Chromosoma (Berl.) 72, 53–66 (1979)

CHROMOSOMA

Ecdysone-induced Changes in Glycoprotein Synthesis and Puff Activities in *Drosophila virilis* Salivary Glands

Horst Kress

Zoologisches Institut der Universität, Luisenstr. 14, D-8000 München 2, Federal Republic of Germany

Abstract. During five hours after the injection of α-ecdysone into the hemolymph of *D. virilis* late third instar larvae the formation of larval glycoproteins in the salivary glands is terminated and the synthesis of a different set of glycoproteins which is characteristic for the prepupal gland is initiated. The data presented suggest that products from early puffs inhibit the formation of larval glycoproteins while the induction of late puffs may be responsible for the appearance of prepupal glycoproteins.

Introduction

In insects, fully differentiated tissues with a specific function, often assume a new function in the course of metamorphosis (reviewed by Kafatos, 1976). In a few cases it has been shown that this "reprogramming" is independent of DNA synthesis and cell division (Selman and Kafatos, 1974; Kastern and Krishnakumaran, 1975). It is conceivable that these tissues acquire the new function by a change in transcriptional activity.

At the biochemical level, the change of cell function may be reflected by the reprogramming of protein synthesis. This is seen in *Drosophila* salivary glands at the time of puparium formation (Tissières et al., 1974; Korge, 1977a). It has been suggested that the synthesis of different macromolecular products in larval and prepupal salivary glands is due to the reprogramming of puff activities as observed during the larval/prepupal transition (Kress, 1972). Two puffs, which become inactive prior to puparium formation have been shown to code for two larval secretion proteins (Korge, 1975, 1977b; Akam et al., 1978). However, a relationship between puffs and prepupal proteins has not yet been established.

Since puparium formation in *Drosophila* is induced by the molting hormone ecdysone (Vogt, 1943; Becker, 1962; Poels, 1970) it may be suspected that the changes in protein synthesis observed in salivary glands at that stage are also triggered by this hormone.

0009-5915/79/0072/0053/$02.80

The aim of the present work is to see whether the injection of ecdysone into late third instar larvae of *D. virilis* induces the reprogramming of protein synthesis in the salivary glands and whether the sequence of events can be attributed to corresponding changes in puff activities.

Materials and Methods

Animals. Female larvae or prepupae of a *Drosophila virilis* wild type stock were used in all experiments. They were reared and timed as described earlier (Kress, 1974).

Injections. Between 132 and 134 h after oviposition (about 13 h prior to ecdysone release) larvae were collected from the food, washed in water and injected with 0.2 µl of Poels' (1972) medium containing 2×10^{-4} M α-ecdysone (0.02 µg/0.2 µl). For inhibitor studies 2.4×10^{-4} M actinomycin D (60 ng/0.2 µl) and 5.3×10^{-2} M cycloheximide (3 µg/0.2 µl) were injected. At these concentrations the inhibitors reduce RNA and protein synthesis to less than 2% of the initial rate (Kress, 1977). After injection the larvae were kept individually at 25° C in small vessels covered with moist Kleenex paper.

Cytological Preparations. Isolated salivary glands were fixed in ethanol/acetic acid (3:1) for 5 min on a microscope slide. After swelling in 45% acetic acid for 2 min the glands were squashed under a cover slip. The preparation was frozen on a mixture of dry ice/methanol, the cover slip removed and the slide transferred into 100% isopropanol. The preparations were mounted in Entellan (Merck, Darmstadt) for microscopic inspection and phase contrast microphotography.

Autoradiographies. Isolated salivary glands were incubated at 25° C for 15 min in 25 or 50 µl of the medium described by Schmidt (1975) containing ^{3}H-uridine (26.7 Ci/mmol; Amersham Buchler) as specified in the legend to Fig. 7. Squash preparations were made as described above and processed for autoradiography as detailed by Pelling (1964). The preparations were exposed to Kodak AR stripping film and finally treated as described by Korge (1970).

Labelling of Glycoproteins in vitro. Glands from three animals were incubated at 25° C for 1 h in 50 µl of medium (Poels, 1972) containing 5 µCi ^{14}C-glucose (327 mCi/mmol; Amersham Buchler) or for 10 min in 25 µl of medium containing 2.5 µCi ^{14}C-proline (285 mCi/mmol; Amersham Buchler) and 10 µCi ^{14}C-glucose. Proline was omitted from the original medium and glucose replaced by mannose. The glands were fixed in ethanol/acetic acid (3:1) and stored at −20° C until electrophoresis.

Acrylamide Gel Electrophoresis. The isolated glands were heated at 56° C for 1.5 h in 40 µl of a solution of 0.1 M dithiothreitol, 0.2 M TRIS, pH 8, 0.01 M EDTA and 1.25% SDS. Electrophoresis was carried out on SDS-containing polyacrylamide gels according to Laemmli (1970). The separation gel ($115 \times 100 \times 1.5$ mm) contained a linear gradient of acrylamide increasing from 5.5 to 19.8% from top to bottom. Electrophoresis was run in the cold at 7 or 10 mA (constant current) until the brompenolblue front had migrated for 9 cm in the separation gel. Gels were stained with 0.1% Coomassie Brilliant Blue R 250 (Serva, Heidelberg) in 25% TCA (w/v) for at least 3 h and destained in 36% methanol + 7% acetic acid.

For electrophoresis on urea containing gels the gland proteins were reduced and alkylated as described by Korge (1977a). Linear gradients of 3.8 to 5.2% acrylamide from top to bottom were formulated on the basis of Grossbach's (1969) urea system, however, the gel buffer was diluted 1:1 and the stacking gel omitted. The size of the gel was $115 \times 120 \times 1.5$ mm. Electrophoresis was run for 6 h in the cold at 8 mA constant current. Gels were stained with 0.25% Coomassie Brilliant Blue R 250 in 7% acetic acid overnight and destained in 10% methanol + 7% acetic acid (Korge, 1977a).

Autoradiography and Autofluorography. Gels containing labelled proteins were dried in vacuum at 70° C and exposed to Kodak Royal X-Omat film at −20° C. For autofluorography the gels

were treated according to the method of Bonner and Laskey (1974). The X-Omat film was preexposed to 0.15–0.2 A_{578} according to Laskey and Mills (1975).

All chemicals were commercial products. α-ecdysone was a gift of Dr. P. Hocks, Schering AG, Berlin.

Results

Effects of Ecdysone on Glycoprotein Synthesis

Injection of ecdysone into the hemolymph of 133 h old larvae produced a change in the pattern of ^{14}C-glucose incorporation into glycoproteins in the salivary glands during the subsequent 5 h (Fig. 1), which is identical to the change observed in normal development at the time of puparium formation (Kress and Enghofer, in prep.). Among the fractions labelled in total glands from untreated larvae, two are also found in the isolated secretion (Fig. 1, LGP 1 and LGP 2). They are discharged from the gland within the first 5 min after puparium formation and serve as glue for the fixation of the puparium to the substrate. The synthesis of the component LGP 1 involves the formation of a protein precursor LGP 1_p with a molecular weight of approximately 138,000 daltons, which is modified by carbohydrate attachment in two steps to an intermediate product LGP 1_i and the final secretion product LGP 1 (Kress and Enghofer, in prep.).

The termination of the synthesis of glycoprotein LGP 1 under the influence of ecdysone was studied in more detail by incubating glands from ecdysone-treated larvae in medium containing ^{14}C-proline and ^{14}C-glucose for 10 min. Under these conditions 80–85% of the label found in fractions LGP 1_p + LGP 1_i originates from ^{14}C-proline, while LGP 1 is exclusively labelled by ^{14}C-glucose (Fig. 2). In this way the synthesis of LGP 1 can be examined independently from the synthesis of its precursors within the same sample. It was found that

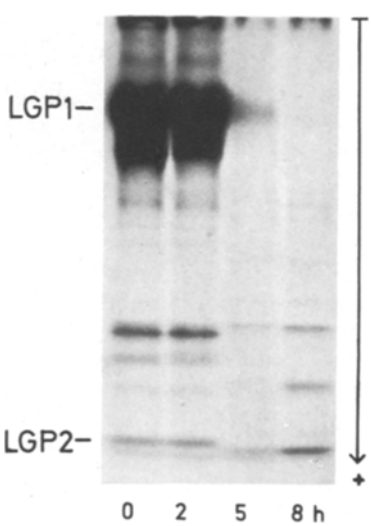

Fig. 1. Ecdysone-induced reprogramming of glycoprotein synthesis in *D. virilis* salivary glands. 0.02 µg α-ecdysone in 0.2 µl medium (2×10^{-4} M) was injected into 133 h old larvae. After different times the salivary glands were isolated and incubated for 1 h in medium containing ^{14}C-glucose. The glands were fixed at the times indicated (h post-ecdysone) and prepared for SDS-polyacrylamide gel electrophoresis. After elctrophoresis labelled bands were detected by autoradiography. 38 day exposure. LGP 1 and LGP 2 = larval glue proteins 1 and 2

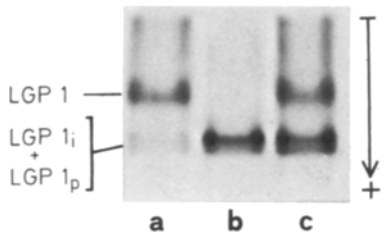

Fig. 2. Differential labelling of precursors and final product in the case of larval glue protein 1 (LGP 1). Salivary glands from 3 larvae were incubated for 10 min in 25 µl of medium containing ^{14}C-glucose (**a**), ^{14}C-proline (**b**) or ^{14}C-glucose + ^{14}C-proline (**c**). For details see Materials and Methods. The glands were fixed and prepared for SDS-polyacrylamide gel electrophoresis. After electrophoresis labelled bands were detected by autofluorography (8 h exposure). LGP 1_p = precursor protein; LGP 1_i = intermediate product; LGP 1 = larval glue protein 1

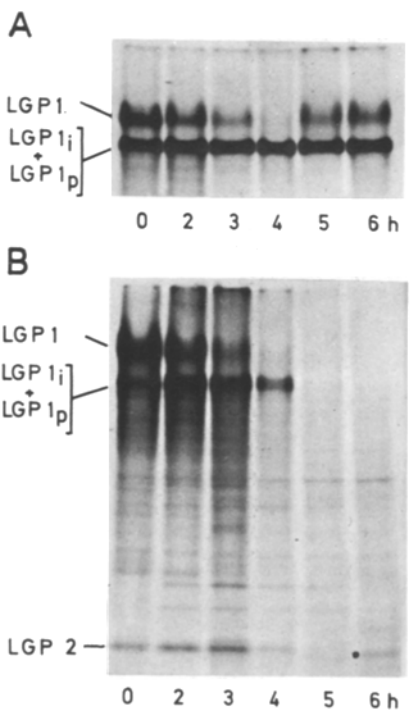

Fig. 3 A and B. Effects of ecdysone on the synthesis of precursors and final product of larval glue protein 1 (LGP 1). 0.2 µl of medium (**A**) or 0.02 µg α-ecdysone in 0.2 µl of medium (**B**) was injected into 133 h old larvae. At the times indicated the salivary glands were isolated and incubated for 10 min in medium containing ^{14}C-glucose and ^{14}C-proline. The glands were fixed and prepared for SDS-polyacrylamide gel electrophoresis. After electrophoresis labelled bands were detected by autofluorography. **A** 1 day exposure. **B** 15 days exposure. For symbols see legend to Fig. 2. LGP 2 = larval glue protein 2. The dot between the 5 and 6 h slot in (B) indicates a prepupal protein. It corresponds to the fast migrating glycoprotein that is strongly labelled in the 8 h sample of Fig. 1

incorporation of radioactivity into fractions LGP 1_p + LGP 1_i decreased between 3 and 5 h after injection of ecdysone; in control experiments the labelling pattern was not significantly altered (Fig. 3). The incorporation of glucose into the final product decreased between 2 and 4 h post-ecdysone both in the experiment and in the control, but recovered again in the control during the subsequent 2 h. The transitory decrease of LGP 1 labelling in the control in spite of continuous synthesis of its precursors can not be explained so far, but might indicate a general sensitivity of the second glycosylation step to experimental manipulations.

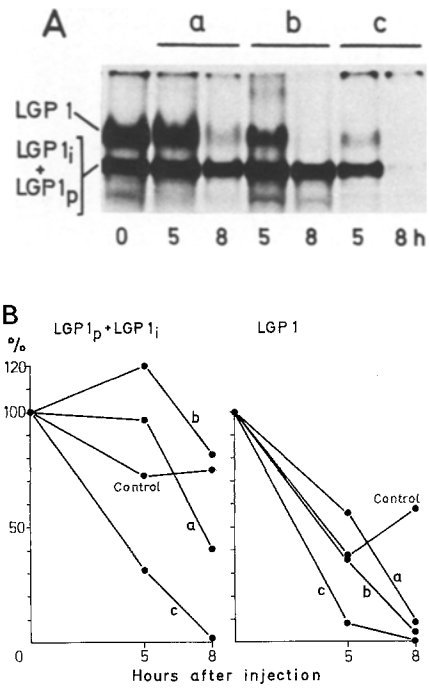

Fig. 4A and B. Effect of actinomycin D on the ecdysone-induced termination of larval glue protein 1 synthesis. **A** 60 ng actinomycin D (**a**) or 60 ng actinomycin D + 0.02 µg α-ecdysone (**b**) was injected into 133 h old larvae. In experiment **c** the drug was injected 2 h after ecdysone injection. At the times indicated (h after onset of experiment) the salivary glands were isolated and incubated for 10 min in 25 µl of medium containing ^{14}C-glucose and ^{14}C-proline. The zero-hour samples are from untreated larvae. For further details see legends to Figs. 2 and 3: 6 days exposure. **B** For the quantitation of radioactivity fluorographs (18 h exposure) were scanned and A_{578} of fractions LGP 1_p + LGP 1_i (left panel) and LGP 1 (right panel) determined for experiments **a**, **b** and **c**. In controls 0.2 µl of medium was injected. Values were normalized to zero-hour samples. Means of two experiments

In contrast to the termination of LGP 1_p and LGP 1_i formation within 5 h after ecdysone injection, the simultaneous injection of ecdysone and Actinomycin D (Act D), or injection of Act D alone did not reduce the incorporation of ^{14}C-proline into these fractions during this interval (Fig. 4, experiments a and b). Injection of Act D at 2 h post-ecdysone, however, did not inhibit the decrease in the rate of precursor formation, which starts about 1 h later (compare Fig. 3B). It is concluded, therefore, that Act D does not impair this process per se and that the result of experiment b must be explained by the lack of RNA synthesis during the first 2 h after ecdysone injection. This suggests that ecdysone induces the synthesis of specific RNA (and protein?) which inhibits the translation of the otherwise stable messenger for the protein LGP 1_p.

The labelling of the final product LGP 1 decreased to 37% of the initial value during the first 5 h after the injection of medium (compare the 0 and 5 h sample in Fig. 3A) and recovered during the subsequent hours (Fig. 4B, right panel). In contrast to this, labelling of LGP 1 continued to decrease if either Act D or ecdysone + Act D were injected (experiments a and b). This would indicate that LGP 1 synthesis is mediated by RNA (and protein?) with a short half life and, therefore, depends on continuous RNA synthesis which is inhibited by Act D. In addition to this effect the inhibition of precursor formation is responsible for the strong reduction of LGP 1 labelling in experiment c.

While 2 h of RNA synthesis after the injection of ecdysone were sufficient for the inhibition of larval secretion protein synthesis (Fig. 4, exp. c), the synthesis of prepupal glycoproteins, which starts at about 5 h post-ecdysone (com-

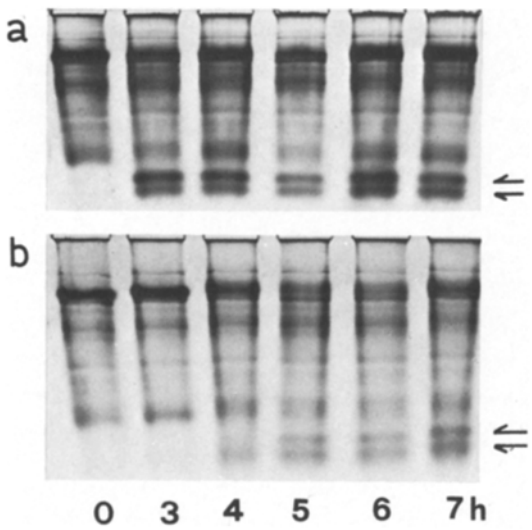

Fig. 5a and b. Inhibition of ecdysone-induced formation of prepupal proteins by actinomycin D. 0.02 µg α-ecdysone in 0.2 µl of medium (2×10^{-4} M) was injected into 133 h old larvae and the salivary glands isolated and fixed 12 h later. At the times indicated (h post-ecdysone) either 0.2 µl of medium (**a**) or 60 ng actinomycin D in 0.2 µl of medium (**b**) was injected. 0 h samples are from untreated larvae. Glands were prepared for urea-polyacrylamide gel electrophoresis. After electrophoresis the proteins were identified by staining with Coomassie Blue. Prepupal proteins are indicated by arrows

pare Figs. 1 and 3), was not observed under these conditions at 8 h post-ecdysone. In order to define the time interval of RNA synthesis required for the induction of these proteins, Act D was injected at different times after the injection of ecdysone and the glands fixed 12 h after the first injection. The gland proteins were reduced and alkylated and separated by electrophoresis on urea-containing polyacrylamide gels. In this way two prepupal proteins could be identified by staining with Coomassie Blue. The results are shown in Figure 5. In the controls (gel a) the two protein bands were always strongly visible. If Act D was injected at 3 h the induction of prepupal proteins was significantly inhibited (gel b). In the 4 h-experiments the faster migrating fraction was present at variable amounts, while the other one was still missing (it is the same prepupal protein marked in Fig. 3B; evidence not shown). Injection of Act D at 5 h or later did no longer prevent the synthesis of both prepupal proteins. Since their synthesis commences at about 5 h (compare Figs. 1 and 3) unspecific interference of the antibiotic with translation can be excluded and the observed effects attributed to the inhibition of RNA synthesis.

Effects of Ecdysone on Puff Activities

The results described above suggest that RNA synthesis is required for both the inhibition of larval glycoprotein synthesis and the induction of prepupal

glycoprotein synthesis. It was assumed that the RNA involved is induced de novo by the hormone. Therefore, the changes in puff activities during 5 h post-ecdysone were examined in more detail.

In cultured *D. melanogaster* salivary glands β-ecdysone inactivates preexisting "intermolt puffs" and induces "early puffs" which are supposed to be responsible for the induction of "late puffs" (Ashburner et al., 1974). In principle, the same patterns of activity were found in the present in vivo studies using α-ecdysone. The time relationships between these different patterns are schematically shown in Figure 6 by a few representative examples.

Among the intermolt puffs examined there were two puffs (97 E and 84 A) that did not immediately respond to ecdysone, but did so 1.5 h later, and furthermore, their regression was inhibited by cycloheximide (Fig. 7). Since puffs of this type have not been described in *Drosophila* so far, the effects of cycloheximide (CH) on ecdysone-induced regression of puff 97 E will be described in more detail. The injection of CH alone did not significantly change the morphology of this puff during the interval of observation (not shown). The injection of CH either simultaneously with the hormone or 1 h later inhibited puff regression, which was normally observed between 1.5 and 2 h post-ecdysone (Fig. 8, a–c). If CH was injected at 1.5 h (experiment d) a significant decrease in puff size was observed between 1.5 and 2 h (compare the 1.5 h sample in experiment a and the 2 h sample in experiment d; confidence intervals (*t*-test) did not overlap for $P=0.1$; $n=13$ in either case). During the subsequent hours the puff increased in size again, while in controls (injection of medium at 1.5 h; experiment e) puff regression proceeded. From the results of experiment d two conclusions can be drawn: i) The decrease in puff size in the presence of CH indicates that the process of regression per se is not affected by the antibiotic. It is concluded, therefore, that between 1 and 1.5 h proteins are synthesized de novo which inactivate puff 97 E. ii) The regression of this puff is reversible.

For *D. melanogaster* it has been suggested that the induction of late puffs depends on prior protein synthesis (Ashburner, 1974; Ashburner and Richards, 1976). In the present experiments late puffs were induced between 2.5 and 3 h or later (Fig. 6, puffs 43 F and 76 DE). In order to define the time interval of protein synthesis required for their induction, CH was injected at different times prior to the appearance of late puffs. In Figure 9 the results for the late puff 76 DE are shown. After the injection of ecdysone this puff was induced between 3 and 3.5 h (Fig. 9a). Puff formation was suppressed if CH was injected either simultaneously with the hormone or 2 h later (Fig. 9b and c). Injection of CH at 2.5 h post-ecdysone inhibited the activation of band 76 E_1 (from which the puff originates) in part, while CH injection at 3 h was almost without effect (Fig. 9d and e). Obviously, for puff induction protein synthesis is required which starts at about 2.5 h after the application of the hormone, that is 0.5–1 h before onset of puff formation. Similar results were obtained for the late puff 43 F.

One pecularity of puff 76 DE should be mentioned: 1 h after the injection of CH alone, a small puff was observed at the band 76 E_1, which was similar in size to that found in experiment d of Fig. 9. At 1.5 h or later the puff

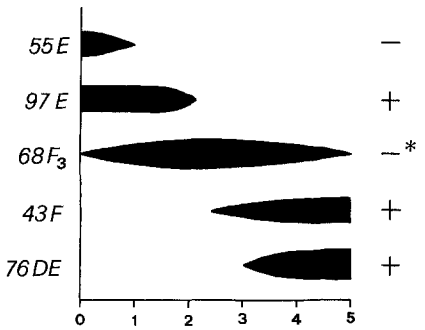

Fig. 6. Characteristic patterns of puff activities observed during the first 5 h after the injection of ecdysone (abscissa) into 133 h old larvae. Puff symbols are shown on the left side. Effect of cycloheximide on ecdysone-induced change is indicated on the right side. – = No inhibition, + = inhibition, * regression is inhibited by cycloheximide

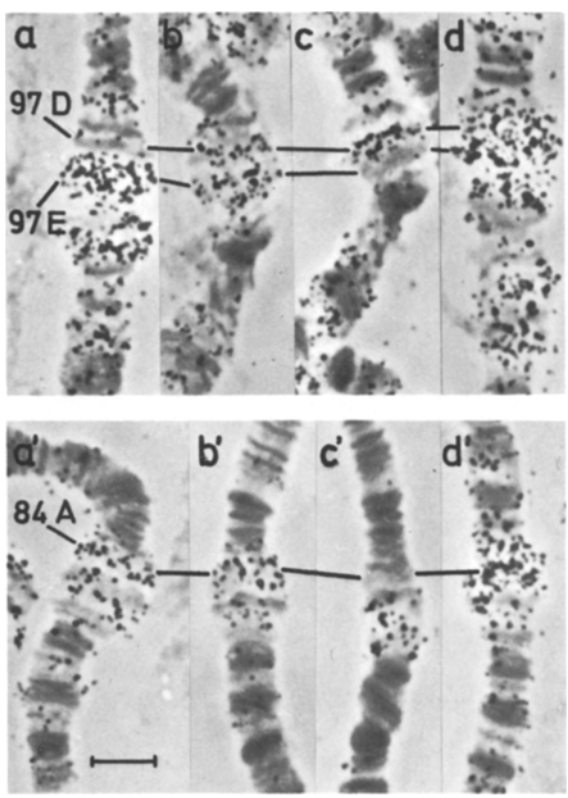

Fig. 7a, a'–d,d'. Effects of ecdysone and cycloheximide on RNA synthesis in the intermolt puffs 97 E and 84 A. Puffs were labelled in vitro by incubating isolated salivary glands for 15 min in medium (Schmidt, 1975) containing 1 μCi ^{3}H-uridine/50 μl (**a, a'**) or 2 μCi/25 μl (**b–d, b'–d'**). The glands were fixed and processed as detailed in Materials and Methods, **a, a'** Untreated larvae; **b, b'** incubation between 80 and 95 min after the injection of 0.02 μg α-ecdysone; **c, c'** incubation between 130 and 145 min after the injection of 0.02 μg α-ecdysone; **d, d'** incubation between 289 and 304 min after the injection of 0.02 μg α-ecdysone + 3 μg cycloheximide. The presence of ecdysone is indicated by the induction of the early puff 97 D. 7–19 days exposures. Bar = 5 μm

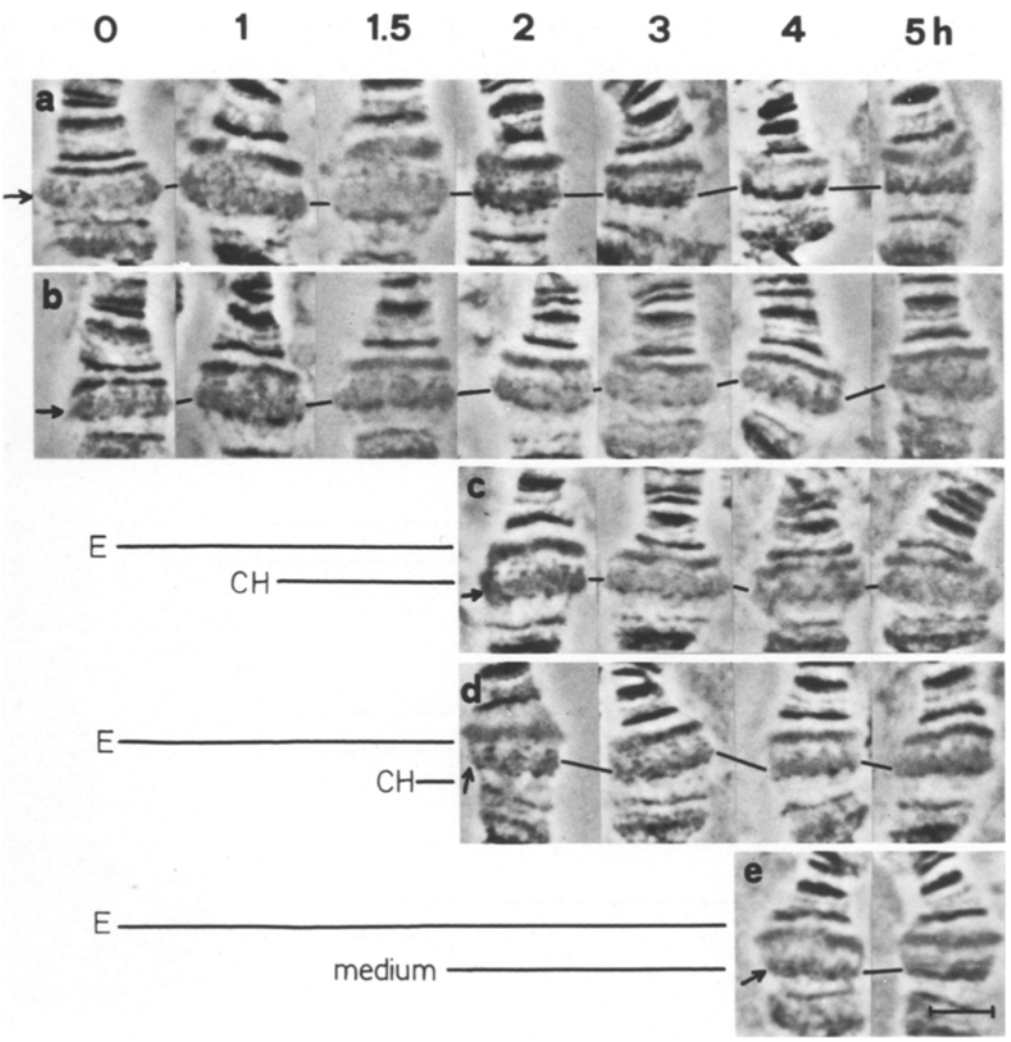

Fig. 8a–e. Effect of cycloheximide on the ecdysone-induced regression of the intermolt puff 97 E. 0.02 µg α-ecdysone in 0.2 µl of medium (2×10^{-4} M) was injected into 133 h old larvae. Squash preparations of salivary gland chromosomes were made at different times during the subsequent 5 h. The site observed is indicated by a small arrow. **a** After the injection of ecdysone, **b** after the injection of ecdysone + 3 µg cycloheximide. In the experiments **c–e** 3 µg cycloheximide (*CH*) in 0.2 µl of medium or medium alone were injected separately after the injection of exdysone (*E*) at the times indicated. Bar = 5 µm

was no longer observed. This transitory activation was also observed after the injection of water and, therefore, is interpreted as an experimental artefact. Since it was never observed in experiments that included exdysone, this effect was not relevant to the present experiments.

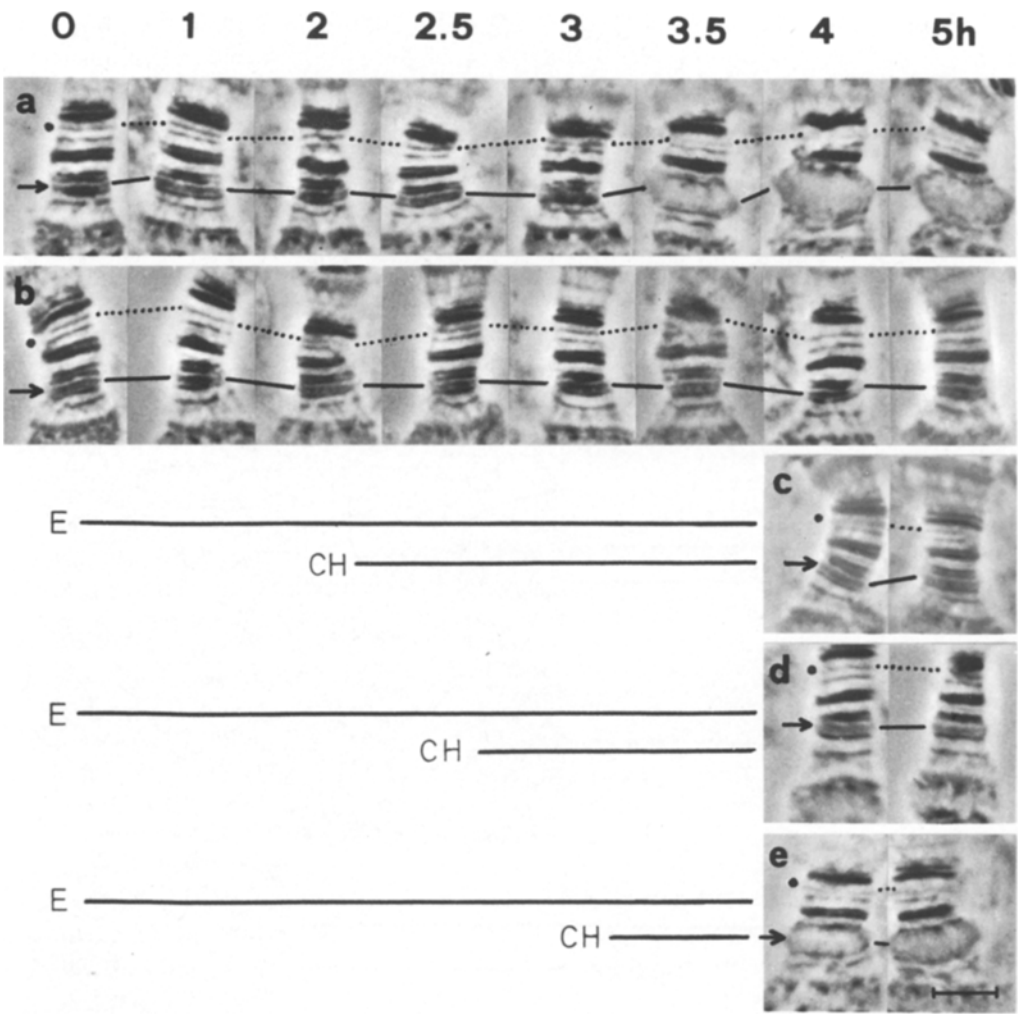

Fig. 9. Effect of cycloheximide on the induction of the late puff 76 DE by ecdysone. The experiments were done as described in Figure 8. For the explanation of symbols see legend to Figure 8. Dotted line indicates the late puff 76 A which will be referred to in the discussion

Discussion

The injection of α-ecdysone into late third instar larvae promotes the termination of larval glue protein synthesis between 3 and 5 h post-ecdysone. The simplest way to explain this observation is the ecdysone-stimulated inactivation of intermolt puffs that contain the genes for these proteins. Indeed, it has been shown in *D. melanogaster* that two intermolt puffs code for two proteins of the larval secretion (Korge, 1975, 1977b; Akam et al., 1978), and in *D. virilis* there is indirect evidence suggesting that the intermolt puff 55 E might be engaged in the synthesis of the larval secretion (Kress 1973, 1975). However, this primary

effect of ecdysone seems to be only part of a rather complex process. In the present work it was found that the injection of Act D, which leads to complete inhibition of all RNA synthesis within 1 h (Kress, 1977), does not entail a significant decrease in the rate of glue protein synthesis during the subsequent 5 h (Fig. 4, exp. a). It is concluded that the mRNA for glue protein LGP 1 is stable for more than five hours. Similar observations in other dipteran salivary glands that intensely synthesize glue proteins support this contention (Clever et al., 1969; Daneholt and Hosick, 1973; Zhimulev and Kolesnikov, 1975). Therefore, an additional control at the level of translation is required which provides for the termination of secretion protein synthesis between 3 and 5 h post-ecdysone.

Obviously, this control becomes effective under the influence of ecdysone by a process that is initiated by RNA synthesis during the first 2 h post-ecdysone. In *Drosophila* salivary glands there are still other cytoplasmic processes that are controlled in a similar way. The ecdysone-controlled inactivation of the enzyme glutamine-fructose-6-phosphate aminotransferase, which is engaged in larval glycoprotein synthesis, occurs only, if RNA synthesis is allowed to proceed for at least 1.5 h after the injection of ecdysone and the same holds true for the induction of the extrusion of the secretion proteins into the gland lumen (Kress, 1977). In *D. hydei* and in *D. melanogaster* 3 h of RNA synthesis after β-ecdysone treatment in vitro are required for the extrusion to occur (Poels, 1972; Boyd and Ashburner, 1977). At least for these three cytoplasmic processes, therefore, the action of ecdysone requires RNA synthesis during a period in which the induction of early puffs is typically observed. It is attractive to speculate that products from these puffs (RNA and/or protein) control and integrate the complex pattern of events that is associated with the termination of the synthesis of larval secretion proteins and their release into the gland lumen.

This hypothesis implies that sites which form early induced puffs contain regulatory genes that control cytoplasmic processes. In addition, there is evidence that they also might control the activity of genes (see also Ashburner et al., 1974). It is shown in this paper that late puffs are only induced if protein synthesis is allowed to proceed during an interval that precedes puff formation by 0.5–1 h (see Fig. 9). Presumably the proteins required for late puff induction are translated from transcripts from early induced puffs. A similar mechanism may be proposed for the regression of late intermolt puffs like 97 E and 84 A described in this paper. In accord with their regulatory function, such proteins should be unstable and thus disappear soon after the inactivation of early induced puffs. Correspondingly, the regression of late intermolt puffs should be transitory. Indeed, we find that the puffs 97 E and 84 A are reactivated during the prepupal stage in normal development (Fig. 10a and b). The activity of these puffs in the prepupal salivary gland suggests that they are not specifically involved in the synthesis of larval secretion proteins, but might serve more general cellular functions. It has been demonstrated that the late intermolt puff 97 E contains sequences that are complementary to 5 S rRNA (Cohen, 1976; Wimber and Wimber, 1977).

Finally, we are left to ask for the physiological role of late induced puffs. The present study shows that the synthesis of prepupal proteins, which starts

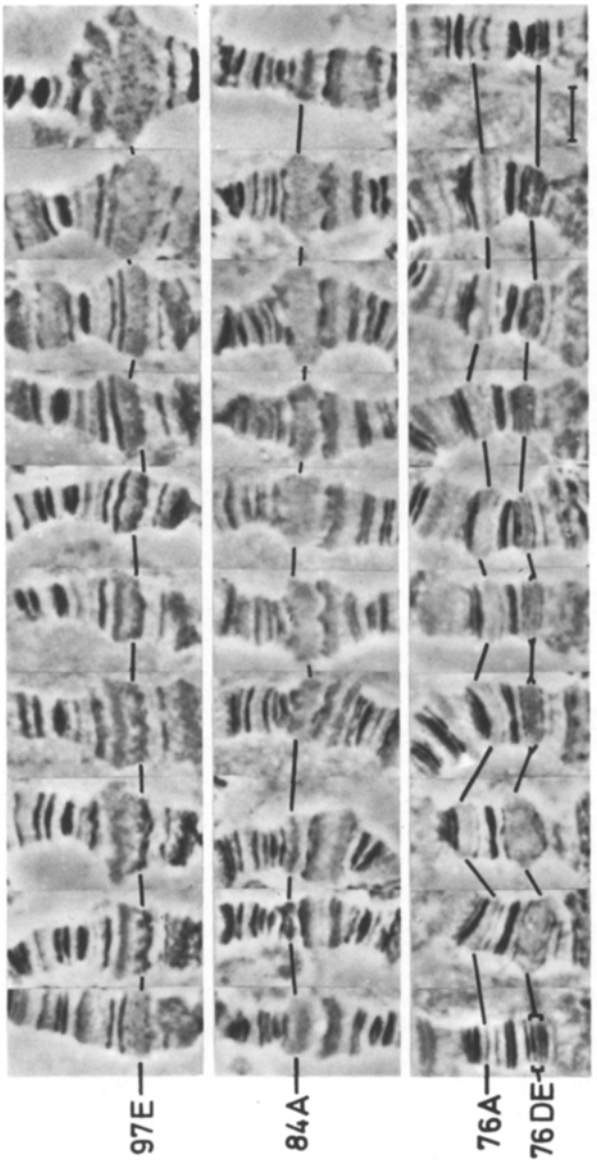

Fig. 10a–c. Ontogeny of late intermolt and late induced puffs in *D. virilis* salivary gland chromosomes. At the times indicated (hours at 25° C before and after puparium formation) squash preparations of salivary gland chromosomes were made and the following sites observed for developmental changes in activity: late intermolt puffs 97 E (**a**) and 84 A (**b**), late induced puffs 76 A and 76 DE (**c**). Bar = 5 µm

at about 5 h post-ecdysone (see Fig. 3B), requires RNA synthesis between 3 and 5 h after the injection of ecdysone (see Fig. 5). Since late puffs become active at about 3 h post-ecdysone (see Fig. 9) it is likely that the mRNAs for prepupal proteins are transcribed from sites that form late puffs. These puffs should at least be active as long as prepupal proteins are synthesized. In *D. virilis* their synthesis is terminated between 10 and 15 h after puparium formation (Kress and Enghofer, in preparation). In Fig. 10c the small prepupal puff 76 A is marked which becomes inactive during the same interval. This puff is induced between 3 and 3.5 h after the injection of ecdysone and requires protein synthesis for its activation (see Fig. 9). This example demonstrates the existence of late induced puffs that fit the hypothetical time-table. However, a direct correlation between late induced puffs and prepupal glycoproteins remains to be established.

Acknowledgements. I would like to thank Professors H.J. Becker and M. Ashburner for their comments on the manuscript and Dr. G. Korge for helpful discussions. I am indebted to Prof. B. Linzen for his interest and to Mrs. Helga Geisert for excellent technical assistance.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 51).

References

Akam, M.E., Roberts, D.B., Richards, G.P., Ashburner, M.: Drosophila: the genetics of two major larval proteins. Cell **13**, 215–225 (1978)

Ashburner, M.: Sequential gene activation by ecdysone in polytene chromosomes of Drosophila melanogaster. II. The effects of inhibitors of protein synthesis. Develop. Biol. **39**, 141–157 (1974)

Ashburner, M., Richards, G.: Sequential gene activation by ecdysone in polytene chromosomes of Drosophila melanogaster. III. Consequences of ecdysone withdrawal. Develop. Biol. **54**, 241–255 (1976)

Ashburner, M., Chihara, C., Meltzer, P., Richards, G.: Temporal control of puffing activity in polytene chromosomes. Cold Spring. Harb. Symp. quant. Biol. **38**, 655–662 (1974)

Becker, H.J.: Die Puffs der Speicheldrüsenchromosomen von Drosophila melanogaster. II. Mitt. Die Auslösung der Puffbildung, ihre Spezifität und ihre Beziehung zur Funktion der Ringdrüse. Chromosoma (Berl.) **13**, 341–384 (1962)

Bonner, M., Laskey, A.: A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Europ. J. Biochem. **46**, 83–88 (1974)

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

Clever, U., Storbeck, I., Romball, C.G.: Chromosome activity and cell function in polytenic cells. I. Protein synthesis at various stages of larval development. Exp. Cell Res. **55**, 306–316 (1969)

Cohen, M., Jr.: Evolution of 5 S ribosomal RNA genes in the chromosomes of the virilis group of Drosophila. Chromosoma (Berl.) **55**, 359–371 (1976)

Daneholt, B., Hosick, H.: Evidence for transport of 75 S RNA from a discrete chromosome region via nuclear sap to cytoplasm in Chironomus tentans. Proc. Nat. Acad. Sci. (Wash.) **70**, 442–446 (1973)

Grossbach, U.: Chromosomen-Aktivität und biochemische Zelldifferenzierung in den Speicheldrüsen von Camptochironomus. Chromosoma (Berl.) **28**, 136–187 (1969)

Kafatos, F.C.: Sequential cell polymorphism: a fundamental concept in developmental Biology. Advanc. Insect Physiol. **12**, 1–15 (1976)

Kastern, W.H., Krishnakumaran, A.: Reprogramming in the absence of DNA synthesis in Galleria larval epidermis. Cell Differentiation **4**, 45–53 (1975)

Korge, G.: Dosiskompensation und Dosiseffekt für RNS-Synthase in Chromosomen-Puffs von Drosophila melanoaster. Chromosoma (Berl.) **30**, 430–464 (1970)

Korge, G.: Chromosome puff activity and protein synthesis in larval salivary glands of Drosophila melanogaster. Proc. nat. Acad. Sci. (Wash.) **72**, 4550–4554 (1975)

Korge, G.: Larval saliva in Drosophila melanogaster: production, composition and relationship to chromosome puffs. Develop. Biol. **58**, 339–355 (1977a)

Korge, G.: Direct correlation between a chromosome puff and the synthesis of a larval saliva protein in Drosophila melanogaster. Chromosoma (Berl.) **62**, 155–174 (1977b)

Kress, H.: Das Puffmuster der Riesenchromosomen in den larvalen Speicheldrüsen von Drosophila virilis: seine Veränderungen in der Normalentwicklung und nach Injektion von Ecdyson. Sitz.-Ber.-Bayer. Akad. Wiss., Math. Naturw. Kl. **1972**, 129–149

Kress, H.: Specific repression of a puff in the salivary gland chromosomes of Drosophila virilis after injection of glucosamine. Chromosoma (Berl.) **40**, 379–386 (1973)

Kress, H.: Temporal relationships between leaving food, ecdysone release, mucoprotein extrusion and formation in Drosophila virilis. J. Insect Physiol. **20**, 1041–1055 (1974)

Kress, H.: Correlation between puffing activity and synthesis of secretion in the larval salivary glands of Drosophila virilis. Verh. Dtsch. Zool. Ges. 1974, 92–95, Stuttgart: G. Fischer, 1975

Kress, H.: Transcriptional control of developmental processes by ecdysone in Drosophila virilis salivary glands. Roux' Archives **182**, 107–116 (1977)

Laemmli, U.: Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature (Lond.) **227**, 680–685 (1970)

Laskey, R.A., Mills, A.D.: Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Europ. J. Biochem. **56**, 335–341 (1975)

Pelling, C.: Ribonucleinsäure-Synthese der Riesenchromosomen. Chromosoma (Berl.) **15**, 71–122 (1964)

Poels, C.L.M.: Time sequence in the expression of various developmental characters induced by ecdysterone in Drosophila hydei. Develop. Biol. **23**, 210–225 (1970)

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

Schmidt, J.: Die Haltung von larvalen Malphigischen Gefäßen der Fruchtfliege Drosophila hydei unter in vitro-Bedingungen. I. Die Entwicklung einer Kulturlösung. Diplom-Arbeit, University of Giessen 61975)

Selman, K., Kafatos, F.C.: Transdifferentiation in the labial gland of silk moths: is DNA required for cellular metamorphosis? Cell Differentiation **3**, 81–94 (1974)

Tissières, A., Mitchell, H.K., Tracy, U.M.: Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. J. molec. Biol. **84**, 389–398 (1974)

Vogt, M.: Zur Produktion und Bedeutung metamorphosefördernder Hormone während der Larvalentwicklung von Drosophila. Biol. Zbl. **63**, 395–446 (1943)

Wimber, D.E., Wimber, D.R.: Sites of the 5 S ribosomal genes in Drosophila. I. The multiple clusters in the virilis group. Genetics **86**, 133–148 (1977)

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

Received December 5–20, 1978 / Accepted December 21, 1978 by W. Beermann
Ready for press January 7, 1979