

BRIEF NOTES

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INDUCED ACCUMULATION OF NONHISTONE PROTEINS IN POLYTENE NUCLEI OF *DROSOPHILA HYDEI*

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INTRODUCTION

In Diptera, the polytene chromosome puffs, regarded to be morphological manifestations of active genes (1, 2, 9), contain an increased quantity of protein if compared with their inactive state. By means of experimental induction of puff formation of certain groups of loci in *Drosophila hydei*, it was demonstrated that a local change in protein-dye binding capacity is one of the first events in puff formation (3). This change in dye binding capacity due to a local increase in protein concentration (10) occurs in the virtual absence of *de novo* protein synthesis (7). Radioautographic analyses of chromosomal amino acid incorporation during induced puff formation failed to provide evidence for enhanced incorporation in puff-forming loci (10). Because the same puff in active and inactive state displays an identical histone content (8), the local increase in protein concentration should result from an accumulation of preexisting nonhistone protein(s). In an attempt to characterize the changes occurring at the genome level upon experimental gene activation, in the first instance nonhistone protein patterns of salivary gland nuclei in which specific puffs were induced *in vivo* or *in vitro* by a temperature treatment or a hormone (ecdysone) treatment were compared with those of nuclei of untreated glands.

MATERIALS AND METHODS

From a batch of synchronized mid-third instar larvae of *D. hydei* (50 g = 10,000 larvae) reared at

25°C, 25 g was submitted to a 30 min temperature treatment at 37°C. Immediately after this treatment the salivary gland nuclei were isolated (4). The same procedure was applied to the remaining 25 g of untreated larvae. The nuclei of these two preparations differ with regard to the chromosomal puffing pattern in at least five loci at which distinct puffs have formed in nuclei of temperature-treated larvae (5). For comparison, the same loci have been activated by a 45 min CO₂ treatment (anaerobiosis) followed by a period of recovery (6).

For induction of hormone-specific puffs, isolated salivary glands of mid-third instar larvae were incubated with 1 mg/ml β -ecdysone in the presence of cyclic-adenosine monophosphate in the medium (13). As a control, nuclei of salivary glands incubated in a medium deprived of the steroid were used. For comparison, the nonhistone protein pattern of salivary gland nuclei of very late third instar larvae, displaying naturally induced ecdysone-specific puffs, was determined.

Nonhistone proteins were extracted according to a modified phenol-sodium dodecyl sulfate (SDS) extraction procedure described by Konings et al. (11). The nuclear pellet was suspended in 1 ml Ringer's solution containing 0.16 mM ethylenediaminetetraacetic acid and 1% SDS. This suspension was mixed with 3 vol freshly distilled, 0.25 M Tris-HCl (pH 7.8) saturated phenol, shaken for 10 min and centrifuged for 10 min at 16,000 g. This procedure was repeated with the resulting interphase and water-phase. The phenol phases of the two runs were subsequently mixed with 2 vol ice-cold acetone containing 0.1 M acetic acid. The precipitate that formed after 2 hr at 0°C was pelleted, thoroughly washed with ethanol/ether (1:1) and ether, dried, and dissolved in 200 μ l of a 0.01 M sodium phosphate buffer (pH

7.6) containing 0.1% SDS, 0.14 M β -mercaptoethanol and 4 M urea. After storing for 2 hr at 37°C, the preparation was heated to 100°C for 1.5 min (12) and applied on top of a 15% polyacrylamide gel. Electrophoresis was performed according to Weber and Osborn (17), applying 5 mA per gel during 3 hr at room temperature.

For protein labeling, an L-amino acid mixture NET-250 (NEN) was used. After staining, the gels were frozen sectioned and each 1 mm slice was oxidized in a Packard tritium oxidizer. Liquid scintillation analysis was performed in dimethyl-POPOP-PPO-toluene mixed with methyl Cellosolve (7:3 v/v) in a Philips scintillation analyzer. Counting efficiency was 25%.

RESULTS AND DISCUSSION

The phenol-SDS soluble nuclear proteins of polytene nuclei separate into 15–18 principal bands. A comparison of the electrophoretic pattern of these proteins extracted from nuclei displaying experimentally induced “temperature” puffs, resulting from either a temperature treatment (Fig. 1 *b*) or anaerobiosis (Fig. 1 *c*), reveals the presence of a distinct protein band at a position in the gels at which no obvious band could be detected in the controls (Fig. 1 *a*). This difference was consistently obtained in a number of replicate experiments.

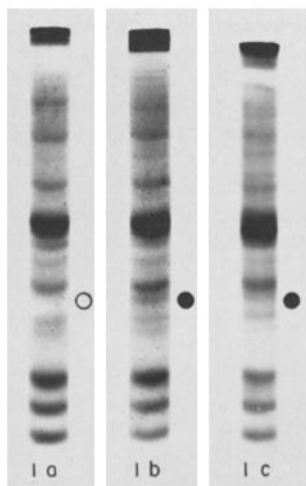


FIGURE 1 Electrophoretic separation of phenol-SDS-soluble protein of polytene salivary gland nuclei of *D. hydei*. (a) nuclei from mid-third instar glands; (b) as *a* after temperature treatment in vivo; (c) as *a* after CO₂ treatment. Points indicate location of new species of protein with approximate mol wt of 23,000 in nuclei with temperature puffs.

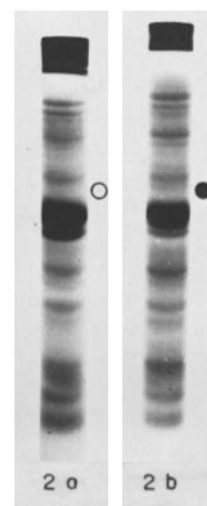


FIGURE 2 Separation of phenol-SDS-soluble protein of polytene salivary gland nuclei of *D. hydei*. (a) nuclei from mid-third instar glands; (b) nuclei from mid-third instar glands incubated in vitro with 1 mg/ml β -ecdysone (45 min).

In order to determine whether the particular protein band is merely a result of any treatment affecting the pattern of genome activity, or is specifically related to the induction of temperature puffs, the electrophoretic pattern of phenol-SDS-soluble proteins of nuclei carrying ecdysone-specific puffs was established.

As compared with nuclei in which these puffs are absent, the protein pattern of nuclei displaying ecdysone puffs, irrespective of their natural or experimental origin, reveals one specific protein band which is absent in the controls (Fig. 2, *a*, *b*). Also, this band was consistently present in replicate experiments. The location of this protein band is definitely different from that found in extracts of nuclei of temperature-treated larvae.

The molecular weights of the new protein species found in nuclei with temperature puffs and in nuclei with ecdysone puffs were estimated by comparing their migration distances with those of cytochrome *c* (12,500), bovine pancreas ribonuclease A (13,900), bovine pancreas trypsin (24,000), pepsin (34,500), and human hemoglobin (64,450) (14). From this comparison it appeared that the new protein occurring in nuclei of temperature-treated animals has a molecular weight of approximately 23,000, whereas the specific protein occurring in nuclei displaying ecdysone

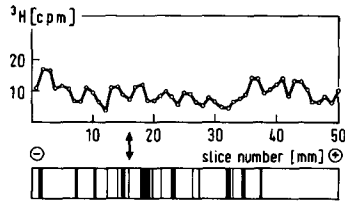


FIGURE 3 Distribution of radioactivity in gel after separation of phenol-SDS-soluble proteins extracted from mid-third instar salivary glands of *D. hydei* after 45 min incubation with 1 mg/ml β -ecdysone and 20 μ Ci of a mixture of tritiated L-amino acids. The arrow indicates the ecdysone band.

puffs has an approximate molecular weight of 42,000.

In order to establish whether or not the specific protein present in nuclei with an ecdysone-induced puffing pattern is synthesized in response to the hormone treatment, 20 μ Ci of a mixture of tritiated L-amino acids was supplied to the medium at the onset of the incubation with the hormone. The distribution of radioactivity in the gels displaying the characteristic protein band revealed no indication for a specifically labeled protein fraction (Fig. 3).

The data presented indicate that concurrent with the activation of a particular set of genes, a specific preexisting nonhistone protein accumulates in the nucleus. The *in vitro* experiments with β -ecdysone further indicate that the protein accumulating in the nucleus concurrent with the formation of ecdysone-specific puffs originates from the cytoplasm rather than from the hemolymph. The question whether or not this protein is identical with that accumulating at the genome sites which respond with puff formation requires further study.

Recently, it was reported that the steroids, hydrocortisone, (15) and estradiol (16), are effective in the induction of a specific change in the pattern of acidic chromosomal proteins in their respective target organs. However, in contrast to β -ecdysone which induces accumulation of a specific nonhistone protein in the salivary gland nuclei of *Drosophila* within 45 min, hydrocortisone and estradiol stimulate *de novo* synthesis of an acidic chromosomal protein within some hours after their application.

SUMMARY

Different treatments effective in the induction of specific changes in the chromosomal puffing pat-

tern of *Drosophila hydei* polytene chromosomes stimulate the accumulation of a treatment-specific phenol-soluble protein in the nucleus. In response to a temperature treatment a protein of 23,000 mol wt, and upon an ecdysone treatment a protein of 42,000 mol wt, migrate into the nucleus.

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REFERENCES

1. BEERMANN, W. 1952. Chromomerenkonstanz und spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von *Chironomus tentans*. *Chromosoma*, 5:139.
2. BERENDES, H. D. 1965. Salivary gland function and chromosomal puffing patterns in *Drosophila hydei*. *Chromosoma*, 17:35.
3. BERENDES, H. D. 1968. Factors involved in the expression of gene activity in polytene chromosomes. *Chromosoma*, 24:418.
4. BERENDES, H. D., and J. B. BOYD. 1969. Structural and functional properties of polytene nuclei isolated from salivary glands of *Drosophila hydei*. *J. Cell Biol.* 41:591.
5. BERENDES, H. D., F. M. A. VAN BREUGEL, and T. K. H. HOLT. 1965. Experimental puffs in *Drosophila hydei* salivary gland chromosomes. *Chromosoma*, 16:35.
6. BREUGEL, F. M. A. VAN., 1966. Puff induction in larval salivary gland chromosomes of *Drosophila hydei* Sturtevant. *Genetica*, 37:17.
7. CLEVER, U., and C. G. ROMBALL. 1966. RNA and protein synthesis in the cellular response to a hormone, ecdysone. *Proc. Nat. Acad. Sci. U. S. A.* 56:1470.
8. GOROVSKY, M. A., and J. WOODARD. 1967. Histone content of chromosomal loci active and inactive in RNA synthesis. *J. Cell Biol.* 33:723.
9. GROSSBACH, U. 1969. Chromosomen-Aktivität und biochemische Zelldifferenzierung in den Speicheldrüsen von *Camptochironomus*. *Chromosoma*, 28:136.
10. HOLT, T. K. H. 1971. Local protein accumulation during gene activation. II. Interferometric measurements on the amounts of solid material in temperature induced puffs of *Drosophila hydei*. *Chromosoma*, 32:428.

11. KONINGS, R. N. H., R. WARD, B. FRANCKE, and P. H. HOFSCHEIDER. 1970. Gene order of RNA bacteriophage M12. *Nature (London)*. **226**:604.
12. LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)*. **227**:680.
13. LEENDERS, H. J., G. J. WULLEMS, and H. D. BERENDES. 1970. Competitive interaction of adenosine 3',5'-monophosphate on gene activation by ecdysterone. *Exp. Cell Res.* **63**:159.
14. SHAPIRO, A. L., E. VINUELA, and J. V. MAIZEL. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. *Biochem. Biophys. Res. Commun.* **28**:815.
15. SHELTON, K. R., and V. G. ALLFREY. 1970. Selective synthesis of a nuclear acidic protein in liver cells stimulated by cortisol. *Nature (London)*. **228**:132.
16. TENG, C. S., and T. H. HAMILTON. 1970. Regulation by estrogen of organ-specific synthesis of a nuclear acidic protein. *Biochem. Biophys. Res. Commun.* **40**:1231.
17. WEBER, K., and M. OSBORN. 1969. The reliability of molecular weight determinations by dodecylsulphate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**:4406.