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Genetics

Parallel changes in puffing activity and patterns of protein synthesis in salivary glands of *Drosophila**

(polytene chromosomes/puffs/heat shock/gene activity/RNA synthesis)

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ABSTRACT **The changes in protein synthesis of salivary glands of *Drosophila* resulting from a brief exposure to 37° have been analyzed on sodium dodecyl sulfate–acrylamide gels. In *D. melanogaster* and *D. hydei* this treatment induces nine and six new puffs, respectively, in the polytene chromosomes. After 20 min treatment seven new proteins are synthesized by the glands of *D. melanogaster* and six by those of *D. hydei* as detected by [^{35}S]methionine labeling. Other agents, e.g., recovery from anaerobiosis, induce the same puffs and the same proteins. The extent of protein induction and the degree of puff induction are related to the severity of the temperature treatment. The new proteins are detected after 10 min treatment at 37° and their synthesis is inhibited by actinomycin D. Actinomycin D added 5 min after the start of temperature treatment has little effect on subsequent protein synthesis. The induced proteins are not tissue specific. Electrophoretic differences of two proteins exist between *D. melanogaster* and *D. simulans*, encouraging attempts to map the proteins' gene loci and to test directly whether or not the puffs code for them.**

The pattern of puffing activity in the polytene chromosomes of *Drosophila*, and other Diptera, changes in a complex way during development. During late larval and "prepupal" development, at least, these changes are under hormonal control (1, 2). Puffing activity can also be controlled by certain environmental treatments; in particular a brief exposure of salivary glands, either in the intact animal or in culture, to high temperature induces specific puffs (3–5).

We report the changes in salivary gland protein synthesis that result from temperature shock and the correlated changes in puffing activity. They will enable a formal proof of the hypothesis that puffs are active gene loci (6), a proof that demands the genetic mapping of a putative puff product to the puff itself and the demonstration that mutations which specifically result in the loss of the puffs also result in the absence of its putative product.

MATERIALS AND METHODS

Animals. *D. melanogaster:* Canton-S wild type; *D. hydei:* standard laboratory strain of Nijmegen laboratory. Other species were from the Cambridge stock collection.

Salivary Gland Incubation. Salivary glands were dissected from surface sterilized third instar larvae or prepupae and cultured in a modified Grace's insect tissue culture medium according to Ashburner (7) (*D. melanogaster*) or in Poels' (8) medium (*D. hydei*). Incubations were done in the wells of disposable plastic microtiter plates. Four pairs of salivary glands, freed of as much adhering fat body as possible were cultured in 50 μl of medium. For heat shock experiments the control temperature was 25° and the shock temperature, 37°. The duration of heat treatment was 20 min unless stated to the contrary. Dissection of larvae and setting up of the organ cultures was done under sterile conditions in a laminar flow hood.

Labeling. Following experimental treatment the glands were rapidly washed in medium lacking methionine and then labeled for 20 min in 8 μl of medium containing [^{35}S]methionine (Amersham: specific activity between 57 and 175 Ci/mmol) at a final concentration of 1.66 mCi/ml (*D. melanogaster*) or in 5 μl of medium containing [^{35}S]methionine at a final concentration of 3.0 mCi/ml (*D. hydei*). All labeling was done at 25°. Under these experimental conditions the incorporation of [^{35}S]methionine into trichloroacetic-acid-precipitable material by larval salivary glands of *D. melanogaster* is linear for at least 6 hr.

Preparation of Samples. At the end of the labeling period the radioactive medium was removed with a fine-tipped glass pipette and the glands were flooded with medium containing a 100-fold excess of nonradioactive methionine. If necessary they were then stored at −70° to await further processing. After the wash in nonradioactive medium the glands were washed in cold 10% trichloroacetic acid and then transferred into small glass test tubes. Then they were washed with 10% cold trichloroacetic acid (20 min), 95% ethanol (20 min), chloroform:methanol 1:1 (20 min) and dried at 37° (30 min). The dried glands were dissolved by methods described in the legends to Figs. 3 and 8 for *D. melanogaster* and *D. hydei*, respectively.

Gel System and Autoradiography. The samples were analyzed by vertical sodium dodecyl sulfate–acrylamide gel electrophoresis as described in the legends to Figs. 3 and 8 for *D. melanogaster* and *D. hydei*, respectively. The gels were dried onto Whatmann 3MM paper on a porous polythene drying board under vacuum and autoradiographs were made by exposing to Kodak Kodirex x-ray film for 24–48 hr. The films were developed according to Kodak's directions.

Control Experiments. The extraction procedure we used is essentially that of Tissières *et al.* (9). We have varied the extraction method (for example by using boiling 10% trichloroacetic acid or prolonged chloroform:methanol extraction at 60°) without affecting the results. We have also studied the possible effects of sodium dodecyl sulfate on protease activity by treatment of samples with either phenyl methyl sulfonyl fluoride (10) or L-1-tosylamido-2-phenylethylchlo-

* *In memory of Hans D. Berendes*

Abbreviation: MW, molecular weight.

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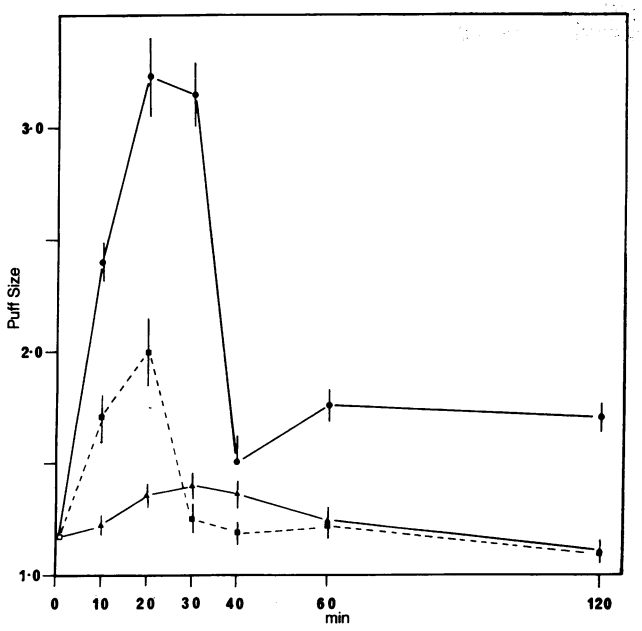

FIG. 1. Activity of the temperature-induced puff 3R:87B of *D. melanogaster* after transfer of third instar larvae to 29° (▲), 33° (■), or 37° (●). The ordinate is puff size expressed as the ratio of the diameter of the puff to that of an unpuffed reference band (3R:87D1.2); the abscissa is time after transfer of larvae to the higher temperature from 25°.

romethylketone (11) and by prolonged treatment of the samples with 1% sodium dodecyl sulfate (20 min at 90° or 2 hr at 25°), again without affecting the pattern of bands after electrophoresis. No effect on the pattern of protein synthesis by salivary glands was seen in the presence of chloramphenicol (1.3 mM); cycloheximide (0.71 mM) inhibited all but 3% or so of methionine incorporation.

RESULTS

Puffing. The induction of puffs in the salivary gland chromosomes of *Drosophila* larvae by a temperature shock was first described by Ritossa (5). A temperature shock, from 25° to 37° for example, induces new puffs at nine different sites in *D. melanogaster* (3) and at six sites in *D. hydei* (12, 13). A variety of other agents induce the same puffs, these

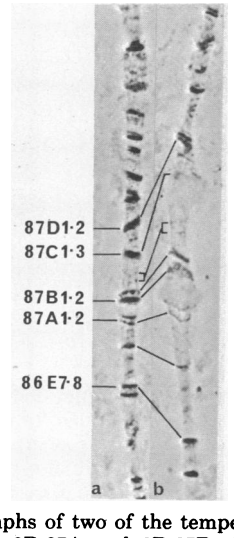

FIG. 2. Photographs of two of the temperature-induced puffs of *D. melanogaster*, 3R:87A and 3R:87B. (a) is from a control larva, at 25°, (b) is from a larva after treatment at 37° for 20 min.

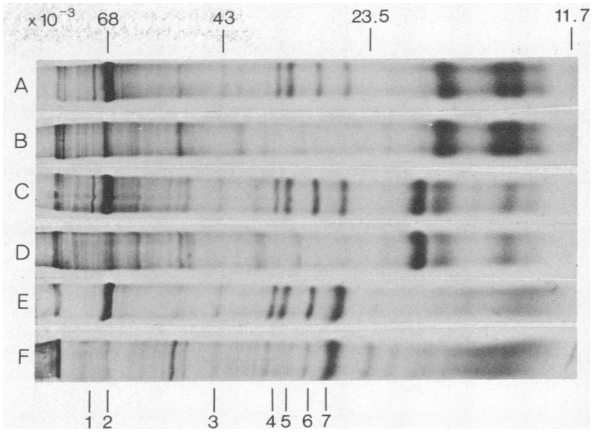

FIG. 3. Autoradiographs of sodium dodecyl sulfate–acrylamide gels of proteins labeled in salivary glands of third instar *D. melanogaster* larvae (E, F), 0 hr prepupae (C, D), and 6 hr prepupae (A, B). The glands were labeled with [^{35}S]methionine for 20 min after culture at 25° for 20 min (F, D, and B) or after culture for 20 min at 37° (E, C, and A). The seven temperature-induced proteins are numbered 1 to 7. Protein 7 is synthesized by the larval glands but not by prepupal glands at 25°, it is induced in prepupal glands by the temperature treatment. The fact that the pattern of protein synthesis in glands at 25° changes in a characteristic manner with development can be seen by comparison of gels F, D, and B. Molecular weight markers (bovine serum albumin, ovalbumin monomer, γ-globulin light chain, and cytochrome *c*) are indicated above the photograph. Salivary glands were cultured and labeled as described in the *Materials and Methods*. After lipid extraction the dried glands were dissolved in 50 μl of sample buffer (0.0625 M Tris·HCl, pH 6.8, 1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 10% (w/v) glycerol, and about 0.001% bromophenol blue) at 90° for 10 min. Aliquots (10 μl) were taken for counting in a Packard 3375 Spectrometer, in 4 ml Triton X-100, 2,5-diphenyloxazole–1,4-bis[2(5-phenyloxazolyl)]benzene (PPO-POPOP) cocktail. Samples were loaded on 12.5% acrylamide–sodium dodecyl sulfate gels (0.8% bismethylene acrylamide) with a 6% acrylamide stacking gel and run in the discontinuous buffer system of Laemmli (22), using the apparatus described by Studier (20). The gels were run at 25 mA until the bromophenol blue dye front had migrated 80 mm (about 4 hr).

include: recovery from prolonged anaerobiosis [e.g., 2 hr under nitrogen (13)], treatment of isolated glands with uncouplers of oxidative phosphorylation [e.g., 2,4-dinitrophenol (14, 15)], and treatment of glands with inhibitors of electron transport [e.g., rotenone (4)].

In parallel with an experiment to be described below in which glands were treated at 29°, 33°, or 37° for up to 6 hr we have reinvestigated the activity of the temperature-inducible puffs in *D. melanogaster* third instar larvae. Data for one of these puffs is shown in Fig. 1, and two of the puffs themselves are illustrated in Fig. 2. The major features of the puffing response to heat are: (*i*) puffs appear very quickly after transfer of animals from 25° to the higher temperature, reaching their maximum sizes 20–30 min afterwards and then regressing; (*ii*) the size of the induced puffs is a function of the severity of the temperature shock.

Protein Synthesis in Salivary Glands after Heat Shock. Tissières *et al.* (9) discovered that as a consequence of heat shock larval salivary glands incorporated radiolabeled precursors into new proteins. We confirm this important observation. In a standard experiment cultured salivary glands, or whole animals, were incubated at 37° for 20 min and the salivary glands were then labeled for 20 min with [^{35}S]methionine at 25°. Comparison of the proteins made by salivary glands after 37° treatment with control glands shows that the treatment results in the synthesis of seven new proteins

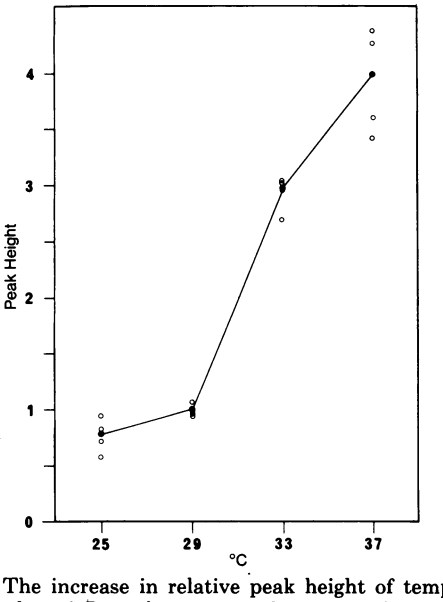

FIG. 4. The increase in relative peak height of temperature-induced band 2 of *D. melanogaster* after a 20 min temperature shock at 29°, 33°, and 37°. The height of band 2 was measured from densitometric traces of the autoradiographs and is expressed as a ratio to the height of a band whose activity is unaffected by temperature treatment. Each point (●) is the mean of four independent samples (○) of salivary glands run on the same gel.

in *D. melanogaster* and six in *D. hydei*. In prepupae, at least, none of these proteins are synthesized in significant amounts by salivary glands at 25°. In larvae of *D. melanogaster* (at 25°) glands synthesize a protein with mobility identical to that of the smallest of the induced proteins. In *D. hydei* the synthesis of one protein (molecular weight, MW, 43,000) ceases after temperature treatment.

For convenience we will number the induced bands of *D. melanogaster* 1 to 7, in order of decreasing molecular weight. Fig. 3 is a photograph of a typical autoradiograph showing the induced proteins in salivary glands from animals of different ages. The pattern of induced proteins is almost identical to that found by Tissières *et al.* (9) despite the differences in culture media. These authors report only six new proteins but it is clear from their Fig. 3 that their "38 mm" band has a pronounced shoulder. This band corresponds to our bands 4 and 5 which indeed often fail to resolve as two bands. Otherwise the only difference in the protein pattern is that Tissières *et al.* do not see our band 1 but find an additional band slightly smaller than our band 2 (which corresponds to their "9.5 mm" band).

Variations in Duration and Intensity of Heat Treatment. We have exposed both cultured salivary glands and whole larvae (of *D. melanogaster*) to 29°, 33°, and 37° for varying periods of time before labeling salivary gland proteins for 20 min at 25°. There is a clear relationship between the severity of the heat shock and the degree of induction of the protein bands. In Fig. 4 we show how the relative peak height of band 2 varies as a function of temperature. Even a heat shock of 4° results in some synthesis of this band.

At 29° bands 2 and 6 are the only bands to be induced. They can first be detected after 20 min heat treatment. At 33° all six bands (these experiments were with larvae, band 7 being active in the controls) are synthesized in the 20 min period following 10–20 min of heat treatment, but as the time of treatment continues they gradually cease synthesis, band 2 after 1 hr, bands 4 and 5 after 4 hr, while synthesis of band 6 is still detectable after 6 hr.

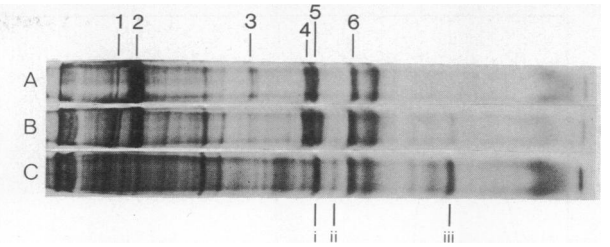

FIG. 5. Autoradiograph of sodium dodecyl sulfate–acrylamide gel showing the decline in heat-induced protein synthesis (bands 1 to 6) and the synthesis of new proteins (bands i to iii) during recovery from heat shock. Larval salivary glands of *D. melanogaster* were treated at 37° for 20 min and then allowed to remain at 25° for 10 min (A), 1 hr (B), or 4 hr (C) before labeling with [^{35}S]methionine for 20 min at 25°.

Intense induction of all bands is seen after a 10 min shock at 37°. The maximum rates of synthesis are found after 20 and 30 min heat treatment. The survival of glands, or larvae, is poor beyond 2 hr at 37° so that an accurate study of the duration of synthesis of the bands at this temperature is difficult. However band 2 is still being synthesized by glands after 6 hr exposure at 37°.

In glands of *D. hydei, in vivo* or *in vitro*, the heat-induced proteins appear in sequence at 37°: the 38,000 MW band is made first (at 20–30 min), followed by the 67,000 MW band (30–45 min), the 70,000 MW and 26,000 MW bands (60 min), and finally, after 90 min at 37°, the 25,000 and 20,000 MW bands.

Recovery Experiments. When glands (*D. melanogaster*), exposed to 37° for 20 min, are allowed to recover at 25° for varying periods of time before labeling the synthesis of the induced proteins ceases more rapidly than had the glands remained at high temperature. Band 2 is not made after 1–2 hr at 25° while other bands, especially band 4, may persist for a further hour or so. Recovery at 25° after a short heat shock leads to very rapid regression of the induced puffs.

An unexpected feature of the recovery experiments is that 1–2 hr after the end of high temperature treatment a completely new set of bands appears (Fig. 5). The precise pattern of these "recovery proteins" is rather variable but consistently (in each of seven experiments) we found one protein of very low molecular weight (17,500) and two which migrate between bands 5 and 6.

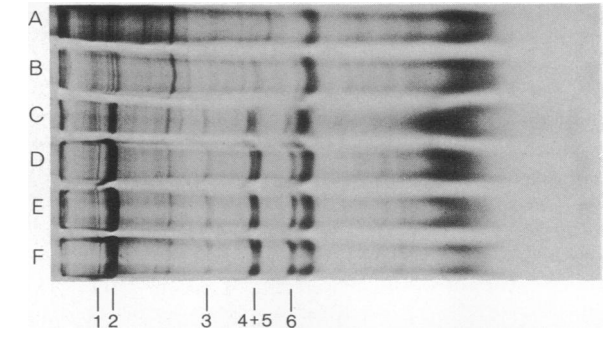

FIG. 6. Autoradiograph of a sodium dodecyl sulfate–acrylamide gel showing the effect, on subsequent protein synthesis by larval salivary glands of *D. melanogaster*, of adding actinomycin D at successive 5 min intervals during a 20 min treatment at 37°. The induced proteins are numbered from 1 to 6. In (A) are proteins made by control glands incubated, before labeling, at 25°, (B), (C), (D), and (E), actinomycin D added at 0, 5, 10, and 15 min, respectively. (F), control temperature shock without actinomycin D.

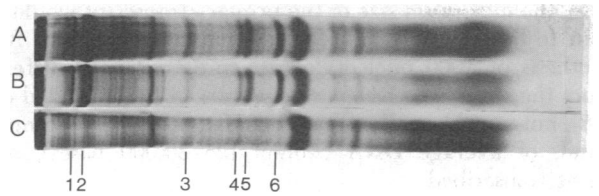

FIG. 7. Autoradiograph of a sodium dodecyl sulfate–acrylamide gel showing that the proteins (numbered 1 to 6) whose synthesis is induced in larval salivary glands of *D. melanogaster* after heat shock and after recovery from anaerobiosis are the same. Third instar larvae of *D. melanogaster* were either (A) kept under oxygen-free nitrogen for 2 hr and then allowed to recover in air for 1 hr, (B) kept at 37° for 20 min, or (C) kept in air at 25°, before their glands were dissected and labeled with [^{35}S]methionine for 20 min.

Turnover Experiments. Salivary glands of *D. melanogaster* were exposed to 37° for 20 min, labeled for 20 min at 25° and then either (*1*) processed immediately, (*2*) cultured for 2 hr at 25° in the presence of cycloheximide at a concentration (0.71 mM) which inhibits over 97% incorporation of methionine into protein, or (*3*) cultured for 2 hr in the presence of a 100-fold excess of nonlabeled methionine. The autoradiographs of the three series gave no indication of turnover, during the 2 hr period, of the induced proteins (results not shown).

Inhibitor Studies. Addition of either actinomycin D (final concentration 1.6 μM) or α-amanitin (28 μM) to cultured glands during the period of heat treatment completely suppresses synthesis of the induced proteins in the subsequent labeling period. Both antibiotics, especially α-amanitin, reduce the rate of protein synthesis, although the pattern of bands is very similar to that found in control, unshocked, salivary glands. They both inhibit puff induction (16, 15).

In order to find out whether the actinomycin-D-sensitive process(es) was required throughout the period of heat treatment the following experiment was done with *D. melanogaster*. Parallel series of glands were cultured at 37° for 20 min and then labeled with [^{35}S]methionine for 20 min. Actinomycin D (1.6 μM final concentration) was added to the samples after 0, 5, 10, and 15 min of heat treatment. In addition, one series had no inhibitor added. The result (Fig. 6) is clear. Addition of actinomycin D 5 min into the shock had little, and at 10 min into the shock period almost no, effect on the subsequent synthesis of the induced proteins. Identical results were also obtained in further experiments using a much higher inhibitor concentration (80 μM).

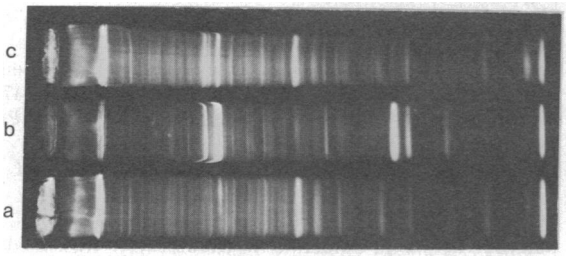

FIG. 8. Autoradiograph of a sodium dodecyl sulfate–acrylamide gel showing the [^{35}S]methionine-labeled proteins of *D. hydei* larval salivary glands. Larvae were kept (a) at 25° in air, (b) at 37° in air for 90 min, or (c) under nitrogen for 2 hr followed by 45 min in air, before their salivary glands were dissected and labeled. The glands were cultured as described in *Materials and Methods* and, after lipid extraction, the dried glands were dissolved by boiling for 1.5–2 min in 50 μl of buffer (0.1 M phosphate, pH 6.9, 1% sodium dodecyl sulfate, 4 M urea, and 1% 2-mercaptoethanol). Aliquots were loaded onto 10% acrylamide–sodium dodecyl sulfate gels according to MacGillivray *et al.* (21) and run at 16 mA.

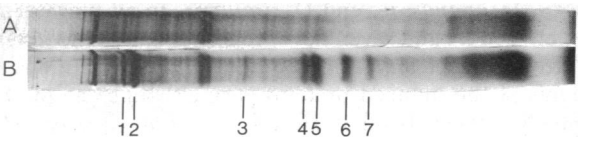

FIG. 9. Autoradiograph of a sodium dodecyl sulfate–acrylamide gel of proteins synthesized by ovaries of 1-day-old adult nonvirgin *D. melanogaster* females. The ovaries were labeled with [^{35}S]methionine for 20 min at 25° after *in vitro* incubation for 20 min at 25° (A) or 37° (B). The heat-induced proteins are numbered 1 to 7.

Other Treatments. Since identical puffs are induced by heat and by 2,4-dinitrophenol, rotenone, and during recovery from anaerobiosis, we have studied the effects of these treatments on subsequent synthesis. In *D. melanogaster* the proteins made following 2 hr nitrogen treatment and 1 hr recovery in air were identical to those seen after heat shock (Fig. 7). In *D. hydei* only the proteins of MW 70–72,000, 67,000, and 38,000 are made after 2 hr nitrogen followed by 45 min in air (Fig. 8). The synthesis of the 43,000 MW protein is not inhibited by this treatment (contrast with heat shock). Treatment of glands with 2,4-dinitrophenol (0.5 mM), rotenone (0.64 mM), antimycin A (0.5 mM), vitamin B_6 plus oligomycin (10 mM) leads to a severe inhibition of protein synthesis although, in *D. melanogaster* with dinitrophenol and rotenone at lower concentrations (0.1 mM and 0.13 mM, respectively, 20 min treatment at 25° before labeling), synthesis of band protein 2 can be detected despite the fall-off in overall protein synthesis (results not shown).

Tissue and Species Comparisons. Identical pattern of induced protein synthesis (although the patterns of proteins made at 25° differ) are found in larval midgut, larval brain, larval imaginal discs, adult ovaries, and adult testes (Fig. 9).

We have compared the heat-induced proteins in different species of *Drosophila* including *D. virilis*, *D. funebris*, *D.*

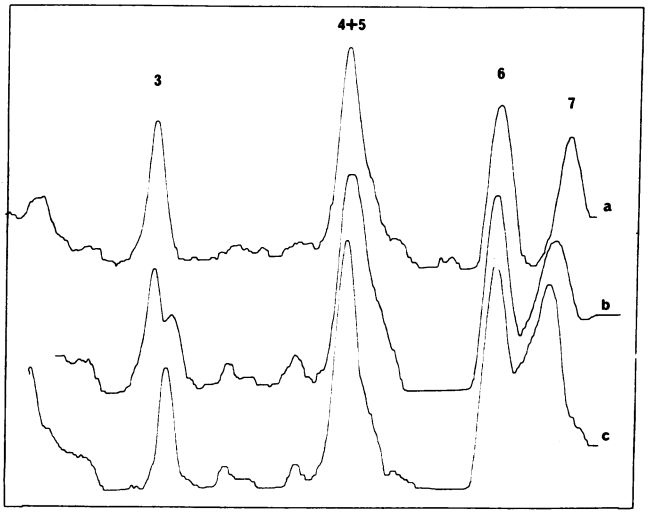

FIG. 10. Densitometric scan (traced at a 1:9 linear expansion) of autoradiographs of [^{35}S]methionine-labeled proteins from larval salivary glands, heat shocked at 37° for 20 min before labeling, of (a) *D. simulans* (strain C.137.32), (c) *D. melanogaster* (Canton-S) and (b) their F1 hybrid (*D. melanogaster* ♀ × *D. simulans* ♂). Only the region of the autoradiograph showing bands 3 to 7 is included. The electrophoretic mobilities of induced bands 1, 2, 4, 5, and 6 are identical in the two species. Bands 3 and 7 differ and the F1 hybrid expresses both parental bands.

buscki, D. ananassae, and *D. simulans.* In all species there are between six and seven new proteins made as the result of 37° treatment of larvae for 20 min. The precise molecular weights of the induced proteins vary although the most intensively labeled band is always one of molecular weight about 70,000 (results not shown).

The pattern in *D. simulans* is of particular interest since it is the closest known relative of *D. melanogaster,* with which it will form (sterile) hybrids. The patterns of induced proteins in these two species are identical except that two bands (3 and 7) differ in their mobilities on sodium dodecyl sulfate gels. These differences are species specific—10 unrelated strains of each species having been tested. We presume these differences in migration to reflect differences in molecular weight of the polypeptides.

In the hybrid between these species we find two bands 3, each band corresponding to a band 3 of the parental species. For band 7 the situation is not as clear. In both the species and hybrid we can only resolve one band 7—it is intermediate in mobility between the parental bands and conspicuously broader than either parental band (Fig. 10). Since band 7 is normally a broader band than band 3 and since the mobility difference between band 7 of *D. melanogaster* and *D. simulans* is slight we interpret this failure to find two bands in either the species mixture or the species hybrid as a failure of resolution.

DISCUSSION

Environmental agents which cause the very rapid induction of specific puffs in salivary gland polytene chromosomes also result in an almost equally rapid change in the nature of the proteins being synthesized by the glands. There is a striking numerical correspondence between the number of puffs induced and the number of new proteins synthesized. In addition to the mere numerical coincidence there is a suggestive similarity between the size of induced puffs and the rate and duration of synthesis of the induced proteins.

The molecular signals which control the induction of the induced puffs are not known, although proteins released from the mitochondria have been suggested (17). We presume that the response of the genome is homeostatic. As Tissières *et al.* (9) point out, the temperatures used are within the range that *Drosophila* might normally be expected to meet, whilst temporary anaerobiosis may be a very common situation in the semifluid conditions of *Drosophila* cultures. The rapidity of the response, both at the puff and protein level, should therefore be viewed in this light; a homeostatic response which took hours to be activated would be of little help to an animal in coping with temporary environmental conditions which, if prolonged, would be lethal.

The experiment in which actinomycin D was added at successive 5 min intervals of a 37° shock suggests that some event is occurring within the first 5 min of high-temperature treatment which is sufficient for induced protein synthesis in the period 15–35 min after the shock ends. The fact that labeled RNA from polysomes of heat-shocked *D. melanogaster* tissue culture cells hybridizes *in situ* to the 87A and 87B regions (18) argues strongly that this event is RNA synthesis. If so, the *maximum* size of the primary transcript would appear to be only 5000 nucleotides (assuming a rate of RNA synthesis of 1000 nucleotides per min), not very different from the size of the RNA in polysomes of heat-shocked cells (18) but much smaller than that expected if a complete band, of average DNA content (25–30,000 bases), were being transcribed.

The correlations between puffs and specific protein synthesis found by Tissières *et al.* (9) and by us encourages the use of genetic techniques to map the loci coding for the proteins and to see whether or not these loci correspond to induced puff sites. The fact that electrophoretic variants for two of the proteins have already been found, albeit between related species, means that such an analysis is possible. Genetic mapping of allelic differences between *D. melanogaster* and *D. simulans,* whose hybrids are sterile, must be unconventional. It should be possible to map the chromosome arm involved by using available compound autosome stocks of *D. melanogaster* and to map within arms by making species hybrids in which the *melanogaster* chromosome complement is deficient for specific regions.

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1. Ashburner, M., Chihara, C., Meltzer, P. & Richards, G. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **38,** 655–662.
2. Clever, U. & Karlson, P. (1960) *Exp. Cell Res.* **20,** 623–626.
3. Ashburner, M. (1970) *Chromosoma* **31,** 356–376.
4. Leenders, H. J. & Berendes, H. D. (1972) *Chromosoma* **37,** 433–444.
5. Ritossa, F. (1962) *Experientia* **18,** 571–573.
6. Beermann, W. (1956) *Cold Spring Harbor Symp. Quant. Biol.* **21,** 217–232.
7. Ashburner, M. (1972) *Chromosoma* **38,** 255–281.
8. Poels, C. L. M. (1972) *Cell Differ.* **1,** 63–78.
9. Tissières, A., Mitchell, H. K. & Tracy, U. (1974) *J. Mol. Biol.* **84,** 389–398.
10. Farney, D. E. & Gold, A. M. (1963) *J. Am. Chem. Soc.* **85,** 997–1000.
11. Schoellman, G. & Shaw, E. (1963) *Biochemistry* **2,** 252–255.
12. Berendes, H. D., Breugel, F. M. A. van & Holt, T. K. H. (1965) *Chromosoma* **16,** 35–46.
13. Breugel, F. M. A., van (1966) *Genetica* **37,** 17–28.
14. Ellgaard, E. G. (1972) *Chromosoma* **37,** 417–422.
15. Ellgaard, E. G. & Clever, U. (1971) *Chromosoma* **36,** 60–78.
16. Berendes, H. D. (1968) *Chromosoma* **24,** 418–437.
17. Leenders, H. J., Berendes, H. D., Helmsing, P. J., Derksen, J. & Koninkx, J. F. J-G. (1974) *Sub-Cell. Biochem.* **3,** 119–147.
18. McKenzie, S. L., Henikoff, S. & Meselson, M. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 1117–1121.
19. Leenders, H. J. & Beckers, P. J. A. (1972) *J. Cell Biol.* **55,** 257–265.
20. Studier, F. W. (1973) *J. Mol. Biol.* **79,** 237–248.
21. MacGillivray, A. J., Cameron, A., Krauze, R. J., Rickwood, H. and Paul, J. (1972) *Biochim. Biophys. Acta* **277,** 384–402.
22. Laemmli, U. K. (1970) *Nature* **227,** 680–685.