REPLICATION IN DROSOPHILA CHROMOSOMES

III. DISPROPORTIONATE REPLICATION OF HETERO- AND EU-CHROMATIN IN WING IMAGINAL DISK CELLS OF D. NASUTA LARVAE

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Wing imaginal disks of 3rd instar larvae of *Drosophila nasuta* were labelled *in vivo* with ^{3}H-thymidine for 46h by a feeding method. Observations on autoradiographs of these imaginal disk cells reveal that nearly all interphase nuclei became labelled with ^{3}H-thymidine. However, on average, while 50% of nuclei show labelling of their heterochromatic chromocentre as well as euchromatic regions, only the euchromatic regions are labelled with the chromocentre remaining distinctly unlabelled in about 46% of the nuclei. It is suggested that many of the wing imaginal disk cells of 3rd instar larvae may be undergoing endo-reduplication cycles with under-replication of heterochromatin.

Introduction

Endo-reduplication and disproportionate replication of hetero- and euchromatin occur in a variety of polytene and non-polytene cells of *Drosophila* larvae and adults (Rudkin, 1964, 1972; Berendes & Keyl, 1967; Gall et al., 1971; Endow & Gall, 1975). Endo-reduplicated cells which contain DNA in excess of 2C/4C levels, usually show an under-replication of heterochromatic regions although in a few cases the heterochromatin may also be over-replicated (Berendes & Keyl, 1967; Endow & Gall, 1975). In view of the widespread occurrence of disproportionate replication of hetero- and eu-chromatin in different tissues of *Drosophila* and its possible significance for differentiation, it is of interest to examine this aspect in the larval imaginal disks which differentiate to form the adult structures during pupal metamorphosis. The undifferentiated imaginal disks of *Drosophila* larvae have generally been considered to contain predominantly diploid cells (Gall et al., 1971; Fristrom, 1972). However, the observations presented here suggest that the undifferentiated imaginal disk cells of 3rd instar larvae of *D. nasuta* may not be typically diploid; instead, it seems that many of these cells are endo-reduplicated with a disproportionate replication of hetero- and eu-chromatic regions.

Material and methods

A wild type strain of *Drosophila nasuta* has been used for these studies. The flies and larvae were reared on standard *Drosophila* food at 24°±1°C. In *D. nasuta*, all the three pairs of larger chromosomes carry prominent blocks of heterochromatin and all these heterochromatic regions form a single compact chromocentre in interphase nuclei of different cell types (Lakhotia & Kumar, 1978). The presence of only one well defined and large chromocentre region in interphase nuclei in *D. nasuta* (Figs. 1-2) facilitates the analysis of replication patterns of heterochromatin and euchromatin in a nucleus.

Mid-third instar larvae of *D. nasuta* were transferred to fresh food supplemented with ^{3}H-thymidine (5μCi/g food; specific activity of ^{3}H-thymidine stock solution, obtained from BARC, Trombay, was 12.4Ci/mM). The larvae were fed on this food for 46h after which the wing imaginal disks were dissected out in Ringer and immediately fixed in freshly prepared aceto-methanol (1:3). Air-dried preparations (Lakhotia & Kumar, 1978) of disks were treated with 5% trichloroacetic acid at 4°-6°C for 10 min, washed, stained with carbol-fuchsin and processed for autoradiography with Ilford L4 emulsion. After an expo-

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sure of 35 days, the autoradiographs were developed with Kodak d19b, fixed, washed, dried and mounted with D.P.X.. The autoradiographic labelling patterns of interphase nuclei were classified into the following four categories on the basis of distribution of silver grains on chromocentre and non-chromocentre regions: (1) Completely-labelled nuclei – with labelling on the heterochromatic chromocentre (cc) as well as on the non-chromocentric (ncc) regions of a nucleus; (2) Euchromatic-labelled nuclei – with less than 3 grains on cc and more than 5 grains on ncc regions; (3) Heterochromatic-labelled nuclei – with more than 3 grains on cc and less than 5 grains on cc regions, and (4) Unlabelled nuclei – with less than 3 grains on cc and less than 5 grains on ncc regions.

Observations and discussion

In most of the labelled nuclei, the grain density was much higher than the minimum grain number required for the nucleus to be considered labelled. Representative examples of completely labelled and euchromatin-labelled type nuclei are shown in Figure 1. In all the autoradiographic preparations, each made from wing imaginal disks of 2 or 3 larvae, these two categories of labelled nuclei were most common. Data on the frequencies of different labelling patterns in 4 different autoradiographic preparations are presented in Table 1. It is seen that in these preparations, the heterochromatin-labelled and unlabelled nuclei are very few or totally absent, except in the preparation no. 4 in which about 11% nuclei are unlabelled. The relative frequencies of completely labelled and euchromatin-labelled type nuclei vary considerably from one preparation to another. Since the larvae used for in vivo labelling with ^{3}H-thymidine were not selected for a specific age, the large differences in the proportion of completely labelled and euchromatin-labelled type nuclei in the four preparations are, presumably, related to variations in the age of larvae from which the wing imaginal disks were obtained (see below). However, the important point is that in all preparations of wing imaginal disks examined, a large number of nuclei have labelling restricted to their euchromatic regions only. It may be mentioned that the absence of any silver grains over the cc region in these nuclei is apparently due to lack of ^{3}H-thymidine incorporation in heterochromatin rather than to any technical limitations. The area of the cc region in *D. nasuta* nuclei is sufficiently large to appear distinctly labelled or unlabelled. Besides, if the cc region had incorporated ^{3}H-thymidine along with euchromatin, it should show a higher autoradiographic grain density because of greater chromatin density in heterochromatin. This is borne out by the distinct and heavy labelling (upto 20-30 silver grains) of the cc region in most nuclei of the completely labelled cate-

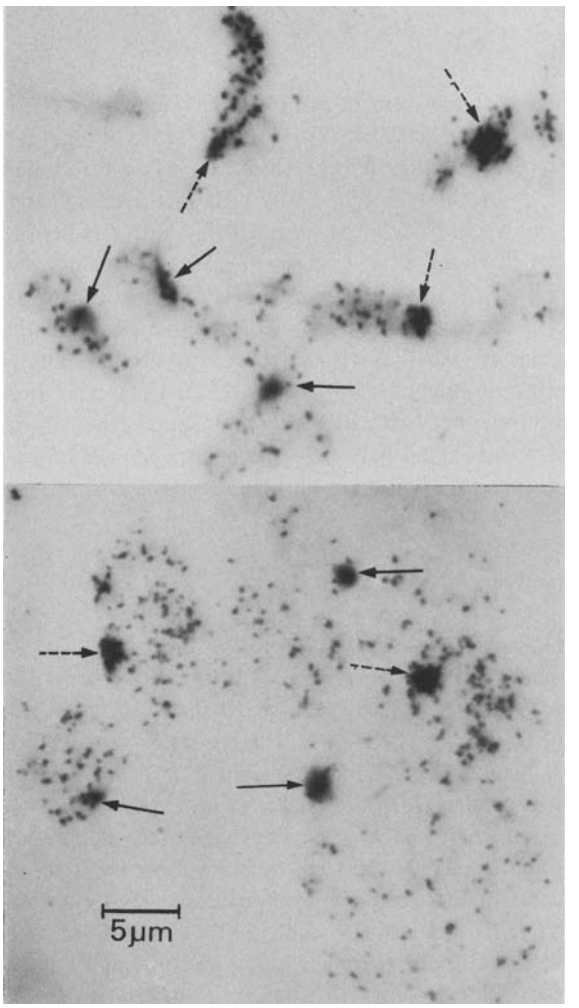

Fig. 1. Autoradiographs of two groups of interphase nuclei from wing imaginal disks of 3rd instar larvae of *Drosophila nasuta* labelled in vivo with ^{3}H-thymidine for 46h. Completely labelled and euchromatin-labelled type nuclei are seen. The euchromatin in all nuclei is moderately to heavily labelled while the chromocentre in some nuclei (⟶) is heavily labelled and in others totally unlabelled (→).

Table 1

Frequency of different types of autoradiographic labelling patterns in wing imaginal disk nuclei from 3rd instar larvae of *Drosophila nasuta* grown on ^{3}H-thymidine supplemented food

Preparation No.	Labelling Patterns (%) Completely labelled	Euchromatin-labelled	Heterochromatin-labelled	Unlabelled	Total nuclei observed
1	11.6	87.4	0.5	0.5	206
2	85.3	14.7	0.0	0.0	258
3	67.5	32.5	0.0	0.0	163
4	37.0	46.5	0.0	11.5	208
Average	50.3	46.5	0.1	3.0	835

gory. In the majority of the euchromatin-labelled type nuclei, the grain density on euchromatin is moderate to heavy and yet the cc region in these nuclei appears entirely devoid of any silver grains.

In this study, ^{3}H-thymidine was made available to growing third instar larvae for a period of 46h. ^{3}H-thymidine administered to *Drosophila* larvae through food gets quickly and specifically incorporated into DNA of various replicating cell populations and its autoradiographic localization provides precise information about the replicative status of different cells or different nuclear components (Lakhotia, 1974; White & Kankel, 1978). During third instar stages, the imaginal disks grow rapidly and, as expected, very few cells were unlabelled with ^{3}H-thymidine in the present material. The average cell cycle duration in imaginal disk of *Drosophila* larvae has been estimated to range from 6 to 15h (Nöthiger, 1972). Thus, during the long period of in vivo labelling (46h) in the present study, most cells of wing imaginal disks would have undergone more than one replication cycle in presence

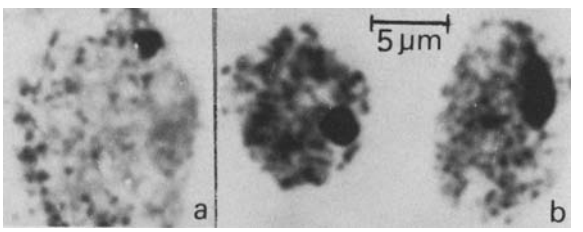

Fig. 2 Giemsa stained interphase nuclei from 6h old embryo (a) and wing imaginal disk of late third instar larva (b) of *D. nasuta*. In both cell types, all the heterochromatic regions form one dark stained chromocentre. Note the much larger chromocentre region in imaginal disk nuclei.

of ^{3}H-thymidine. Normally, the autoradiograms of such cells should show labelling of eu- as well as hetero chromatic regions of nuclei. However, in this study, the ^{3}H-thymidine labelling was seen to be restricted to euchromatic regions in a significant proportion (about 46% on average, see Table 1) of cells. This implies that during the 46h period, the heterochromatic cc region in these nuclei did not incorporate ^{3}H-thymidine while the euchromatic regions did. The possibility that ^{3}H-thymidine was not available to replicating heterochromatin or that heterochromatin utilizes a different pool of thymidine, is unlikely in view of the presence of heavy labelling of heterochromatin with ^{3}H-thymidine taken with food in many other cells of the same disk.

The presence of cells in which only euchromatin incorporates ^{3}H-thymidine during the 46h period is suggestive of occurrence of endo-reduplication cycles in which the heterochromatic regions remain underreplicated. In such cells, euchromatin may replicate more often than heterochromatin, and, therefore, in some such cells, the cc region may appear totally unlabelled with ^{3}H-thymidine. Occurrence of endoreduplication cycles in imaginal disk cells is also suggested by comparison of nuclear areas, and more particularly, the cc area in embryonic cells and in cells from wing imaginal disks of late larvae of *D. nasuta* (Fig. 2). As can be seen in Figure 2, the heterochromatic cc area is considerably larger in wing imaginal disk cells than in embryonic cells which are expected to be at 2C/4C DNA levels. The significant increase in the area of the condensed cc in wing imaginal disk cells suggests an increased DNA content in these nuclei. The increase in the size of the cc and the lack

of ^{3}H-thymidine incorporation in this region in many of the wing imaginal disk cells, suggest that the replicative organization of hetero- and eu-chromatic regions in wing imaginal disk cells of late third instar larvae of *D. nasuta* may be similar in some respects to that noted by Berendes & Keyl (1967) for the larval brain cells in *D. hydei*. As in larval brain cells (Berendes & Keyl, 1967), it is possible that in wing imaginal disk cells also, both the eu-chromatic and heterochromatic regions of a nucleus undergo endo-reduplication cycles, but the heterochromatic cc may replicate fewer times than the ncc regions, so that during the later endo-reduplication cycles, only the ncc regions incorporate ^{3}H-thymidine (as seen in euchromatin-labelled type nuclei). The DNA content of individual nuclei of imaginal disk cells of *Drosophila* has not been measured, but the available information on the total DNA content and the estimated cell numbers in a larval imaginal disk are not incompatible with presence of cells with increased DNA content. As discussed by Fristrom (1972), if each of the wing imaginal disk cells of *D. melanogaster* larvae is assumed to contain DNA at the 2C level, the estimate of cell numbers on the basis of total DNA content of an average imaginal disk is much higher than the estimate based on cell-lineage studies. Fristrom (1972) has considered several factors, such as under-estimate of cell numbers from cell-lineage studies and under-estimate of the actual 2C DNA content, to be the cause for this large discrepancy in the two estimates. Alternatively, however, it may also be suggested that the discrepancy between the total DNA content of an imaginal disk and the estimated cell numbers can be due to the presence of endo-reduplicated cells with DNA content in excess of 2C/4C levels. Occurrence of endo-reduplication cycles in imaginal disks of *Drosophila* larvae satisfactorily explains the present observations on the increased nuclear size and the lack of ^{3}H-thymidine incorporation in cc regions in many cells of the wing imaginal disks of 3rd instar larvae of *D. nasuta*. It is possible that during early stages, cc and ncc regions replicate equally, but as development proceeds, many cells of imaginal disks are triggered to enter endo-reduplication cycles in which the heterochromatic regions replicate fewer times compared to the euchromatic regions. This developmental regulation of disproportionate replication in different cells of a disk may be related to their differentiation and may also explain the large variation in the frequency of completely-labelled and euchromatin-labelled type nuclei in the different preparations examined (Table 1). Further studies on the developmental aspect are in progress. The almost total absence of cells in which the heterochromatin only is labelled, suggests that in wing imaginal disks of 3rd instar larvae of *D. nasuta*, overreplication of heterochromatin does not occur.

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