Indian Biologist, Vol. VIII. No. 1&2, 1976.

Received on 31st March, 1976

BINDING OF ^{3}H-ACTINOMYCIN D WITH POLYTENE CHROMOSOMES OF *DROSOPHILA MELANOGASTER*[1]

S. C. Lakhotia

Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad 380 009.

INTRODUCTION

Actinomycin D (AMD) is frequently used to elucidate the structure and function of the nucleus. The selective action of AMD on the DNA-dependent RNA polymerase (Reich and Goldberg, 1964) has been utilised in numerous studies to examine various aspects of nuclear transcription ; in addition to being used as a metabolic inhibitor, tritium-labelled Actinomycin-D (^{3}H-AMD) has also been used as a probe for locating small amounts of DNA (Brachet and Ficq, 1965) as well as to assess the transcriptive activity of DNA (see Sieger *et al.*, 1971). A few studies on the ^{3}H-AMD binding capacity of eu- and heterochromatin have suggested that the transcriptionally inactive condensed chromatin binds less or no ^{3}H-AMD as compared to the active euchromatin (Brachet and Hulin, 1969 ; Ringertz and Bolund 1969 ; Berlowitz *et al.*, 1969). On the other hand, Simard (1967), Desai and Tencer (1968) and Sieger *et. al.* (1971) have reported equal or more binding of ^{3}H-AMD to the condensed chromatin compared to the disperse euchromatin. In the present studies, ^{3}H-AMD binding in different regions of polytene nuclei of *Drosophila melanogaster* has been examined by EM autoradiography. This system presents special interest since as shown earlier (Lakhotia and Jacob, 1974b), the constitutive heterochromatin in the chromocentre region of these nuclei includes both transcriptionally inactive (the alpha-heterochromatin) and active (the beta-heterochromatin) regions. As such, an analysis of the relative binding of ^{3}H-AMD in these two types of constitutive heterochromatin and the euchromatin regions would be useful.

MATERIAL AND METHODS

Salivary glands from the late third instar larvae of *Drosophila melanogaster* were used for these studies.

1 Paper presented at the All India Symposium on the Effects of Physical and Chemical Agents on Chromosomes (Department of Botany, Calcutta University, 16th to 18th November, 1975).

Excised salivary glands were incubated in dark in *Drosophila* ringer solution containing ^{3}H-AMD (50 μCi/ml ; specific activity : 2.97 Ci/mM) for 20 min at room temperature and the glands were immediately fixed either in acetomethanol (1 : 3) for 30 min or 2.5% glutaraldehyde following the technique described earlier (Lakhotia and Jacob, 1974a). In either case the glands were post-fixed in 1% osmium-tetroxide and the ultrathin sections of glands processed for EM autoradiography by the routine technique (Lakhotia and Jacob, 1974a). The preparations, coated with Ilford L4 emulsion, were exposed in dark at 4°C for 16-22 days and were developed in Kodak D19b for 3 min at 17°C. The preparations were examined in an AEI EM6 electron microscope at the Institute of Animal Genetics, Edinburgh. The grain count data, presented here, were taken from EM autoradiographs exposed for 22 days.

OBSERVATIONS

A. Effect of the fixative on ^{3}H-AMD binding :

It has been noted that after fixation in acetomethanol, the labelling in the autoradiographs of ^{3}H-AMD incubated glands is very poor and generally non-specific. In contrast, the glands incubated in ^{3}H-AMD and fixed in glutaraldehyde and processed further in the same way as the acetomethanol fixed material, show very good and specific labelling in the EM autoradiographs. It, therefore, seems that acetomethanol fixation somehow removes the ^{3}H-AMD bound to the DNA during the previous incubation. In the following, only the data obtained from glutaraldehyde and osmium-tetroxide fixed glands have been considered.

B. Patterns of ^{3}H-AMD binding in different regions of polytene nuclei :

In glutaraldehyde fixed glands, the ^{3}H-AMD binding, as visualised by silver grain localisation in EM autoradiographs, was restricted to nuclear regions. In general, the electron dense band regions show more silver grains than the interband and puff regions (Fig. 1). Occasionally, silver grains were also seen over the nucleolar areas seen in the sections. The "nucleolar segregation" effect of actinomycin D (Simard *et al.*, 1974) was quite apparent in the present material (Fig. 2A-D.) In the chromocentre, grains were seen to be located on both the alpha- and the beta-heterochromatin regions (Figs. 2-3). In order to compare the relative binding of ^{3}H-AMD in euchromatin (band, puff and interband regions included) and the alpha- and beta-heterochromatin regions of chromocentre, an empirical method of grain density analysis has been adopted (for details of the method, see Lakhotia and Jacob, 1974b). Silver grains have been counted in the enlarged prints on the alpha- and beta-heterochromatin areas, as also on a randomly selected euchromatin area in the same print. At the border of the alpha- and

Figs. 1-3

EM autoradiographs of ultrathin sections of *D. melanogaster* larval salivray glands labelled *in vitro* with ^{3}H-Actinomycin D and double fixed with glutaraldehyde and osmium tetroxide. a=alpha-heterochromatin; b=beta-heterochromatin; ne=nuclear envelope; nu=nucleolus; sg secretory granules.

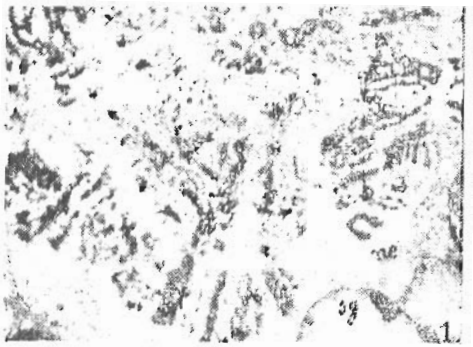

Fig. 1

Part of a nucleus showing the general pattern of labelling in euchromatin of polytene nuclei. The electron-dense bands show greater labelling compared to the interband and puff regions. x4,000.

beta-heterochromatin, a "peripheral" zone, encompassing a 1600Å wide band on either side of the border, has been considered separately to take into account the limits of EM autoradiographic resolution (Lakhotia and Jacob, 1974b). The grain count data from 20 different EM autoradiographs obtained from different salivary glands are presented in Table I. In each of these 20 prints, the chromocentre region included both the alpha- and the beta-heterochromatin areas. It is obvious from the Figs. 1-3 and the data presented in Table I, that the different regions of polytene nuclei bind ^{3}H-

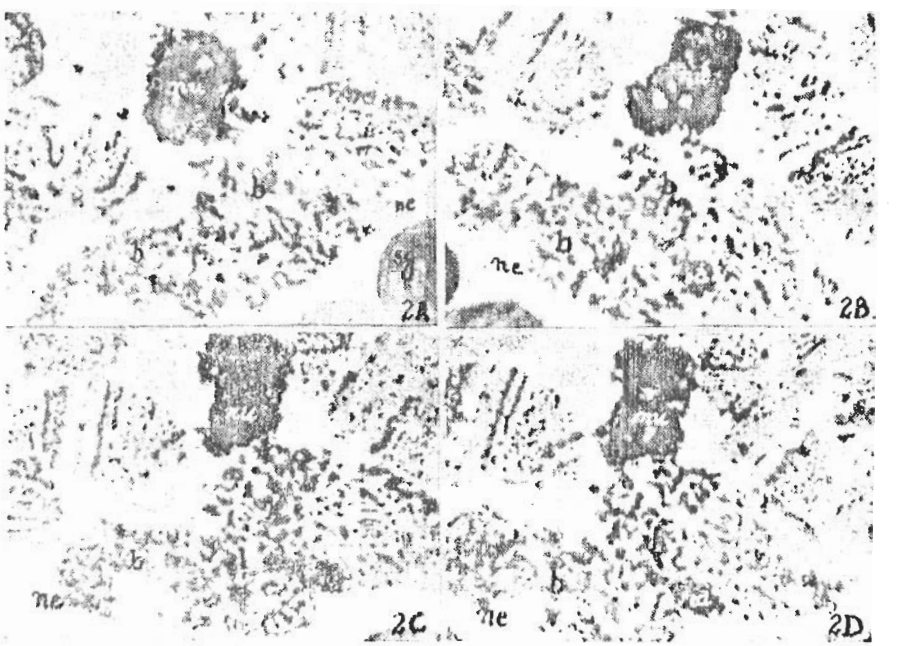

Fig. 2A-D

Serial section of a nucleus through the chromocentre region and nucleolus. In 2A, there is a cluster of grains on the alpha-heterochromatin. The beta-heterochromatin also shows as much labelling as the euchromatic regions in the nucleus. The nucleolus shows typical "segregation" effect as a result of actinomycin D treatment. In 2D, silver grains are also seen over the nucleolar regions. x4,000.

AMD to the same extent. The alpha- and the beta-heterochromatin show similar grain density to each other as well as to the general euchromatin regions. Although a grain density analysis of the degree of labelling on bands, interbands and puffs has not been done, it seems generally, that the dense bands show more labelling than the disperse puff and interband regions.

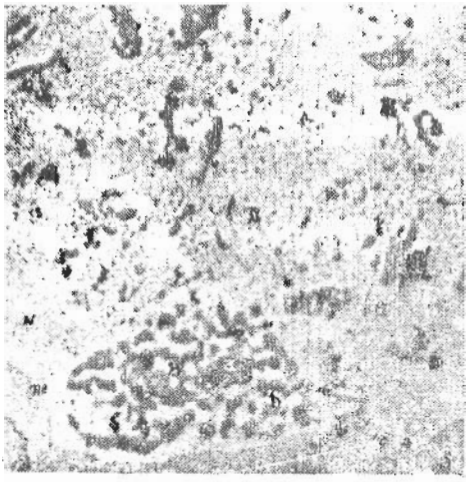

Fig. 3

Part of a nucleus showing labelling over the alpha-hetrochromatin, beta-heterochromatin and euchromatin regions. In all the regions the grain-density appears to be similar. x6,000.

DISCUSSION

The ^{3}H-actinomycin D binding capacity of different regions of the live polytene nuclei of late third instar larvae of *D. melanogaster* has been analysed by EM autoradiography. Of the two different fixation methods followed, acetomethanol has been found to cause loss of the bound ^{3}H-AMD since very poor and unspecific labelling was seen after this fixation method. Glutaraldehyde fixation resulted in very good and specific labelling. The mechanism underlying this effect of acetomethanol in removing bound AMD is not clear but it may be of interest to note here that acetomethanol treatment is routinely used to remove several other agents like Quinacrine Mustard, "Hoechst 33258" fluorescent dye etc. which are probably intercalated in the DNA double helix (Comings *et al.*, 1975) like AMD (see Harold, 1972). Possibly a similar mechanism is involved in the removal of ^{3}H-AMD as a result of acetomethanol fixation.

As noted in the Introduction, the earlier data on ^{3}H-AMD binding in condensed (heterochromatin) and disperse (euchromatin) chromatin are not uniform. However, Sieger *et al.* (1971), considering these conflicting data, have suggested that the facultative and constitutive heterochromatin (Brown, 1966) may differ in this respect—the facultative heterochromatin may fail to bind ^{3}H-AMD in condensed state while the constitutive heterochromatin may bind as much as the euchromatin irrespective of its condensed state. The present data seem to be in agreement with this interpretation of Sieger *et. al.* (1971).

The chromocentre heterochromatin in the polytene nuclei of *Drosophila* is of the constitutive type. However, within this region, two distinct zones can be distinguished—the central alpha-, and the peripheral beta-hetero-

TABLE I

Analysis of silver grains in different areas of the chromocentre and euchromatin after *in vitro* labelling with ^{3}H-Actinomycin D

Region	Total Area μ^2	Total no. of grains	Average no. grains/100 μ^2
Alpha-heterochromatin	33.7	6	18.10
Beta-heterochromatin	1104.3	185	16.75
Peripheral zone	39.7	7	17.63
Euchromatin	958.2	155	16.17

(data collected from 20 different EM autoradiographs)

chromatin (Heitz, 1934 ; Lakhotia and Jacob, 1974b). The alpha- and beta-heterochromatin differ not only with respect to their repetitive DNA content (Rae, 1970 , Gall *et al.*, 1971), but also in other features of their organisation. The alpha-heterochromatin is much more condensed than the beta-heterochromatin and fails to replicate in polytene nuclei while the beta-heterochromatin polytenizes with euchromatin in the nucleus (Gall *et al.*, 1971 ; Lakhotia, 1974). Furthermore, the alpha region is completely inactive in transcription while the beta-heterochromatin is highly active in transcription in the salivary gland polytene nuclei (Lakhotia and Jacob, 1974b). However, inspite of these structural and organisational differences, the present study shows that their ^{3}H-AMD binding capacity is similar and is as much as the typically euchromatic regions. Within the euchromatic regions, the transcriptionally active puff and interband regions do not bind more ^{3}H AMD than the condensed and relatively inactive band regions ; on the contrary, the bands seem to bind more. These results seem to suggest that in the polytene nuclei, the ^{3}H-AMD binding capacity is not related to the transcribing activity of a given region, rather it may be related to the DNA content.

Acknowledgements

This work was carried out at the Institute of Animal Genetics, Edinburgh and was supported by an Overseas Scholarship of the Royal Commission for the Exhibition of 1851, U.K. I express my sincere thanks to Dr. J. Jacob of the Institute of Animal Genetics for providing excellent laboratory facilities and for his keen interest in the work. Financial assistance from the University Grants Commission (Grant no. F. 23-207/75 (SRII) and the Department of Atomic Energy, Govt. of India (Grant no. BRNS/B & M/72/74) is also gratefully acknowledged.

References

Berlowitz, L. Pallotta, D. and Sibley, C. H. 1969. Chromatin and histones : binding of tritiated actinomycin-D to heterochromatin in mealy bugs. *Science 164* : 1527.

Brachet, J. and Ficq, A. 1965. Binding sites of ^{14}C-actinomycin in amphibian ovocytes and an autoradiographic technique for detection of cytoplasmic DNA. *Exp. Cell Res. 38* : 153.

Brachet, J. and Hulin, N. 1969. Binding of tritiated actinomycin and cell differentiation. *Nature (Lond.) 222* : 481.

Brown, S.W. 1966. Heterochromatin. *Science 151* : 417.

Comings, D.E., Kovacs, B.W., Avelino, E. and Harris, D.C. 1975. Mechanism of chromosome banding. V. Quinacrine banding. *Chromosoma 50* : 111.

Desai, L. and Tencer, R. 1968. Effects of histones and polylysine on the synthetic activity of the giant chromosomes of salivary glands of dipteran larvae. *Exp. Cell Res. 52* : 185.

Gall, J.G., Cohen, E.H., and Polan, M.L. 1971. Repetitive sequences in *Drosophila*. *Chromosoma 33* : 319.

Harold, F.M. 1972. Novel antibiotics as metabolic inhibitors. In : Metabolic Inhibitors, vol. III (eds. Hochster, R.M., Kates. M., and Quastel, J.H.), Academic Press, New York, London, p. 305.

Heitz, E. (1934) Uber α-und β-Heterochromatin sowie Konstanz und Bau der Chromomeren bei *Drosophila*. *Biol. Zbl. 54* : 588.

Lakhotia, S.C. 1974. EM autoradiographic studies on polytene nuclei of *Drosophila melanogaster*. III. Localisation of non-replicating chromatin in the chromocentre heterochromatin. *Chromosoma 46* : 145.

Lakhotia, S.C. and Jacob. J, 1974a. EM autoradiographic studies on polytene nuclei of *Drosophila melanogaster*. I. Replication and its relationship with nuclear membrane. *Ind. J. Exp. Biol. 12* : 389.

Lakhotia, S.C. and Jacob, J. 1974b. EM autoradiographic studies on polytene nuclei of *Drosophila melanogaster*. II. Organisation and transcriptive activity of the chromocentre. *Exp. Cell Res. 86* : 253.

Rae, P.M.M. 1970. Chromosomal distribution of rapidly reannealing DNA in *Drosophila melanogaster*. *Proc. nat. Acad. Sci (Wash.) 70* : 773.

Reich, E. and Goldberg, J.H. 1964. Actinomycin and nucleic acid function. In : Progress in Nucleic Acid Research and Molecular Biology (eds. Davidson, J.N. and Cohn, W.E.), Academic Press, New York, p. 183.

Ringertz, N.R. and Bolund, L. 1969. Actinomycin-binding capacity of deoxyribonucleoprotein. *Biochim. Biophys. Acta 174* : 147.

Sieger, M. Garweg, G. and Schwarzacher, H.G. 1971. Constitutive heterochromatin in *Microtus*

agrestis : binding of actinomycin-D and transcriptional inactivity. *Chromosoma 35* : 84.

Simard, R. 1967. The binding of actinomycin D-^{3}H to heterochromatin as studied by quantitative high resolution autoradiography. *J. Cell Biol 35* : 716.

Simard, R., Langelier, Y., Mandeville, R., Maestracci, N. and Royal, A. 1974. Inhibitors as tools in elucidating the structure and function of the nucleus. In : The Cell Nucleus, vol. III, Academic Press, S. F., p. 447.