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A Study of Heterochromatin in *Drosophila nasuta* by the 5-Bromodeoxyuridine–Giemsa Staining Technique

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Abstract. Larval brain ganglia of *Drosophila nasuta* were cultured in vitro in the presence of 5-bromodeoxyuridine for 1 or 5 h at 24° C and the air-dried chromosome preparations stained by the Hoechst 33258-Giemsa technique to reveal bromodeoxyuridine induced sister chromatid differentiation. In 1 h as well as 5 h preparations, 10–15% of well spread metaphase plates show a sister chromatid differentiation in only C-band heterochromatin regions of different chromosomes. We infer that this sister chromatid differentiation in all heterochromatic regions is seen after bromodeoxyuridine incorporation for only one replication cycle and is related to the presence of asymmetric A-T rich satellite sequences in all the C-band regions of *D. nasuta* karyotype.

Introduction

The 5-bromodeoxyuridine-Hoechst 33258-Giemsa staining technique is a sensitive probe for the analysis of some aspects of chromosome organization. One of the interesting application of this technique has been in the study of asymmetry and polarity of satellite DNA in heterochromatic regions of mouse metaphase chrtmosomes (Lin et al., 1974; Holmquist and Comings, 1975). In the mouse, the satellite DNA in C-band heterochromatin is asymmetric since one strand is rich in A and the other in T (Flamm et al., 1967). Because of this satellite DNA asymmetry, it is possible to obtain a differential Giemsa staining of sister chromatids in the C-band regions after growing mouse cells for only one replication cycle in the presence of bromodeoxyuridine (Holmquist and Comings, 1975). On the other hand, chromosome regions containing DNA with a symmetric distribution of A and T bases require two cell cycles in the presence of bromodeoxyuridine to reveal differentiation of sister chromatids (Latt, 1973).

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On the basis of the report of Travaglini et al. (1972) on satellite DNA in *Drosophila nasuta* and our own observations of Hoechst 33258 (H) and Quinacrine Mustard (QM) fluorescence patterns, we suggested earlier (Lakhotia and Kumar, 1978) that all the heterochromatic regions on *D. nasuta* chromosomes contain A-T rich satellite sequences. We have now used the bromodeoxyuridine-Hoechst 33258-Giemsa staining technique to find out if the A-T rich sequences are symmetric or otherwise and if the heterochromatic regions on different chromosomes of *D. nasuta* can be cytologically differentiated on this basis.

Material and Methods

A wild type strain of *Drosophila nasuta* (Varanasi) was used for these studies. Larvae were reared on the standard agar-cornmeal-brown sugar food at 24° ± 1° C. Brain ganglia from mature third instar larvae (post-black spiracle stage; Lakhotia and Roy, 1979) were dissected out under aseptic conditions, freed of adhering imaginal disks etc and transferred to a modified Poels' (1972) medium. In the modified medium, 10% lactalbumin hydrolysate (Sigma) has been added in place of different amino acids and the yeastolate and serum are omitted. 5-bromodeoxyuridine (BrdU) was added to the culture medium at a concentration of 40 μg/ml. The brain ganglia were cultured in dark at 24° C for 1 or 5 h. In the 1 h experiment, the ganglia were not exposed to colchicine while in the 5 h experiments, colchicine (1 μg/ml) was added to the medium at the end of a 4 h period and the culture was terminated after 1 h of colchicine exposure. In both cases, the ganglia were tranferred to a hypotonic solution (0.67% sodium citrate) for 35 min at 24° C following which they were fixed in fresh aceto-methanol (1:3) fixative. Air dried chromosome preparations from individual brain ganglia were made as described earlier (Lakhotia and Kumar, 1978). The chromosome preparations were first stained with Hoechst 33258 (50 μg/ml) for 15 min, rinsed with distilled water, mounted with 2 × SSC and exposed to bright sunlight (mild winter sunlight between 12 noon and 2 p.m.) for 60–70 min. The sunlight exposed slides were washed well with water, stained with 5% Giemsa (pH 7.0) for 7–10 min, rinsed, dried and mounted with D.P.X.

Observations

As described earlier (Lakhotia and Kumar, 1978), metaphase chromosomes from brain ganglia of *D. nasuta*, not exposed to BrdU, show prominent heterochromatic segments on all the three pairs of larger chromosomes (Fig. 1) and all these heterochromatic segments have been shown to be C-band positive and H- and QM-bright. H-Giemsa stained preparations of *D. nasuta* brain ganglia exposed to BrdU for 4–5 h show metaphase plates with varying chromosome morphology. In many metaphases, normal chromosome morphology and staining are retained while in some others, either the euchromatic regions are despiralized to a varying degree or the heterochromatic regions appear extended. In all these plates, the sister chromatids show a similar organization. The significance of such metaphases in BrdU-incorporated preparations is being analysed and will be reported later. However, of interest in the present study are metaphases in which the heterochromatic segments of each of the three pairs of larger chromosomes have the usual dark staining on one chromatid while the corresponding regions on sister chromatids are pale stained and/or attenuated and relatively stretched. We have examined H-Giemsa stained preparations

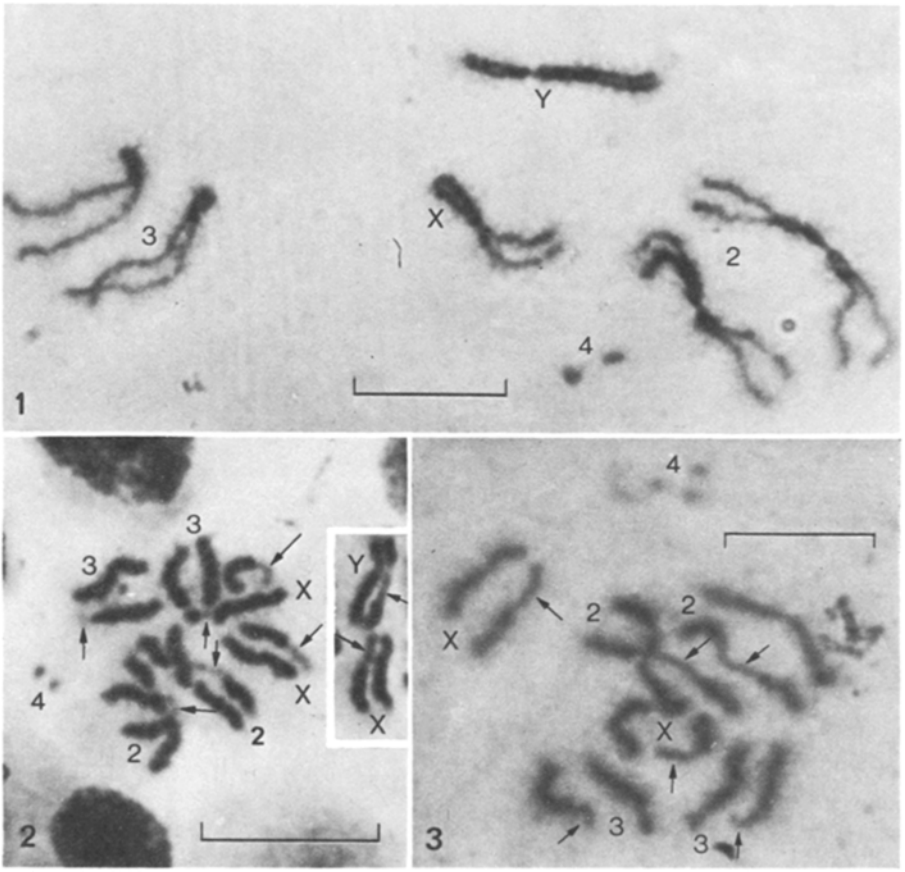

Fig. 1. A metaphase plate from a normal Giemsa stained air-dried preparation of brain ganglia of a male larva of *D. nasuta*. Note the dark stained heterochromatic regions on all chromosomes (*X, Y, 2* and *3*) except the small fourth pair (*4*). The bar on this and all other micrographs represents 10 μm

Fig. 2. TC-banded metaphase plate from 5 h (last 1 h with colchicine) BrdU labelled preparation. The sister chromatids are separated but lying close to each other. Note the pale staining on the heterochromatic regions (arrows) on one of the sister chromatids of X, 2nd and 3rd pair of chromosomes. The euchromatic regions on both chromatids of all chromosomes are normally stained. The inset shows TC-banded X and Y chromosomes from 5 h BrdU labelled preparation. Differential staining (arrows) of sister chromatids on Y chromosome is restricted to the proximal twothirds of the long arm

Fig. 3. A TC-banded metaphase plate from brain ganglia of a female larva cultured in BrdU for only 1 h. In this case also the sister chromatids have separated. Heterochromatic regions in one sister chromatid (arrows) of all the larger chromosomes are attenuated and slightly stretched, although the pale staining is not very clear

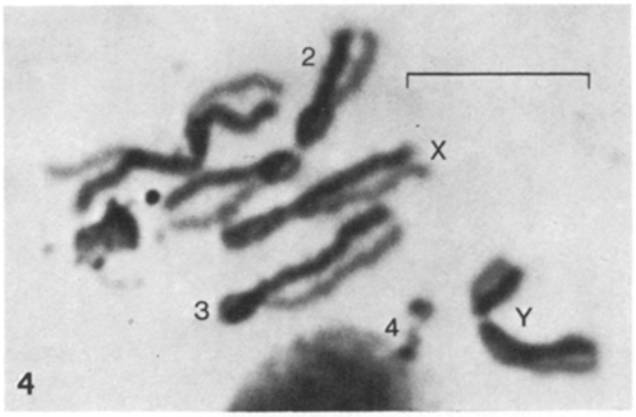

Fig. 4. One of the three exceptional metaphases seen in preparations from brain ganglia (male) exposed to BrdU for 5 h (including 1 h colchicine treatment). In this case, the sister chromatid differentiation extends to the entire length of all the chromosomes. The differential sister chromatid staining in some heterochromatic regions has been obscured in the photomicrograph because of close synapsis. One homologue of chromosome 3 is not seen in this plate

from 11 different brain ganglia exposed to BrdU for 5 h. From these preparations, 200 metaphases, in which the chromosomes were well spread and easily identifiable, were studied. Among these 200 metaphases, 30 plates revealed an unambiguous sister chromatid differentiation in the heterochromatic regions (Fig. 2). The sister chromatids of euchromatic regions of all chromosomes in these plates are normally stained. Metaphases with this type of differential sister chromatid staining or stretching in C-band regions only are referred to as TC-banded following the term used by Holmquist and Comings (1975) in mouse (see Discussion). The submetacentric Y-chromosome, which appears fully heterochromatic in normal Giemsa stained preparations, however, is not differentially stained along its entire length in TC-banded plates. Only the proximal $^{1}/_{2}$ to $^{2}/_{3}$ of the long arm shows sister chromatid differentiation (Fig. 2, inset) with the remaining parts showing normal staining on both chromatids. Interestingly, only this segment of the Y-chromosomes shows typical C-banding and bright fluorescence with both H and QM (Lakhotia and Kumar, 1978). The small 4th pair of chromosomes are C-band negative and also do not show TC-banding. Sister chromatid exchanges in the TC-banded regions are seen only rarely.

H-Giemsa stained preparations from brain ganglia exposed to BrdU for only 1 h also show some (6 out of nearly 60 plates examined from 4 preparations) metaphases in which the C-band regions on chromosomes show sister chromatid differentiation. In these metaphases (Fig. 3), the C-band regions on one chromatid of each chromosome appear narrower and stretched as compared to the corresponding region on sister chromatids; the pale staining of these stretched regions is not as marked as in the 4–5 h preparations. The euchromatic regions in these TC-banded metaphases remain normally stained.

A very unexpected observation made during the present study is that in three metaphases from preparations of two different brain ganglia exposed to BrdU for 4–5 h, the differential sister chromatid staining extended to entire chromosomes (Fig. 4) rather than being confined to C-band regions as in other metaphases. These exceptional metaphase plates give a typical second cycle BrdU-Giemsa staining pattern as reported by Wienberg (1977) in *D. melanogaster* brain cells exposed to BrdU for 15 h. In the 1 h preparations, no such metaphase plates have been observed.

Discussion

The usual BrdU-H-Giemsa differentiation of sister chromatids is seen after two cell cycles in presence of BrdU (Latt, 1973; Perry and Wolff, 1974). In the present study, we have observed a differential staining of sister chromatids in C-band regions of *D. nasuta* chromosomes after only 1 or 4–5 h exposure to BrdU and we presume that during this brief labelling period, the heterochromatic regions would have replicated only once in BrdU. This is very interesting since this may reflect a particular type of base sequence organization of the heterochromatic regions in *D. nasuta* chromosomes. However, before we discuss this, some aspects of the cell cycle patterns in larval brain cells of *D. nasuta* have to be considered.

Our ^{3}H-thymidine autoradiographic studies on the cell cycle in brain cells of third instar larvae of *D. nasuta* (Kumar and Lakhotia, 1977 and other unpublished data) have indicated that the cell cyle patterns vary considerably in different cells of larval brain ganglia. Nevertheless, the majority of the labelled metaphases in samples collected between 1 to 5 h after a brief ^{3}H-thymidine pulse, were seen to have silver grains confined to the heterochromatic regions of both chromatids of all chromosomes. Similar observations have been reported by Barigozzi (1968) for *D. melanogaster*. In view of these observations, in the present study, we labelled *D. nasuta* brain ganglia with BrdU for 4–5 h to get metaphase plates which would have replicated their heterochromatic regions only once in presence of BrdU. However, the presence of some, though rare, metaphases in 4–5 h samples, showing typical 2nd cycle differentiation of sister chromatids along the entire chromosome length may raise doubts about our presumption that the TC-banded chromosomes have incorporated BrdU for only one replication cycle. The presence of metaphases with typical second cycle picture may be due to a very brief cell cycle duration in at least some brain cells of *D. nasuta* larvae under the present culture conditions. On this basis, one may also argue that the metaphases which show sister chromatid differentiation only in C-band regions may have completed more than one cell cycle in the presence of BrdU. Thus if a cell was in late S when BrdU was added and if it completed one more replication cycle and reached the second cycle metaphase during the 4–5 h interval, it would have replicated its heterochromatic regions twice and euchromatic regions only once in BrdU. Such a metaphase cell would show differential sister chromatid staining only in the heterochromatic regions in the usual manner without a need for involving

particular type of base sequences in the C-band regions. However, several considerations suggest that while a few rare cells may have a very short cell cycle duration, the majority of larval brain cells which are dividing would not have completed more than one cell cycle within the 4–5 h labelling period. In our ^{3}H-thymidine autoradiographic studies, referred to above, we did not see many metaphases with single chromatid labelling in 4–5 h sample which would have been expected if the metaphases were in the second cycle after the pulse. Pimpinelli et al. (1976) and Wienberg (1977) have also found that on an average, larval brain cells of *D. melanogaster* complete two cell cycles in about 15 h. More importantly, we have seen that a significant number of metaphases show sister chromatid differentiation specifically in C-band regions even when labelled with BrdU for only 1 h. Certainly, it is very unlikely that these metaphase cells would have completed more than one cell cycle in BrdU.

The above considerations, therefore, permit us to conclude that the differential staining of sister chromatids in C-band regions of *D. nasuta* chromosomes seen in 1 and 4–5 h BrdU labelled brain ganglia preparations, is occurring after only one cycle incorporation of BrdU. Thus we can compare the present observations with those on TC-banding in mouse chromosomes. The differential sister chromatid staining of C-band regions in mouse chromosomes after one replication cycle in BrdU has been interpreted to be due to an asymmetric distribution of A and T bases in the two strands of satellite DNA (Lin et al., 1974; Holmquist and Comings, 1975). There are sufficient reasons to believe that all the C-band regions of the *D. nasuta* karyotype contain A-T rich satellites (Travaglini et al., 1972; Lakhotia and Kumar, 1978). The present observations on TC-banding in all the C-band positive and H- and QM-bright segments of different chromosomes of *D. nasuta* further suggests, in analogy with mouse, that all these regions share A-T rich satellite/s with an asymmetric distribution of A and T bases.

The pattern of TC-banding in the chromosome 2 of *D. nasuta* is significant. The chromosome 2 of *D. nasuta* is metacentric and carries large heterochromatic blocks on both arms adjacent to centromere. In TC-banded metaphases, both these segments of one chromatid are either darkly stained or lightly stained. This is unlike the situation observed in some mouse cell lines which carry metacentric marker chromosomes: the differentially stained heterochromatic segments on two sides of centromere in these chromosomes are located on opposite chromatids (Lin et al., 1974; Holmquist and Comings, 1975). Therefore, the evolutionary origin of the metacentric chromosomes of *D. nasuta* karyotype may be different than that of mouse metacentrics.

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