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Extent of polyteny in the pericentric heterochromatin of polytene chromosomes of pseudonurse cells of *otu* (*ovarian tumor*) mutants of *Drosophila melanogaster*

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Abstract. In the polytene nuclei of germ-line cells (ovarian pseudonurse cells) of *Drosophila melanogaster* females mutant for *otu*^{11} (*ovarian tumor*), the pericentric heterochromatin is much more abundant than in somatic salivary gland cells. This is due to the degree of heterochromatin compaction (and consequently the level of underreplication) being lower in the nurse cells than in the salivary gland cells. The lower level of compaction probably results in a very low degree of position effect gene inactivation in the ovarian nurse cells.

Key words: Polytene pseudonurse cell chromosomes – Underreplication – α- and β-heterochromatin – Position effect variegation

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E. Heitz (1934) distinguished two kinds of heterochromatin, α and β, in the polytene nuclei of *Drosophila*. α-Heterochromatin is very dense, compact and stains heavily. β-Heterochromatin is seen as a net-like granular mass. Heitz (1934) proposed that the β-heterochromatin of mitotic chromosomes is completely represented in polytene chromosomes, but that α-heterochromatin is underrepresented. All subsequent cytogenetic and molecular data support his proposal. Rudkin (1965) has shown by cytophotometry that heterochromatin in polytene nuclei is underreplicated; Gall and collaborators (1971), using in situ hybridization, demonstrated that the quantity of satellite DNA in the heterochromatin of mitotic and α-heterochromatin of polytene chromosomes is similar. The latter was also shown for the quantity of material stained with Hoechst 33258 (Lakhotia 1984).

The generally accepted view of β-heterochromatin is that it is completely (or almost completely) replicated in

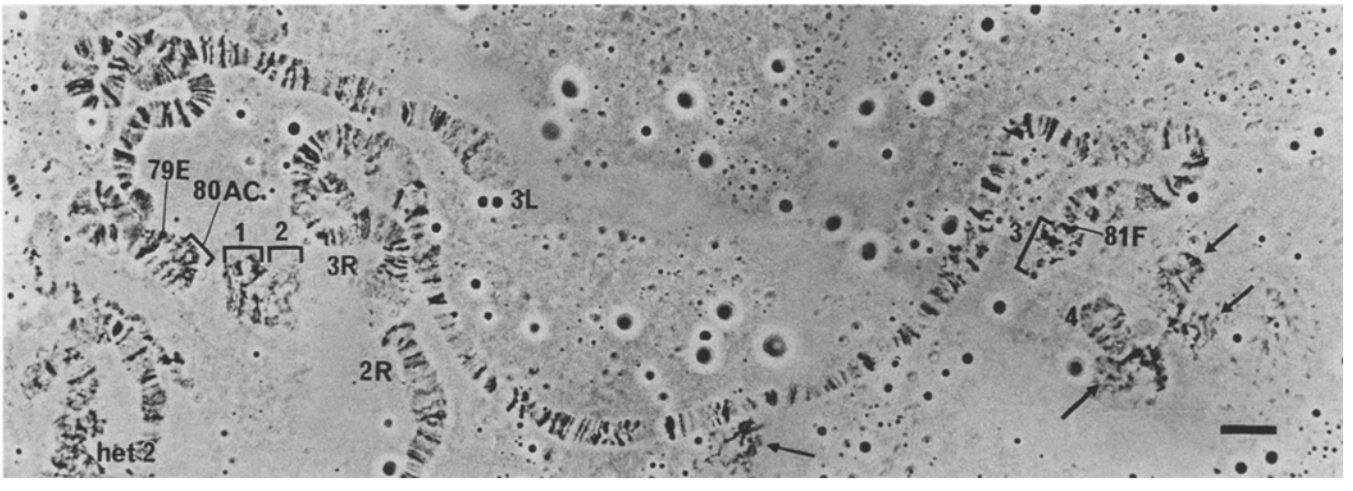

Fig. 1. General view of the third and fourth chromosomes in a nurse cell nucleus of female *y w otu*^{11} *sn*^{3}/*y w otu*^{11} *sn*^{3}/*T(1;Y)y*$^{+}$. *Brackets* mark additional blocks of pericentric heterochromatin. *Arrows* indicate blocks of heterochromatin; het2 is the heterochromatin of the second chromosome. *Bars* in all figures represent 10 μm

polytene nuclei (Gall 1973; Yamamoto et al. 1990), at least in salivary gland chromosomes. There is, however, evidence that in ovarian pseudonurse cells of *Drosophila* the level of underreplication of "heterochromatic" DNA sequences is lower than is salivary glands: (i) According to Endow and Gall (1975) satellite DNA in ovaries of *Drosophila* imago and prepupae is present in much higher proportion then even in diploid cells of larval brains. (ii) The degree of underreplication of 18S and 28S ribosomal RNA cistrons in ovaries is significantly lower than in salivary glands: in polytene chromosomes of *Drosophila,* the relative quantity of these genes is about 20% of that in ovaries (Hennig and Meer 1971; Spear 1974), in nurse cells it is about 80% (Jacob-Lorena 1980) and in *Calliphora erythrocephala* about 130% (Renkawitz and Kunz 1975) of their proportion in diploid cells. (iii) In *Drosophila melanogaster* ovaries, expression of position effect variegation is not found (Slobodyanyuk and Serov 1987). (iv) In the pseudonurse cell polytene chromosomes of *D. melanogaster* females mutant for *otu (ovarian tumors)* or *fs(2)B,* the frequency of "weak points" and, consequently, the degree of underreplication of intercalary heterochromatin, is lower than in the salivary gland chromosomes (H. Gyurkovics, personal communication; Heino 1989).

Polytene chromosomes with a clear-cut banding pattern can be found in the pseudonurse cells of *D. melanogaster* females homozygous for the mutation otu^{11} (Fig. 1). The frequency of ovaries showing well banded chromosomes (Figs. 1, 2) is markedly increased in XXY females.

The chromosome arms 3L and 3R and practically all heterochromatic elements of the other chromosomes (except the X) from otu^{11} pseudonurse cells are shown in Fig. 1. On chromosome arm 3L, there are two chromosomal blocks proximal to region 80AC, the most proximal euchromatin recognisable in salivary gland chromosomes (Figs. 1, 2). The first of these blocks has several bands and a puff, the second is more diffuse. The blocks are separated from each other, and from region 80C, by weak points.

In chromosome 3R of the pseudonurse cells, a large mass of granular heterochromatin is observed proximal to region 81F (Fig. 1). This is always absent from salivary gland chromosomes (Fig. 3a). The pattern of additional heterochromatin in proximal parts of both arms of the third chromosome is highly reproducible (Figs. 2 and 3).

In addition to these blocks of heterochromatin at the bases of the third chromosome arms in pseudonurse cell nuclei, other heterochromatic blocks are also visible. The chromocentral mass on the second chromosome (het2 in Fig. 1) is somewhat larger than in salivary glands. On the X chromosome, the distal part of division 20 has a clear banding pattern. This is very difficult to see in salivary gland chromosomes (data not shown). Several unattached heterochromatic blocks, of unknown origin, are also seen in *otu/otu* pseudonurse cell chromosomes. In all, the mass of the chromocentral heterochromatin is much greater in the pseudonurse cells than in the salivary glands.

These heterochromatic blocks, both those attached to the chromosome arms and those that are unattached, are

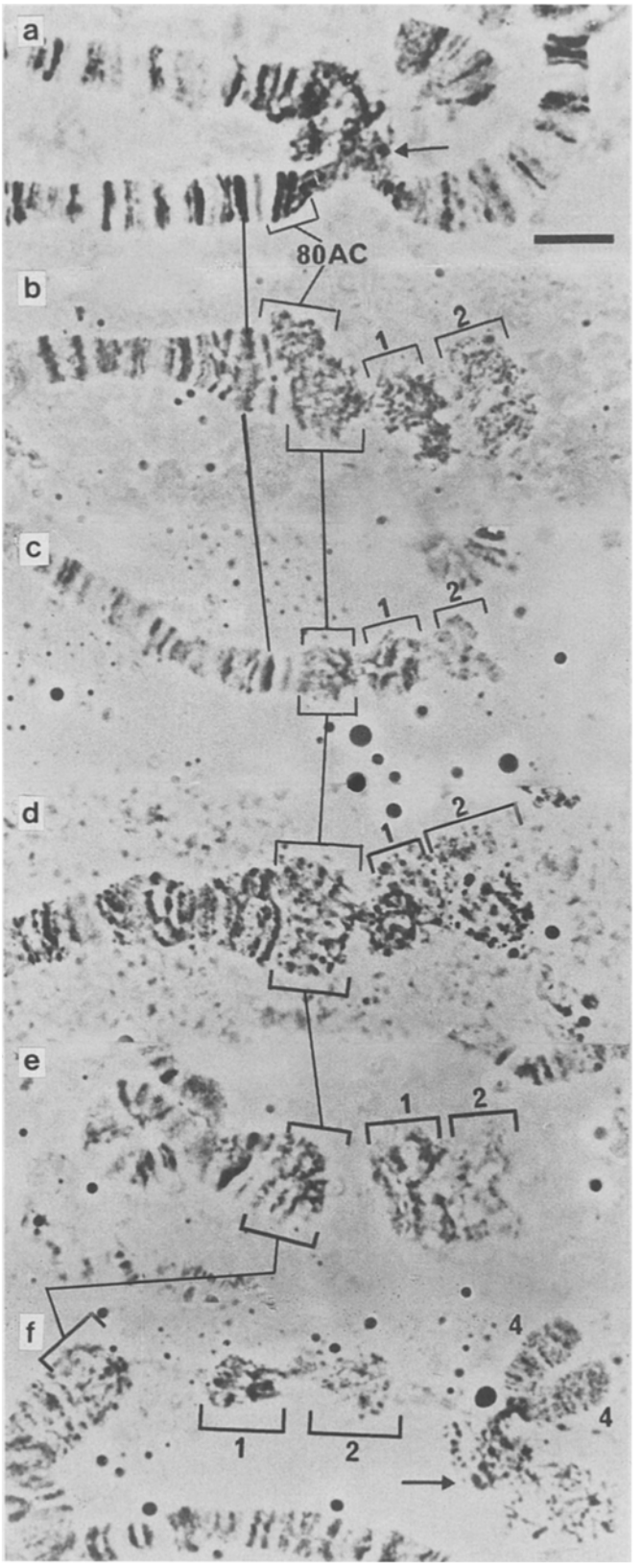

Fig. 2a–f. Pericentric heterochromatin in chromosome 3L of the salivary gland cells of larvae of the genotype $XY^{L}Y^{S}$, *y su(w^{a})w^{a}* (a) and in the nurse cells of *y w* otu^{11} sn^{3} homozygotes (b, d, f) and *y w* otu^{11} $sn^{3}/T(1;Y)y^{+}$ (c, e). The *brackets* mark additional heterochromatic blocks

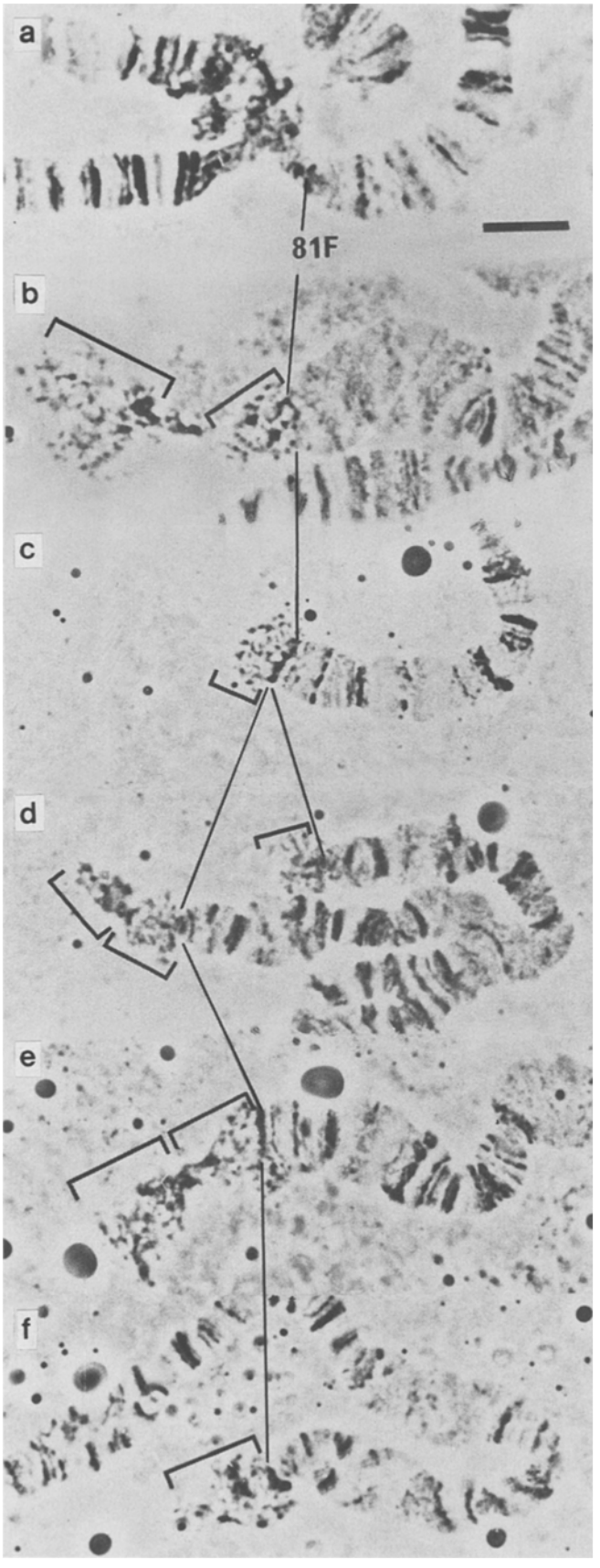

Fig. 3a–f. Pericentric heterochromatin in chromosome 3R of the salivary gland cells of larvae of the genotype $XY^{L}Y^{S}$, *y su(w^{a})w^{a}* (a) and in the nurse cells of *y w otu^{11} sn^{3}* homozygotes (c–e) and *y w otu^{11} sn^{3}/y w otu^{11} sn^{3}/T(1;Y)y^{+}* (b, f)

seen in all nuclei with well banded polytene chromosomes available for analysis.

What is the origin of the additional heterochromatic material seen in pseudonurse cell nuclei? There are at least two possible sources. (i) The α-heterochromatin in these cells could be partially polytenized. If so, the extra material would consist largely of satellite DNA sequences. (ii) The new material could be β-heterochromatin, and would be enriched in moderately repetitive DNA sequences. If this were true, it would imply that the β-heterochromatin of the salivary gland nuclei is, in fact, underreplicated, in contrast to the prevailing view (Heitz 1934; Gall 1973; Yamamoto et al. 1990). Perhaps the β-heterochromatin represents a much greater part of the heterochromatin seen in mitotic chromosomes, and not just the junction between euchromatin and α-heterochromatin (Yamamoto et al. 1990).

Heino (1989) has described the ovarian pseudonurse polytene chromosomes in *otu^{11}* mutants, but does not mention the existence of additional heterochromatin blocks, and indeed they are not apparent in his Figures. One of the possible reasons for this is the very frequent breakage of these blocks. As was mentioned above, there are "weak points" at the junctions between them and euchromatin parts of the chromosomes.

These data show that the systems controlling the process of heterochromatinization in germ-line cells (nurse cells) are different from those in somatic cells (salivary glands). The less marked heterochromatinization, in terms of compaction, at the junction between euchromatin and heterochromatin seen in the nurse cells may account for the absence of position-effect inactivation in these cells.

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