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Localisation of Non-replicating Heterochromatin in Polytene Cells of *Drosophila nasuta* by Fluorescence Microscopy

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Abstract. A simple fluorescence technique is decribed to localise in situ the non-replicating alpha heterochromatin in the chromocentre region of *Drosophila nasuta* polytene nuclei. After incorporating 5-bromodeoxyuridine in larval salivary gland cells for one or two cycles of replication, the polytene nuclei are examined for Hoechst 33258 flourescence at pH 7.0. The non-replicating alpha heterochromatin remains brightly fluorescing as it does not incorporate any 5-bromodeoxyuridine while the rest of the replicating chromatin shows dull fluorescence due to the quenching of Hoechst 33258 fluorescence by the bromodeoxyuridine substituted DNA.

Introduction

It is known that a part of the mitotic heterochromatin of *Drosophila* does not replicate in the polytene cells of larval salivary glands and this can be recognised as the alpha heterochromatin (Heitz, 1934; Rudkin, 1965; Gall et al., 1971; Lakhotia, 1974). However, a precise localisation of the non-replicating chromatin at light microscope level is obscured by the beta heterochromatin which replicates and envelopes the alpha heterochromatin in fully developed polytene nuclei (Lakhotia, 1974; Lakhotia and Jacob, 1974). We report here a simple technique to precisely locate the non-replicating alpha heterochromatin in *Drosophila* polytene cells. The technique is based on the quenching of the UV fluorescence of Hoechst 33258 by 5-bromodeoxyuridine (BrdU) substituted DNA in replicating chromosomes (Latt, 1973).

Material and Methods

A wild strain of *Drosophila nasuta,* raised on the standard cornmeal agar food at $20° \pm 0.5°$ C, was used for these studies. About 25 third instar larvae were transferred to one gram of food containing 50 µg of BrdU (Calbiochem, Sweden). A parallel control set was also maintained without

BrdU. These larvae were permitted to grow at 20° C in total darkness. At intervals of 10, 20 and 28 h, larvae were taken out and their salivary glands fixed in aceto-methanol (1:3) for 40–50 s and immediately squashed in 50% acetic acid. The coverglasses were separated from the slides following the method of Lakhotia and Mukherjee (1969). Both the slides and coverglasses, containing the material, were stained with Hoechst 33258 solution (5 μg/ml dist. water) for 2 min. After washing in dist. water, the preparations were mounted with a pH 7.0 buffer (0.16 M disodium hydrogen phosphate and 0.04 M trisodium citrate) and sealed with wax. The fluorescence patterns were examined with a CZ (Jena) fluorescence microscope using a HBO 200 mercury vapour pressure lamp, BG12/2 and BG3/2 as excitation filters and OG1 and GG9 as suppression filters. Photomicrographs were taken on a high contrast OrWo DK 5 film.

Observations

The chromocentre region in fully developed polytene nuclei of *D. nasuta* larval salivary glands is relatively small. However, neither the normal aceto-orcein

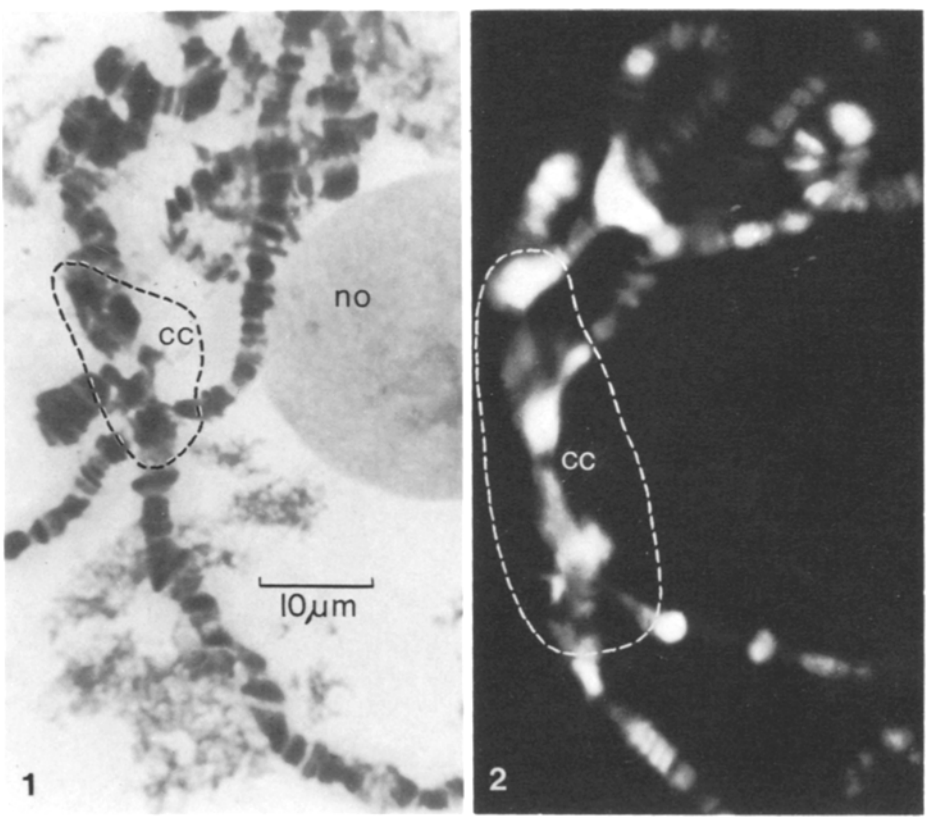
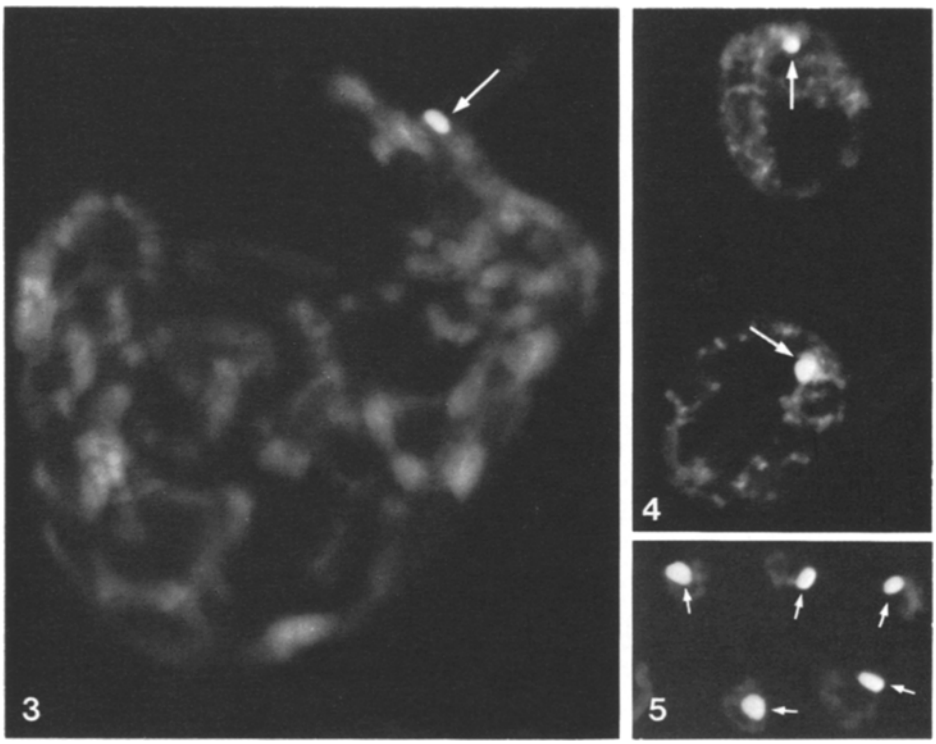


Fig. 1. Part of a fully grown polytene nucleus from normal larval salivary glands of *D. nasuta* stained with Giemsa, showing the chromocentre region (*cc* = chromocentre; *no* = nucleolus). Magnification in this and other figures × 1500. The bar represents 10 μm

Fig. 2. Hoechst 33258 fluorescence of the chromocentre region of a normal fully grown polytene nucleus from *D. nasuta*. Note the typical banded morphology of the chromosome arms and the uniformly bright fluorescence of the chromocentre region (stretched in this nucleus)



Figs. 3–5. Hoechst 33258 fluorescence (at pH 7.0) of nuclei of various levels of polyteny from a larva fed on 5-BrdU containing food (50 μg/gm) for 28 h. In all nuclei, only one brightly fluorescing mass (arrows) is seen in the chromocentre; the size of this mass is similar in the nuclei of different polyteny levels (the slight variation in size is explainable by the different orientations of the rather elliptical alpha heterochromatin region)

nor Giemsa staining (Fig. 1) reveals the alpha heterochromatin in these nuclei. The chromocentre region in mature polytene nuclei shows very bright flouresence with Hoechst 33258 (Fig. 2). However again, the alpha heterochromatin can not be distinguished from the uniformly bright beta heterochromatin of the chromocentre.

The polytene nuclei, which have incorporated BrdU in the experimental larvae are not well spread and show a rather diffuse organisation of bands and other regions and show relatively dull fluorescence (Figs. 3–5). In the 10 h sample, some nuclei still have the normal bright fluorescence and banded morphology with good spreading of chromosome arms; in the 20 h BrdU sample, fewer nuclei show the normal appearance. On the other hand, in the 28 h sample, almost all nuclei have the diffuse appearance with ill-defined bands and poor spreading. In all the three samples, the nuclei with ill-defined bands and poor spreading, show dull flourescence when compared to the normal polytene nuclei. However, in all these nuclei of high level of polyteny, a specific area in the chromocentre region remains very bright and clearly stands out from the rest of the dully flourescing regions (Fig. 3). The polytene nuclei

from BrdU fed larvae, having normal morphology, do not show a comparable specific bright region in the chromocentre, which is uniformly bright.

In squash preparations of *D. nasuta* larval salivary glands, in addition to the nuclei with high levels of polyteny, many nuclei with very low polyteny levels are also seen. Giemsa staining of these nuclei from normal larvae often shows a darkly stained mass in the chromocentre, comparable in size to the brightly fluorescing region in BrdU incorporated nuclei noted above. The homology of this body in the two types of nuclei is demonstrated by the Hoechst 33258 fluorescence of low level polytene nuclei from BrdU fed larvae. Most of the low level polytene nuclei in 10, 20 and 28 h samples show only one bright fluorescent region in the chromocentre, while the rest of the nucleus is very dull (Figs. 4, 5). The size of the bright mass in these nuclei is same as seen in the high level polytene nuclei (Fig. 3) and also compares with the darkly stained (Giemsa) mass in low level polytene nuclei from normal larvae referred to above.

Giemsa staining of BrdU incorporated and Hoechst 33258 stained polytene nuclei has also been done following the technique of Goto et al. (1975). In nuclei of high level of polyteny, Giemsa staining failed to reveal or specifically stain the brightly fluorescing mass seen with Hoechst 33258. Apparently, the large amount of beta heterochromatin obscures the identification of the alpha heterochromatin by Giemsa staining although the bright fluorescence is easily visualised. In low level polytene nuclei from BrdU fed larvae, however, the same mass which is brightly fluorescent with Hoechst 33258, remains darkly stained with Giemsa while the rest of the chromatin takes little stain.

Discussion

The BrdU incorporation in combination with Hoechst 33258 fluorescence has been used in recent years as a very sensitive probe to study the replication patterns in mammalian chromosomes with a resolution better than ^{3}H-thymidine autoradiography (Latt, 1975; Stubblefield, 1975). In the present study, we have made use of the same principle to detect the nonreplicating chromatin in polytene nuclei of *Drosophila*. EM autoradiographic studies in *D. melanogaster* (Lakhotia, 1974) have revealed that the alpha heterochromatin (Heitz, 1934) does not replicate at all during polytenization in larval salivary gland nuclei. In the present study third instar larvae of *D. nasuta* have been fed on BrdU food upto 28 h. The incorporation of BrdU in salivary gland nuclei is indicated by dull Hoechst 33258 fluorescence (at pH 7.0) of these nuclei and also by their poor chromosome morphology. However, a small but specific region in the chromocentre continues to show bright fluorescence even when the rest of the nucleus is dull due to BrdU incorporation. The most likely explanation for the persistent bright fluorescence is that this mass is not replicating and therefore, not incorporating BrdU, which otherwise would have quenched Hoechst 33258 fluorescence at pH 7.0 (Latt, 1973). It could also be suggested that the persistent bright fluorescence of a part of chromocentre in polytene nuclei from BrdU fed larvae is due to a high G-C content of this region so

that little or no BrdU incorporation would occur even if this region were replicating. This however, can be ruled out in view of the following points: (i) Hoechst 33258 is known to be brightly fluorescing only with A-T rich DNA (Comings, 1975), (ii) the size of the bright region is same in nuclei with low and high polyteny – this itself shows that this particular region is not replicating during the increase in polyteny level, and finally, (iii) in our studies on BrdU incorporation in mitotic chromosomes of *D. nasuta*, we could not locate any heterochromatic segment which does not incorporate BrdU (unpublished data). In view of these considerations, it may be surmised that the brightly fluorescing region seen after BrdU incorporation represents the non-replicating alpha heterochromatin of *D. nasuta* polytene nuclei.

The present technique thus provides a simple way to identify and locate the non-replicating alpha heterochromatin in polytene nuclei of *Drosophila* and using this procedure, it would be possible to do a comparative study of non-replicating chromatin in polytene cells of different organs as well as in different species.

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