DIFFERENTIAL INCORPORATION OF 5-BROMODEOXYURIDINE INTO DNA PUFFS OF LARVAL SALIVARY GLAND CHROMOSOMES IN RHYNCHOSCIARA[1]

William S. Bradshaw[2] and John Papaconstantinou
Biology Division, Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

Received September 9, 1970

SUMMARY

An autoradiographic analysis of the incorporation of 5-bromo-2'-deoxyuridine (BUdR) into salivary gland chromosomes of Rhynchosciara larvae has demonstrated differential synthesis of DNA in a specific set of chromosomal puffs. DNA isolated from larval salivary glands at the specific stage in development when these DNA puffs occur has also been subjected to isopycnic centrifugation in CsCl. The profiles of these gradients are heterodisperse and exhibit regions of very high density shifted away from the bulk DNA. The technique makes possible the isolation and purification of sequences unique to a specific set of chromosomal loci.

INTRODUCTION

The polytene chromosomes of the salivary glands in late fourth instar larvae of the sciarid fly Rhynchosciara are characterized by a regular pattern of band-specific puffs (1, 2). Autoradiography utilizing tritiated thymidine (3) and microspectrophotometric studies (4, 5) have implicated a specific set of these puffs as sites of differential synthesis of DNA. It is presumed that this results in a gene amplification of the puff loci (6), and may constitute a unique developmental mechanism for regulating the expression of genetic information. In this paper we report additional evidence for differential DNA synthesis including the resolution by means of buoyant density centrifugation of density labeled sequences from these DNA puffs.

---

[1]Research sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

[2]AEC Postdoctoral Fellow under appointment by Oak Ridge Associated Universities. Present Address: Department of Zoology and Entomology, Brigham Young University, Provo, Utah.

METHODS

Individual larvae from synchronous cultures of Rhynchosciara hollaenderi maintained in this laboratory were injected with 2 µl of either methyl-[^{3}H]thymidine (1 µCi; 11.2 Ci/mmole) or 5-bromo-2'-deoxyuridine-6-[^{3}H] (0.5 µCi; 11 Ci/mmole). For autoradiography, sections of salivary glands were fixed in ethanol:acetic acid (3:1) and squashed under a cover slip in 45% acetic acid. After freezing in liquid nitrogen, the cover slips were removed from the slides and air-dried. Slides were then coated with Kodak NTB emulsion, dried, and after storage in light-tight boxes at 4°C, developed in Kodak D-19.

Partially purified DNA preparations from larval tissues which had been dissected in insect Ringers solution (7) and frozen on dry ice were obtained as follows: Tissues from 50-200 larvae were homogenized in a conical, ground glass homogenizer in 1-2 ml of 0.14 M phosphate buffer, pH 7.0, 0.01 M EDTA 1% sodium lauryl sulfate, 1 M sodium perchlorate. Homogenates were then extracted in an equal volume of chloroform:octanol (10:1), and centrifuged for 30 min at 10,000 rpm. Two volumes of cold ethanol were then added, and the resulting precipitate was dissolved in 0.15 M NaCl, 0.015 M sodium citrate (1 x SSC).

These DNA samples were added to 6-7 ml CsCl gradients in 1 x SSC which were centrifuged in a Spinco No. 40 rotor at 35,000 rpm for 48-72 hr at 25°C. Following centrifugation, fractions of 0.1 ml were collected and diluted with 0.4 ml 1 x SSC for optical density measurements. Radioactivity in DNA was monitored by applying aliquots of these fractions to Whatman 3-mm filter paper disks which were processed according to Bollum (8) and counted in toluene-fluors at a counting efficiency for tritium of about 35%. Buoyant densities of sample fractions from each gradient were determined by measurements of their refractive index.

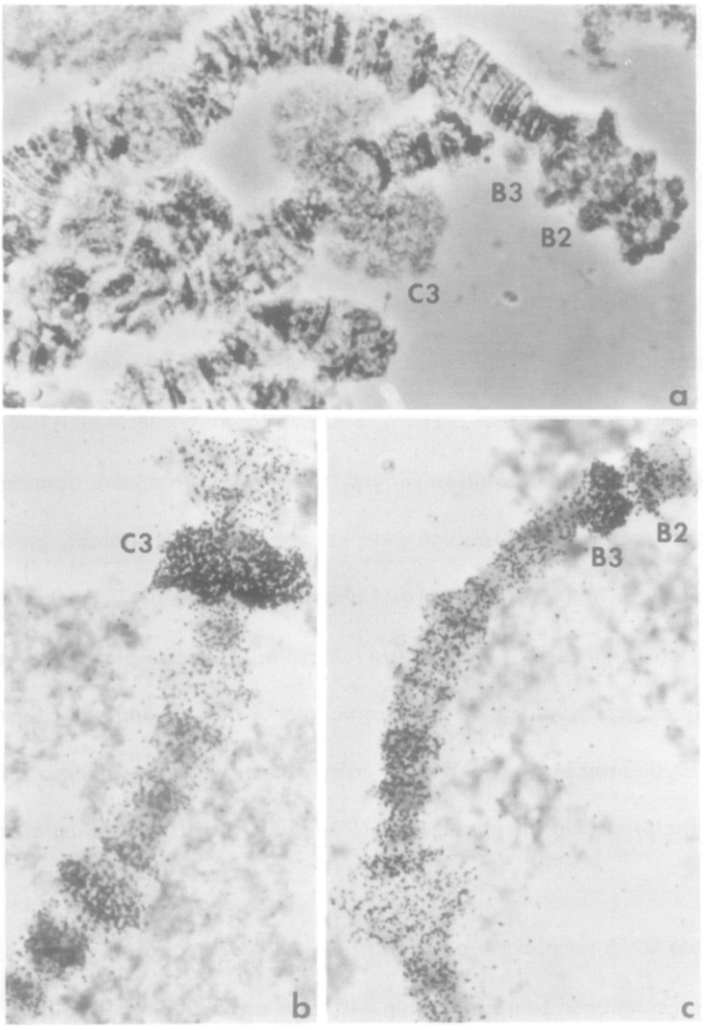

Fig. 1. (a) Phase-contrast micrograph of a squash of salivary glands from a 54-day-old larva of Rhynchosciara hollaenderi. X 640. (b) Autoradiograph of chromosome C from a larva at the same stage of development as in a. Exposure 7 days. X 640. (c) Chromosome B. As in b.

RESULTS

Somatic cell nuclei of Rhynchosciara contain four chromosomes. Figure 1a shows a light micrograph of the distal ends of two of these, the B and C chromosomes, from salivary glands of 54-day larvae. Day 54 designates a very late stage in the development of the fourth larval instar, immediately preceding the prepupal molt. This stage also marks the end of a sequence of DNA puffs which occur in the salivary gland chromosomes. The C-3 puff is at its maximum size. Puffing at the B-2 and B-3 loci precedes that at C-3 (B-2 begins to enlarge at day 52), and these two puffs have receded a great deal at this time. Autoradiographs showing a 3-hr incorporation of BUdR into the C and B chromosomes at this same stage are shown in Figures 1b and 1c, respectively. While it is clear that DNA synthesis is occurring at sites along the entire length of both chromosomes, there is a markedly greater grain density at the DNA puff sites (B-2, B-3, and C-3). A similar indication of differential synthesis at these three sites has been obtained in a large number of autoradiographs made throughout the 2-day period during which the entire sequence of puffing occurs.

This conclusion is further supported by the results of isopycnic centrifugation in CsCl of partially purified DNA preparations from larval tissues, four of which are shown in Figure 2. The positions of the DNA peaks along the abscissa have been drawn on a density scale which is the same for each of the four profiles. The assignment of absolute buoyant densities for each of the insect DNA preparations shown here was made by reference to the density of E. coli DNA centrifuged in the same tube. The bacterial marker DNA is shown for only one in Figure 2a. This figure shows that [^{3}H]thymidine-labeled salivary gland DNA from 53-day-old larvae bands as a single, homogeneous peak with a density of 1.694. Since autoradiography using thymidine as a DNA precursor also shows

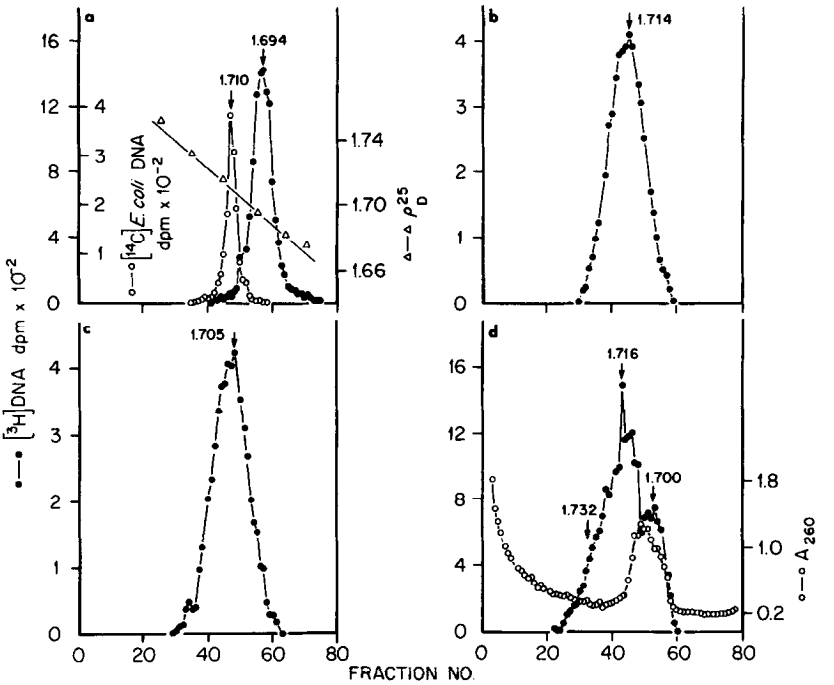

Fig. 2. Buoyant density centrifugation of DNA from larval tissues of Rhynchosciara hollaenderi. (a) Salivary gland DNA from 53-day larvae, labeled with [^{3}H]thymidine. (b) Salivary gland DNA from 43-day larvae, labeled with [^{3}H]BUdR. (c) Fat body DNA from 53-day larvae, labeled with [^{3}H]BUdR. (d) Salivary gland DNA from 53-day larvae, labeled with [^{3}H] BUdR.

differential incorporation into the DNA puffs (3), this result indicates that the average base composition of the DNA sequences at the puff loci is not different from that of total Rhynchosciara DNA.

A similar profile is obtained with BUdR-labeled salivary gland DNA from 43-day-old larvae, a pre-puff stage of development, shown in Figure 2b. The mean density of this peak, however, shows an increase to 1.714 g/cm^3. Based on the data of Erikson and Szybalski (9) this represents a 15% replacement of thymine by BUdR in this DNA. Figure 2c shows that a single, homogeneous DNA

peak also results from the fat body of larvae injected with BUdR at day 53. Fat body chromosomes in Rhynchosciara are not polytene. The relatively low specific activity DNA from this tissue shows a smaller shift in density to 1.705 g/cm^3. These two experiments indicate that a uniform, proportionate synthesis of DNA occurs in the absence of DNA puffing.

The results obtained with BUdR-labeled salivary gland DNA from 53-day-old larvae are shown in Figure 2d. A very heterodisperse profile of radioactivity is evident, which is remarkably reproducible from preparation to preparation. The bulk of the DNA, as shown by the position of the optical density peak, has a density of 1.700. In addition, radioactive DNA which is not coincident with the bulk DNA, whose density has been dramatically shifted to the heavy side of the gradient, is also present. A shift to a density of 1.732 for this very high specific activity DNA represents a 40% replacement of thymine by BUdR.

DISCUSSION

We believe that the heavy DNA resolved by buoyant density centrifugation after BUdR labeling, as shown in Figure 2d, represents the sequence synthesized at the sites of chromosomal puffing shown in Figure 1. It is resolved only by density labeling, is specific to polytene chromosomes of the salivary glands, and is present only in that tissue at the stage in larval development when specific puffs occur. The purification of DNA sequences unique to the DNA puffs make it possible to analyze further at the molecular level differential DNA synthesis and its significance in the regulation of development in this system.

Acknowledgments. — We thank Mr. Curtis Parker and Mrs. Shirley P. Ogle for excellent technical assistance.

REFERENCES

1. Breuer, M. E., and Pavan, C., Chromosoma 7, 371 (1955).
2. Mattingly, E., and Parker, C., Chromosoma 23, 255 (1968).
3. Ficq, A., and Pavan, C., Nature 180, 983 (1957).
4. Rudkin, G. T., and Corlette, S. L., Proc. Nat. Acad. Sci. U. S. A. 43, 964 (1957).
5. Swift, H., in The Molecular Control of Cellular Activity, J. M. Allen, ed. McGraw-Hill, New York, 1962, p. 73.
6. Pavan, C., Genetics 61 (Suppl.), 121 (1969).
7. Ephrussi, B., and Beadle, G. W., Amer. Natur. 70, 218 (1936).
8. Bollum, F. J., in Procedures In Nucleic Acid Research, G. L. Cantoni and D. R. Davis, eds. Harper and Row, New York, 1966, p. 296.
9. Erickson, R. L., and Szybalski, W., Radiat. Res. 20, 252 (1963).