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Repeated Sequences in the DNA of *Drosophila* and their Localization in Giant Chromosomes

Wolfgang Hennig, Ingrid Hennig and Hans Stein (1970)
Max-Planck-Institut für Biologie, Abt. W. Beermann, Tübingen

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Abstract. It is shown by isopycnic density gradient centrifugation that the DNAs of the sibling species *Drosophila hydei*, *Drosophila neohydei* and *Drosophila pseudoneohydei* differ regarding the numbers and proportions of satellite DNA bands. An overwhelming proportion of all repetitive nucleotide sequences of the DNA is contained in these satellite fractions. The majority of the satellites are species specific despite the close phylogenetic and cytological relationship between the three species studied. — By *in situ* hybridization experiments it is demonstrated that the various satellite sequences occupy different positions within the chromosomes. All types of localization patterns, from a wide spread occurrence in all chromosomes to an apparent restriction to kinetochore regions of single chromosomes, have been observed. Main band DNA, on the other hand, in its hybridization behavior reflects the DNA distribution according to the banding pattern in giant chromosomes. Generally satellite sequences seem to be included in α-heterochromatic chromosome regions but no relation to the heterochromatin of the Y-chromosome was found. — Renaturation studies support various evidence that satellite sequences occur in tandemly repetitious units. At least some of this repetitious material seems to be linked to non-satellite DNA sequences or to DNA of other satellites.

Introduction

Since repetitive DNA sequences in the genome of higher organisms have been described by Britten and his coworkers (Britten, 1965, 1967, 1968, 1969; Britten and Kohne, 1968, 1969a, b) many investigations dealing with the occurrence and nature of such sequences have been started. Nevertheless the distribution of repeated nucleotide sequences within the genome and their biological significance is still rather obscure. Only in the case of mouse satellite DNA has some evidence been obtained regarding its position within metaphase chromosomes (Jones, 1970; Pardue and Gall, 1970). By *in situ* hybridization it was demonstrated that mouse satellite DNA is highly concentrated in the kinetochore regions of the chromosomes and similarly Jones and Robertson (1970) gave evidence for an enrichment of highly repetitive DNA in the kinetochore regions of *Drosophila melanogaster*.

The only way to extend our knowledge on repeated DNA sequences and their biological role is the isolation of biologically defined DNA fractions. *Drosophila* is especially useful for any approach in isolating specific

fractions of the genome since the genome is relatively small and a wide background of genetical and cytological data is available which could be useful for understanding the biological role of specific DNA sequences. In light of earlier studies on the morphogenetic processes in the spermatogenesis of *Drosophila hydei* (Hennig, 1968) we became interested in the DNA composition of *D. hydei* and two related species or subspecies which give a small number of offspring in crossing them with *D. hydei* or with one another.

The existence of various different density satellites within the three species prompted a detailed study of the properties and chromosomal localization of such sequences since the possibility to obtain species hybrids may be especially useful in elucidating the biological function of satellite sequences. As will be shown in this first communication the sequences of some satellites are widely spread throughout the chromosomes, while others are strictly limited to specific chromosome regions. Homologous satellite sequences also seem to have different distributions in the different species.

Materials and Methods

1. Genetical and Cytological Methods

In our experiments stocks of *Drosophila hydei*, *Drosophila neohydei* and *Drosophila pseudoneohydei* were used. The stocks of *D. neohydei* and *D. pseudoneohydei* were initially thought to be identical. Later it was found that the spermatocyte nuclei are different in their Y-chromosome structures (cf. Hess and Meyer, 1968). It also turned out that the formation of genetic hybrids between both stocks is difficult. Therefore the name *Drosophila pseudoneohydei* was introduced for the stock differing in its Y-chromosome structures from the originally described *D. neohydei*. So far no taxonomical description is available. A detailed cytological description of the hybrids between the different stocks will be published separately (I. Hennig, in preparation).

In all interspecific crosses a *w lt* mutant of *D. hydei* was used to check the origin of offspring. Breeding was carried out as described earlier (Hennig, 1967).

Salivary gland squash preparations were obtained by dissecting third instar larvae and removing the glands in hemolymph. The glands were fixed with ethanol acetic acid (3/1, vol/vol) for 15 seconds and then transferred to 45% acetic acid. After 5 min the glands were squashed on a gelatinized slide, frozen on dry ice and the coverslip removed with a razor blade. Slides then were dipped into 70% ethanol and transferred to 70%, 80%, 90% and absolute ethanol. After drying slides were kept in a desiccator.

2. Preparation of DNA

For DNA isolation flies were kept for two days on food lacking fresh yeast and then starved for at least 24 h before freezing at −70° C. About 40 g of frozen flies were ground in a mortar, transferred to a Dounce homogenizer and homogenized with 250 ml cold glycine buffer (glycine NaCl, pH 9.6, Na^+ concentration 0.15 M). After extensive homogenization at low temperature the suspension was filtered through 1100 µ mesh nylon gauze. The residue was again homogenized and filtered, and the whole suspension sieved through a 800 µ mesh gauze to remove chitin