Heterochromatin in mitotic chromosomes of Drosophila nauta

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Abstract

The heterochromatin in mitotic cells of larval neural ganglia of Drosophila nauta has been analyzed by Giemsa staining and by fluorescence studies. All chromosomes, except the 'dusky'-like 4th chromosome pair, carry large blocks of heterochromatin which are brightly stained by Giemsa, and which fluorescence uniformly bright with Hoechst 33258, quinacrine mustard, and acridine orange. These heterohromatic segments make up about 40% of the total metaphase chromosome length. The heterochromatic segments also fluoresce brightly with all the dyes at the anaphase stage. In interphase nuclei, all the heterochromatic segments form a condensate, granular and homogeneous nuclei which fluoresce brightly with the three fluorochromes used. The size of the bright condensate is similar with all the dyes. It is suggested that the heterochromatic segments of different chromosomes are relatively homogenous in their DNA base compositions, which are likely to be A-T rich in view of their bright fluorescence with Hoechst 33258 as well as quinacrine mustard.

Introduction

Heterochromatin in Drosophila cells has received considerable attention in recent years in view of its disproportionate replication in different cell types, particularly the polytene cells (Rudomin, 1965; Gall et al., 1971; Lathokida, 1974), and a possible involvement of heterochromatin in cellular transcription and other functions (Lakhdara and Jäckh, 1974; Lindquist et al., 1975; Spradling et al., 1975; Sandler, 1974). The differential replication of heterochromatin in different cell types may be related to the process of cellular differentiation (Rudomin, 1973; Lakhdara, 1974). We are studying this aspect in Drosophila nauta, a member of the immigrant species group. In this paper, we present our observations on the localization and the fluorescence patterns of heterochromatin in the mitotic cells of Drosophila nauta.

Materials and methods

A wild strain of Drosophila nauta, collected from the University campus, was used for these studies. The larvae were grown at 20° ± 0.5°C in standard corn meal agar food. The neutral ganglia from the late third instar larvae were dissected out in Drosophila Ringer solution (pH 7.2) and azo-dyed chromosome preparations were made following the technique of Stock et al. (1972) with some modifications. The ganglia were treated with colchicine in Ringer (1 mg/ml) for 1 hr at 20°C following which they were transferred to a hypotonic solution (0.5% trisodium citrate) for 30 min. The ganglia were then fixed in freshly prepared aceto-ethanol (4:1) for 2 hr with three changes.

In some specific instances, the colchicine and/or hypotonic pretreatments were omitted and the ganglia were fixed directly in aceto-ethanol. A small drop of warm 60% acetic acid was taken on a warm (35°C) slide, and the fixed ganglia from one vial were placed onto this drop. Within a very short time the cells dissociate from
Coping
After several trials, the following procedure was adapted for C-coupling of D. annae
brain ganglia chromosome. Air-dried preparations were treated with 0.2%
N2H4 in 2 x SSC at 20°C for 150 min and washed with three changes (2–3 min each)
of 2 x SSC. After passing through three changes of 70%, and two changes of 90% alcohol
(12 min each), the slides were dried and stained with 5% Giemsa stain (pH 7.5)
for 7.5 min.

Fluorescence handling
Koppe's 33258 (H3), quinacrine mustard dihydrochloride (QM) and acetate orange
(AO) have been used for fluorescence handling. Prior to staining with the fluorochromes,
the air-dried slides were hydrated by passing through 90% (10 min) and
70% (10 min) alcohol, and then two changes of distilled water. In the case of
QM staining, a buffer (pH 5.5) was used in place of distilled water. The dye concentrations
in distilled water and staining time for the fluorochromes were as follows:
H - 5 µg/ml for 10 min, QM - 0.5 mg/ml for 30 min, and AO - 0.1 mg/ml for 10
min.

Following the staining, the preparations were washed in distilled water except
for QM, where buffer (pH 5.5) was used instead. The slides were mounted in
Mowiol buffer (pH 4.0 or 5.5) with or without glycerol. The mounting medium did not
significantly modify the fluorescence pattern. The fluorescence patterns were exam-
ined by incident illumination in a Carl Zeiss (Jena) Fluoro! Fluorescence microscope
with HBO 200 burner, and BG 12/4 excitation and GG4 barrier filters. Micrographs
were taken at an initial magnification of x 400 on OrWo DKS or OrWo NPI 5 films.

Observations
Miotic ganglia preparations, metaphase plates showed eight chromosomes including
a pair of sex chromosomes (XX in female and XY in male), a pair of small "dot"
chromosomes and two pairs of large autosomes (Figure 1). The X chromosome was
a large acrocentric with the proximal half being heterochromatic. The Y chromosome,
of similar size as the X, was sub-metacentric and in normal Giemsa stained preparations,
the top of both arms of the Y chromosome appeared lightly stained compared to the
middle region which was typically heterochromatic. Occasionally in very extended
chromosomes, this region gave a banding appearance (Figure 1a); however, this
differentiation was obscured in condensed plates (Figure 1c). One of the large auto-
somal pair was metacentric (chromosome 2), and the other pair was acrocentric
(chromosome 3). The metacentric chromosome pair carried very large blocks of
darkly stained heterochromatin on either side of the centromere. The acrocentric
pair had a small heterochromatic region near the centromere.

On all the chromosomes, the junction of heterochromatic and euchromatin was
marked by a constriction; this functional region always remained symmetrical even when
the sister chromatids in the rest of heterochromatic region were desynapsed (Figure 1b).
It also appeared that the heterochromatin, and euchromatin junction on the

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Figure 1. Giemsa stained metaphase chromosomes of testis nevrotelost cells of *Drosophila nasuta*. (a) Highly extended chromosomes with a banded appearance of heterochromatin segments, noticeably the Y chromosome; (b) extended chromosomes with a prominent constriction at the junction of heterochromatic and euchromatic segments on all chromosomes; (c) highly condensed chromosomes; the heterochromatic segments, particularly of the Y chromosome, do not show any internal differentiation as in (a); (d) part of a metaphase plate in which the heterochromatic segment has broken away from the euchromatic segment in one X chromosome at the 'peak point' (arrow). Figure 1a (top), Figure 1b (center), Figure 1c (bottom, left), Figure 1d (bottom, right). x 2,000.

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C-banding

In general, the C-band positive regions coincided with the darkly stained regions seen with normal Giemsa staining. The proximal half of the X chromosome took a uniformly dark stain with C-banding (Figure 2). The sub-metacentric Y chromosome had two blocks of C-positive heterochromatin, one on either arm close to the centromere; the tips of both arms were relatively lightly stained. Among the autosomes, the metacentric pair had two large pericentric blocks of C-positive segments. The C-positive region on one arm was smaller than that on the other arm.

The centromeric pair had a small segment near the centromere. Occasionally, this C-positive segment on theacrocentric chromosome pair showed heteromorphism: one chromosome having a smaller block of C-positive heterochromatin than the homologue (Figure 2). The small 4th chromosomes were almost unstained with C-banding.

Fluorescence pattern

Mitotic chromosomes

All the three fluorochromes, namely, Hoescht 33258 (H), quinacrine mustard (QM) and acridine orange (AO), used in the present study gave a differential fluorescence of heterochromatin and euchromatin. The brightly fluorescing regions corresponded, in general, to the heterochromatic regions localized by C-banding (Figure 3). i.e. all the C-banded regions (except the Y chromosome, see below) were uniformly bright with all the three dyes. However, the overall brightness of fluorescence varied with the dye used: H-staining gave maximum bright fluorescence and the differentiation of the heterochromatin was also very pronounced; AO gave minimum differential fluorescence of heterochromatin and euchromatin; with QM the differential fluorescence of heterochromatic segments was well marked, but the total intensity of fluorescence was less. With all the three dyes, the euchromatic segments showed uniform dull fluorescence.

Figure 2 C-banded metaphase chromosomes of female (a) and male (b) larval neuroblast cells of D. melan. Note the lack of C-banding on the tips of both arms of the Y chromosome in (b). Figure 2a (left); Figure 2b (right). ¥ x 2,500.

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The size difference in the heterochromatic segment on chromosome pair 3, noted with C-banding, was also seen in many plates after fluorescence staining (Figures 3a and 3b). The 4th chromosome pair, which was C-negative, manifested dull fluorescence with all three dyes, and being very small in size was occasionally almost invisible after fluorescence staining.

The Y chromosome gave characteristic fluorescence patterns, particularly in extended plates (Figures 3a and 3b). The C-positive block in the short arm had a bright fluorescence with H as well as QM, while the distal region of the short arm was dull. The C-banded region on the long arm was uniformly bright with H and QM. In some plates an indication of two H-dull bands in this segment of the Y chromosome was seen.

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A few anaphase plates were also examined after fluorescence staining (Figure 4). It was seen that at anaphase stage also the heterochromatic regions of all chromosomes were brightly fluorescing with HI, QM as well as with AO. An estimate of the heterochromatin content of metaphase chromosomes was obtained by measuring the relative area occupied by the positive or bright regions. Cells treated with HI showed about 40% of total metaphase chromosome length to be heterochromatic; with QM fluorescence, the proportion of the bright regions was about 38% of the total length of all the chromosomes. The values for male and female nuclei were similar.
Interphase nuclei

Interphase nuclei from brain ganglia cells of third instar larvae had a prominent, darkly stained (with normal Giemsa as well as with C-banding) region, in both the sexes. This region was always associated with the nuclear envelope on one side, and presumably represents the common chromosome formed by the heterochromatic segments of different chromosomes. It is to be noted that in brain cells of third instar larvae of D. melanogaster, interphase nuclei of different sizes, including some typical polytene nuclei, were seen, and in these nuclei the size of the chromatid showed considerable variation. The nuclear size variation and its possible significance will be discussed separately.

In the present analysis, we have selected only the nuclei of small dimensions. The fluorescence patterns of these smaller interphase nuclei have been studied from larval brain ganglia fixed without prior hypotonic and colchicine treatment. In these preparations, the chromatid was nearly always bright with all the three dyes in both the sexes (Figure 5). The fluorescence of the euchromatic regions was very dull with QM as compared to that with H and AO. The bright chromatid in most nuclei was a compact mass associated with the nuclear envelope; no subdivisions within this mass could be identified with any of the three dyes. However, in nuclei from male larvae, stained with H or QM, a small region with relatively less bright fluorescence (compared to the rest of the chromatid) was observed associated

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Discussion

The karyotype of *Drosophila nitidis* has been described by Ray Chaudhuri and Jha (1969), Sajjan and Krishnamurthy (1971), Wakahama and Kitagawa (1972, quoted in Sajjan and Krishnamurthy, 1972), Kumar et al. (1975) and Kumar and Lakhota (1977). Ray Chaudhuri and Jha (1969) described a karyotype with 2n = 12 chromosomes; however, the identity of species described by these authors is doubtful (Sajjan and Krishnamurthy, 1971). The karyotype of *D. nuda* described by Sajjan and Krishnamurthy (1971), and Wakahama and Kitagawa (1972), is essentially similar to that observed by us (Kumar et al., 1975; and present observations) in *D. nuda* collected from Varanasi in Northern India. Sajjan and Krishnamurthy (1971) have identified the Y chromosome as metacentric (V-shaped) while our observations, and those of Wakahama and Kitagawa (1972), show the Y to be subtelocentric. It may be noted here that in very extended prophase and metaphase plates, the X chromosomes and chromosome 3 often gives an impression that they are metacentric and subtelocentric, respectively (see Figure 1b). Indeed, in case of our earlier short reports (Kumar and Lakhota, 1977), we erroneously noted the X to be metacentric, and chromosome 3 to be subtelocentric. Now, however, we have also examined several metaphase stages to confirm the anisotropic locations on different chromosomes, and these observations confirm that the X as well as chromosome 3 are subtelocentric.

Our main interest in *D. nuda* karyotype is its heterochromatin content. The proportion of heterochromatin (about 40%) is substantial. The uniformity of all the heterochromatic segments on different chromosomes with respect to their fluorescence patterns is remarkable. A number of *Drosophila* species have been examined for the fluorescence patterns of their chromosomes (Vos, 1970; Ellison and Barr, 1971; Barr and Ellison, 1971; Heng et al., 1972; Montagut et al., 1975), but in none of these species all the C-band heterochromatic segments give a uniform fluorescence with H as well as QM.
In *D. viridis*, which also has a very large amount of heterochromatin, the different heterochromatic segments vary with their fluorescence with H and QM (Holmquist, 1975a). On the other hand, in *D. nanus*, except for the Y chromosome, all heterochromatic segments fluoresce identically with H and QM. In contrast to *D. viridis* (Holmquist, 1975a), in *D. nanus*, there is no segment which is H bright but QM dark or vice versa.

The fluorescence patterns of interphase nuclei of larval brain cells also suggest a homogeneity of different heterochromatic segments of *D. nanus*. The area of the bright chromocenter in nuclei of similar size is comparable after H or QM staining. This is in agreement with similar fluorescence patterns of the metacentric chromosomes with these two dyes. It is also important to note that in *D. nanus* interphase nuclei, the bright chromocenter appears homogeneous with no indication of any subregions as have been noted in nuclei of *D. viridis* and related species (Holmquist, 1975b).

In *D. viridis*, which has three different satellite sequences associated with heterochromatic segments, the interphase nuclei have a tripartite structure (Holmquist, 1975b). Several other studies have also suggested that similar DNA sequences of heterochromatin tend to condense as one mass in interphase nuclei (Barr and Ellison, 1972; Mayfield and Ellison, 1975; Schmid et al., 1975). In view of these, we think that the single chromocenter formed by the different heterochromatic segments of *D. nanus* chromosomes, reflects a relative homogeneity of DNA sequences associated with the heterochromatin on different chromosomes.

On the basis of H and QM fluorescence patterns, some inferences can be drawn about the nature of DNA sequences associated with the heterochromatic regions (Olagnier, 1975a). Although a variety of factors like chromosome condensation, DNA-protein interaction etc., may modify the H or QM fluorescence, it is generally believed that the base sequences play a significant role in causing certain chromosome regions to be very brightly fluorescent. A very bright fluorescent H and QM has been thought to be due to DNA sequences being enriched in A-T base pairs (Barr and Barr, 1972; Kadai et al., 1974; Comings, 1975; Comings et al., 1975; Holmquist, 1975a). In *D. nanus* all the C-band positive segments (except the Y chromosome) are very brightly fluorescent at the metaphase stage with H as well as QM, and also to some extent with AG. Thus, it seems to us that all these heterochromatic segments on the autosomes and the Y chromosome are enriched in A-T base pairs.

The fluorescence of anaphase chromosomes of *D. nanus* is also interesting. Usually it is observed as a result of greater condensation of chromatic at anaphase, the differential fluorescence of heterochromatin, particularly with QM-staining (Holmquist, 1975a). In *D. nanus*, however, H and QM as well as AG, give a differentially bright fluorescence of all the heterochromatic segments at the anaphase stage. This further suggests that in *D. nanus*, the bright fluorescence of heterochromatin is particularly due to A-T rich base sequences rather than other secondary factors. An analysis of DNA sequences of *D. nanus* would be useful in this respect.

The Y chromosome of *D. nanus*, like the Y chromosome of other species of *Drosophila* (Holmquist, 1975a), appears banded with H. However, with QM, the Y chromosome fluorescence is neither as bright nor does it appear so distinctly banded. In addition, some H-bright segments are QM dull. This difference in the fluorescence of the Y chromosome with H and QM, as compared to other chromo-
somal heterochromatin may reflect a difference in the type of repetitive DNA and/or of chromosomal proteins associated with the Y chromosome. It seems that the relatively less bright region (with H and QM) seems to be associated with the chromosome in male meiosis in relation to the Y chromosome with a similar structure is not present in female larvae. Probably this less bright region represents the distal segments of both arms of the Y chromosome which are dull when fluorescing with H and QM at metaphase. It seems that the tips of both arms of the Y chromosome have an organisation different from the typical heterochromatic segments since these are C as well as fluorescent-negative. In addition, it has also been observed (unpublished data) that these tip segments in brain ganglia cells, complete DNA synthesis earlier than the rest of the Y chromosome.

In conclusion it is suggested that the large amount, and relative homogeneity, of heterochromatin in Drosophila melanogaster may be very useful in further studies on the organisation, replication and role of heterochromatin in different cell types. These studies are in progress in our laboratory.

Note added in proof
After submitting the manuscript for publication we have seen an earlier report by Tragagin et al. (1972) on the satellite DNA sequences of D. melanogaster. According to this study, unlike other Drosophila species examined, D. melanogaster also has two types of polytene chromosomes and these account for 30% of DNA. No other types of satellite sequences were found by these authors in D. melanogaster. These data support our inference drawn on the basis of fluorescence patterns observed in  D. melanogaster, the heterochromatin has A-rich sequences and that different heterochromatic segments appear homogeneous with respect to their base composition.

Acknowledgements
The work reported here has been supported by a Department of Atomic Energy (Government of India) sponsored research grant (number BRNSB and M/72/74) to S.C.L. Huchsch 33258 was a generous gift from Dr H. Lewis, Huchsch AG, Frankfurt, Germany, which we gratefully acknowledge.

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