support this suggestion. One explanation for its behaviour is that normally the cell sensitivity to 20-OH ecdysone is ensured by a very "early" low activity of 2B5 at PS1

that is sufficient for the subsequent 20-OH ecdysone induction of the early puff series. Why exogenous hormone is so effective in increasing 2B5 activity in mutant cells remains a puzzle.

In conclusion, by studying defects of the puff 2B5, we have observed effects on the specific reactions of other ecdysteroid responsive loci which in normal development show group behaviour in such general classes as intermoult, early and late puffs. These effects may be classified as (i) reduced response (74EF, 75B), (ii) total loss of response (68C, 62E, 78D, 63E) and (iii) de novo appearance of response (75CD).

Acknowledgements. The authors are grateful to Dr. Istvan Kiss for providing us with *Dp(1)YSz280*. G.R. thanks the Royal Society and the U.S.R.R. Academy of Sciences for arranging an Exchange Fellowship to visit Novosibirsk.

References

Aizenzon, M.G., Belyaeva, E.S.: Genetic loci in the 2A–B interval of Drosophila melanogaster X chromosome. Dros. Inf. Serv. (in press, 1981)

Aizenzon, M.G., Belyaeva, E.S., Zhimulev, I.F.: Cytegenetic analysis of Drosophila melanogaster X chromosome region 2 Bl.2–2B9.10.IV. Phenocrytic and effective lethal phases of mutants. Genetika (U.S.S.R.) (in press, 1981)

Ashburner, M.: Patterns of puffing activity in the salivary gland chromosomes of Drosophila. VI. Induction by ecdysone in salivary glands of Drosophila melanogaster cultured in vitro. Chromosoma (Berl.), **38**, 255–281 (1972)

Ashburner, M.: Sequential gene activation by ecdysone in polytene chromosomes of Drosophila melanogaster. I. Dependence upon hormone concentration. Devel. Biol., **35**, 47–61 (1973)

Ashburner, M., Bonner, J.J.: The induction of gene activity in Drosophila by heat shock. Cell **17**, 241–254 (1979)

Ashburner, M., Chihara, C., Meltzer P., Richards, G.: On the temporal control of puffing activity in polytene chromosomes. Cold Spr. Har. Symp. quant. Biol. **38**, 655–662 (1974)

Belyaeva, E.S., Aizenzon, M.G., Semeshin, V.F., Kiss, I., Koczka, K., Baricheva, E.M., Gorelova, T.D., Zhimulev, I.F.: Cytogenetic analysis of the 2B3–4—2B11 region of the X chromosome of Drosophila melanogaster. I. Cytology of the region and mutant complementation groups. Chromosoma (Berl.), **81**, 281–306 (1980)

Belyaeva, E.S., Aizenzon, M.G., Kiss, I., Gorelova, T.D., Pak, S.D., Umbetova, G.H., Kramers, P.G., Zhimulev, I.F.: Mutations and chromosome rearrangements in the 1E3–4—2B18 region of Drosophila melanogaster X chromosome. Dros. Inf. Serv. (in press, 1981)

Biyasheva, Z.M., Zhimulev, I.F., Kakpakov, V.T., Richards, G., Aizenzon, M.G., Belyaeva, E.S.: Two closely linked loci controlling ecdysone dependent puffing in Drosophila melanogaster. Dokl. Acad. Nauk. U.S.S.R. **256**, 983–986 (1981)

Boyd, M., Ashburner, M.: The hormonal control of salivary gland secretion in Drosophila melanogaster: studies in vitro. J. Insect Physiol. **23**,517–523 (1977)

Holden, J.J., Ashburner, M.: Patterns of puffing activity in the salivary and prothoracic gland chromosomes of a dominant temperature sensitive lethal of Drosophila melanogaster. Chromosoma (Berl.), **68**, 205–227 (1978)

Kiss, I., Molnar, I.: Metamorphic changes of wild type and mutant Drosophila tissues induced by 20-hydroxyecdysone in vitro. J. Insect Physiol., **26**, 391–401 (1980)

Kiss, I., Bencze, G., Fodor, A., Szabad, J., Fristrom, J.: Prepupal-larval mosaics in Drosophila melanogaster. Nature (Lond.) **262**, 136–138 (1976)

Kiss, I., Szabad, J., Belyaeva, E.S., Zhimulev, I.F., Major,J.: Genetic and developmental analysis of mutants in an early ecdysone inducible puffing region in Drosophila melanogaster. In: Development and neurobiology of Drosophila (O. Siddiqi, P. Babu, L.M. Hall and J.C. Hall, eds.), pp. 163–181. New York, London: Plenum Press 1980

Poels, C.L.M.: Mucopolysaccharide secretion from Drosophila salivary gland cells as a consequence of hormone induced gene activity. Cell Different. **1**, 63–78 (1972)

Poluektova, E.V., Mitrafanov, V.G., Kakpakov, V.T.: Effect of insect hormones on chromosome puffing in salivary glands of Drosophila virilis Sturt. cultivated in vitro. I. Changes of puffing upon cultivation of the glands in C-50 medium. Ontogenez (U.S.S.R) **11**, 175–180 (1980)

Richards, G.: Sequential gene activation by ecdysone in polytene chromosomes of Drosophila melanogaster. IV. The mid prepupal period. Develop. Biol. **54**, 256–263 (1976a)

Richards, G.: The in vitro induction of puffing in salivary glands of the mutant l(2)gl of Drosophila melanogaster by ecdysone. Roux' Archiv. Entw. Mech. Org. **179**, 339–348 (1976b)

Richards, G.: The radioimmune assay of ecdysteroid titres in Drosophila melanogaster. Molec. cell. Endocrinol. **21**, 181–197 (1981)

Zhimulev, I.F., Belyaeva, E.S.: ^{3}H-uridine labelling patterns in the Drosophila melanogaster salivary gland chromosomes X, 2R and 3L. Chromosoma (Berl.), **49**, 219–231 (1975)

Zhimulev, I.F., Belyaeva, E.S., Lychev, V.A.: Comparative study of the function of polytene chromosomes in laboratory stocks of Drosophila melanogaster and the l(3)tl mutant (lethal tumorous larvae). II. Changes of banding pattern and transcriptional activity in the salivary chromosomes of l(3)tl. Chromosoma (Berl.), **55**, 121–136 (1976)

Zhimulev, I.F., Kolesnikov, N.N.: Methods of selection of synchronously developing larvae in Drosophila melanogaster. Ontogenez (U.S.S.R.), **6**, 635–639 (1975a)

Zhimulev, I.F., Kolesnikov, N.N.: Synthesis and secretion of mucoprotein glue in the salivary gland of Drosophila melanogaster. Roux' Arch. Entw. Mech. Org. **178**, 15–28 (1975b)

Received June 24, 1981 / Accepted by W. Beermann
Ready for press July 6, 1981