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ULTRASTRUCTURE OF THE CHROMOCENTRE REGION IN SALIVARY GLAND POLYTENE NUCLEI OF *DROSOPHILA HYDEI*

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It is well known that during the process of polytenization in salivary gland nuclei of *Drosophila* larvae, the heterochromatic centric and pericentric regions of different chromosomes fuse together to form the chromocentre. On the basis of condensation and other cytogenetic features, Heitz (1934) distinguished between the 'alpha'- and 'beta'-heterochromatin in these nuclei. Electron microscopic studies have shown that there are two distinct types of heterochromatin organization in the chromocentre region in salivary gland polytene nuclei of *D. melanogaster* (Lakhotia and Jacob 1974; Lakhotia 1974). In the present paper, the ultrastructure of the chromocentre heterochromatin in larval salivary gland nuclei of *Drosophila hydei* is briefly reported.

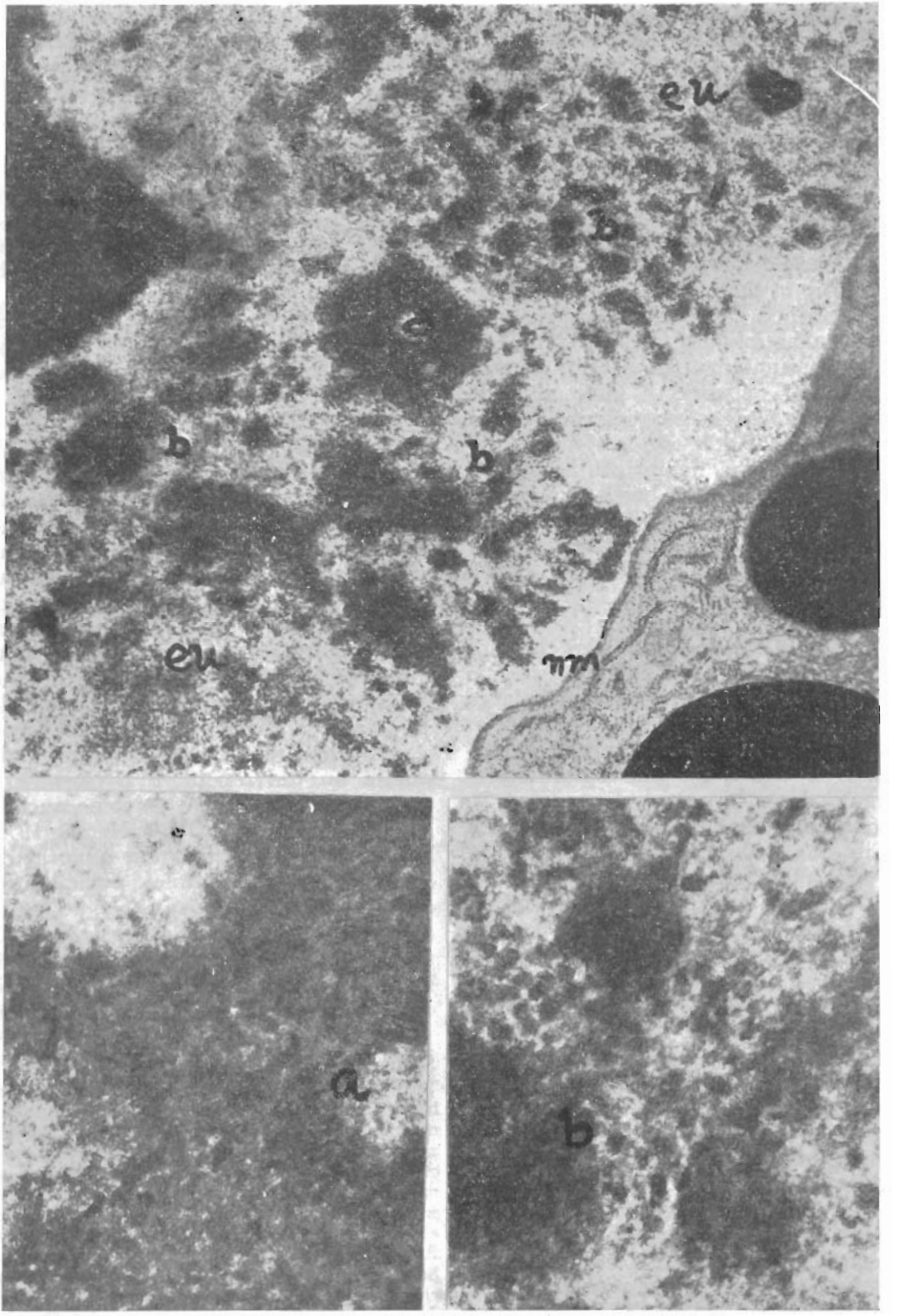
Material and Methods

Salivary glands from third instar larvae of *Drosophila hydei* were double fixed in glutaraldehyde and osmium tetroxide and embedded in Epon. Serial ultra thin sections were double stained with uranium and lead salts by the usual procedure and examined under a Phillips EM 300 electron microscope.

Observations

The general ultrastructure of the chromocentre heterochromatin in *D. hydei* polytene nuclei is strikingly similar to that seen in *D. melanogaster* (Lakhotia and Jacob 1974,

Figs 1-3: Electron micrographs of salivary gland polytene nucleus of a third instar larva of *D. hydei* showing the chromocentre region. a = alpha heterochromatin; b = beta heterochromatin; eu = euchromatin; nm = nuclear membrane; Fig 1: A low magnification micrograph of a section through the chromocentre region. The chromocentre, organized into the alpha and beta heterochromatin, is easily identifiable by its proximity to the nuclear membrane, high electron density and lack of band and interband organization. A large numberof RNP-like particles may be noted in the beta heterochromatin area. × 16,600. Fig 2: A high resolution micrograph of a part of the alpha heterochromatin shown in fig 1. Densely packed fibrils (about 30 Å thick) may be noted. Less electron dense areas are also sometimes seen in the alpha heterochromatin mass. × 1,35,000. Fig 3: A high resolution micrograph of part of the beta heterochromatin area seen in the fig 1. Dense blocks with associated RNP-like particles may be noted. × 75,000.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Sorsa 1969). The chromocentre region is located near the nuclear membrane and is considerably more electron dense than the euchromatic regions (fig 1). Unlike the highly organized band and interband regions, the chromatin fibrils in this region show a disorganized appearance. However, within the chromocentre region, two distinct parts are recognizable (fig 1). In the centre, there is a large single block of densely packed chromatin fibrils (fig 2), comparable to the alpha heterochromatin block described earlier in *D. melanogaster* nuclei (Lakhotia and Jacob 1974). This block is surrounded on all sides by an area of a kind of network of interconnected smaller dense blocks; it corresponds to the beta heterochromatin region described in *D. melanogaster* and finally continues into the euchromatin at the bases of different chromosome arms (fig 1). The constituent fibrils in all the regions of the chromocentre heterochromatin are about 30 Å thick (figs 2, 3).

Again as in *D. melanogaster*, the chromocentre heterochromatin in *D. hydei* is characterized by the presence of a large number of ribonucleoprotein (RNP)—like particles throughout the beta heterochromatin area (figs 1, 3). The alpha heterochromatin lacks any type of particles. So far, only one type of particles has been seen in the beta heterochromatin of *D. hydei* and they measure about 300 Å in diameter. They seem to be more abundant in the immediate vicinity of the electron dense chromatin blocks.

Discussion

The ultrastructure of the chromocentre heterochromatin in the larval salivary gland polytene nuclei of *D. hydei* shows remarkable similarity with the chromocentre organization in *D. melanogaster* polytene nuclei (Lakhotia and Jacob 1974). Both species show a compact block of alpha heterochromatin surrounded by an area of network of dense chromatin masses, referred to as the beta heterochromatin. In both, the beta heterochromatin also shows a large number of RNP-like particles measuring about 250-300 Å in diameter. However, in *D. melanogaster*, another class of RNP-particles (about 450 Å diameter) were also seen to be clustered in a restricted area of beta heterochromatin, presumably the base of the X-chromosome (Lakhotia and Jacob 1974). Comparable particles have not yet been seen in *D. hydei* chromocentre, but more extensive studies are needed for this. Nevertheless, the abundance of at least one type of RNP-like particles in the beta heterochromatin of *D. hydei* suggests that as in *D. melanogaster* (Lakhotia and Jacob 1974), *D. hydei* chromocentre heterochromatin may also be active in transcription.

In *D. melanogaster*, EM autoradiographic studies have very clearly shown that the alpha heterochromatin region does not undergo any replication during the whole of polytenic growth of salivary glands, while the beta heterochromatin replicates in step with the euchromatin in these nuclei (Lakhotia 1974). In view of the remarkable similarity in the organization of the chromocentre heterochromatin in these two species, it seems that in *D. hydei* also, the alpha heterochromatin block represents the non-replicating chromatin. Other studies on the repetitive DNA base sequences in diploid and polytene nuclei of *D. hydei* (Hennig *et al* 1970; Hennig 1972a, b) have also suggested that some heterochromatic regions in diploid nuclei, enriched in repetitive base sequences, do not undergo polytenic replication in salivary gland nuclei. These heterochromatic repetitive DNA-rich nonreplicating regions may correspond to the alpha heterochromatin block.

Summary

The ultrastructural organization of the chromocentre heterochromatin in larval salivary gland polytene nuclei of *Drosophila hydei* has been studied. It is organized into a central large mass of densely packed chromatin (alpha heterochromatin) which is surrounded on all sides by a network of interconnected smaller blocks of dense chromatin (beta heterochromatin). Within the latter, a large number of RNP-like particles (about 300 Å diameter) have been seen, suggesting a transcriptionally active chromocentre heterochromatin. It is also suggested that as in *D. melanogaster*, in *D. hydei* also, the alpha

heterochromatin mass does not undergo polytenization in salivary gland nuclei, while the beta heterochromatin replicates together with the euchromatin.

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J. Derksen from Nijmegen has sent some electron micrographs of *D. hydei* polytene nuclei in which a part of the chromocentre region (beta heterochromatin) displays a large number of RNP-like particles about 450 Å in diameter, showing striking similarity with the larger type of particles (450-500 Å) reported earlier in *D. melanogaster* (Lakhotia and Jacob 1974). The two may be comparable.