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# Electron Microscopic Observations on Interband Fibrils in *Drosophila* Salivary Chromosomes

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*Abstract.* Acetic alcohol squash preparations of salivary chromosomes of *Drosophila melanogaster* stained with uranylacetate during fixation and dehydration were sectioned and examined with the electron microscope. At high magnification the interband regions are seen to be composed of coiled fibrils in the range of 50 Å which often seem to be arranged in pairs. Submicroscopic bodies found along the interband fibrils seem to delimit successive subunits. It is suggested that transverse coiling of the fibrils within the boundaries of each subunit leads to the formation of chromomeres.

## Introduction

The former controversy about the structure of the giant chromosomes has by now been settled on convincing evidence in favour of the polytene hypothesis proposed by KOLTZOFF (1934) and BAUER (1935), and later elaborated especially by BAUER and BEERMANN (1952), BEERMANN (1952, 1956) and BEERMANN and PELLING (1965).

Early electron optical studies of polytene chromosomes, depending on the technique used, gave more or less clear evidence of longitudinal striations in the interband areas (PALAY and CLAUDE, 1949; PEASE and BAKER, 1949; YASUZUMI, ODATE and OTA, 1951; HEILWEIL, HEILWEIL and v. WINKLE, 1952; HERSKOWITZ, 1952). The submicroscopic structure has later been more accurately examined in *Drosophila* (KAUFMANN and McDONALD, 1956; LOWMAN, 1956; SWIFT, 1962; GAY, 1964, 1965) as well as in the puffs of *Chironomus* (BEERMANN and BAHR, 1954; STEVENS and SWIFT, 1966).

## Material and Methods

Salivary glands of *Drosophila melanogaster* (stock "Berlin wild") third instar larvae were dissected out and fixed in acetic alcohol (3:1) saturated with uranyl acetate for 15 minutes. The material was then squashed on a slide in a drop of 45% acetic acid. Dehydration was carried out in a series of alcohols (70, 95, 99.5%) all saturated with uranyl acetate (by E. Merck AG.). The plastic capsules filled with araldite final embedding mixture (Ciba 212) were then adjusted upside-down on the slide. When polymerization had occurred the capsules, thus having the squashed salivary material on top, were pried from the slides after rapid cooling on dry ice. The technique used has been described in an earlier paper (SORSA and SORSA, 1967).

Sectioning was carried out with a Porter-Blum Ultramicrotome MT-1 with glass knives. 200 mesh copper grids coated with formvar film were used.

The sections were examined in a Philips EM 200 electron microscope, 80 kV, with original magnifications from 4000× to 40,000×. Examination and photography were carried out at the Electron Microscope Laboratory, University of Helsinki.

## Observations

Although, as a fixative, acetic alcohol is known to destroy badly the membraneous fine structure of the cytoplasm and also to shrink the fibrillar elements in the chromosome to some extent (Dales, 1960; Montezuma-de-Carvalho and Crawley, 1960; Ris, 1961), it gives a good contrast in the electron microscope when combined with uranyl

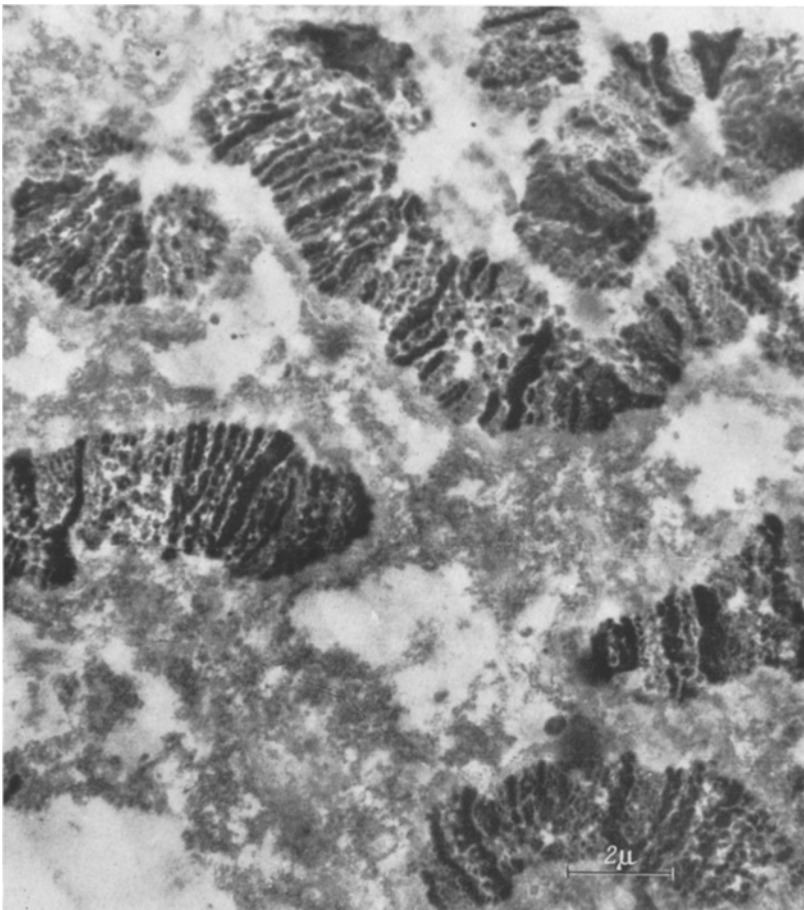


Fig. 1. Low power electron micrograph of *Drosophila* salivary gland chromosomes by squash technique and uranyl acetate staining. ×7,000

Fig. 2. Electron micrograph of a limited region of a salivary chromosome. Longitudinal fibrils and small granular chromomeres are clearly seen in the interband areas. The homology of the bands and the small chromomeres is in most cases beautifully exact. ×71,000

acetate staining, as has been reported earlier (SORSA and SORSA, 1967). Since uranyl acetate is considered to be highly specific stain for DNA (HUXLEY and ZUBAY, 1961; ZOBEL and BEER, 1961), this method seems to be appropriate for chromosome studies at the electron microscope level.

Due to the squash technique used, the chromosomes are mostly sectioned parallel to the plane of the slide. At low magnification (Fig. 1) the electron micrographs of the salivary chromosomes show characteristic dark banding with lighter interband areas. The pattern of banding is mostly beautifully precise even in the smaller details (Fig. 2). The "interband" areas are characterized by numerous small chromomeric granules, which vary in size in different interbands, but are mostly of the same size in homologous chromatids within the same interband. The number of "bands" at the electron microscope level becomes at least doubled as compared with the light microscope picture of the same areas (BEERMANN and BAHR, 1954). The chromomeres and the bands mostly appear too compact in the micrographs to yield good evidence of their structure.

Micrographs of well stretched polytene chromosomes at high magnification (Figs. 2, 3, 4) show distinct fibrillar structure in the interband regions. In most high magnification micrographs the interband fibrils, each about 120—170 Å wide, seem to be composed of two smaller fibrillar subunits, about 50—60 Å wide (Fig. 3, see also Figs. 2 and 4). The pairs of fibrils run parallel between the chromomeres. In some favourable points the interband fibrils are seen to be delicately coiled, the width of the coils being about 130 Å. Small, apparently cylindrical particles, about 200 Å wide, have occasionally been observed along the interband fibrils (Fig. 4). Sometimes a transverse arrangement of such a particle is indicated. We interpret small dots in close contact with chromomeres as representing the same units (cf. Fig. 5).

## Discussion

Two contrary views have been held as to whether or not the interband areas in polytene chromosomes contain DNA (for references, see WOLSTENHOLME, 1965). In a recent paper, WOLSTENHOLME (op. cit.) has, however, convincingly demonstrated by fluorescence microscopy that there are no light-microscopic gaps in the DNA across the interband regions.

Since each of the fibrillar chromosome units in the giant polytene chromosome should be homologous to a single chromatid of a normal diploid cell (e. g. BEERMANN and PELLING, 1965) there is no reason to believe that the basic structure of the polytene chromosome differs in any fundamental respect from that of the ordinary chromosome.

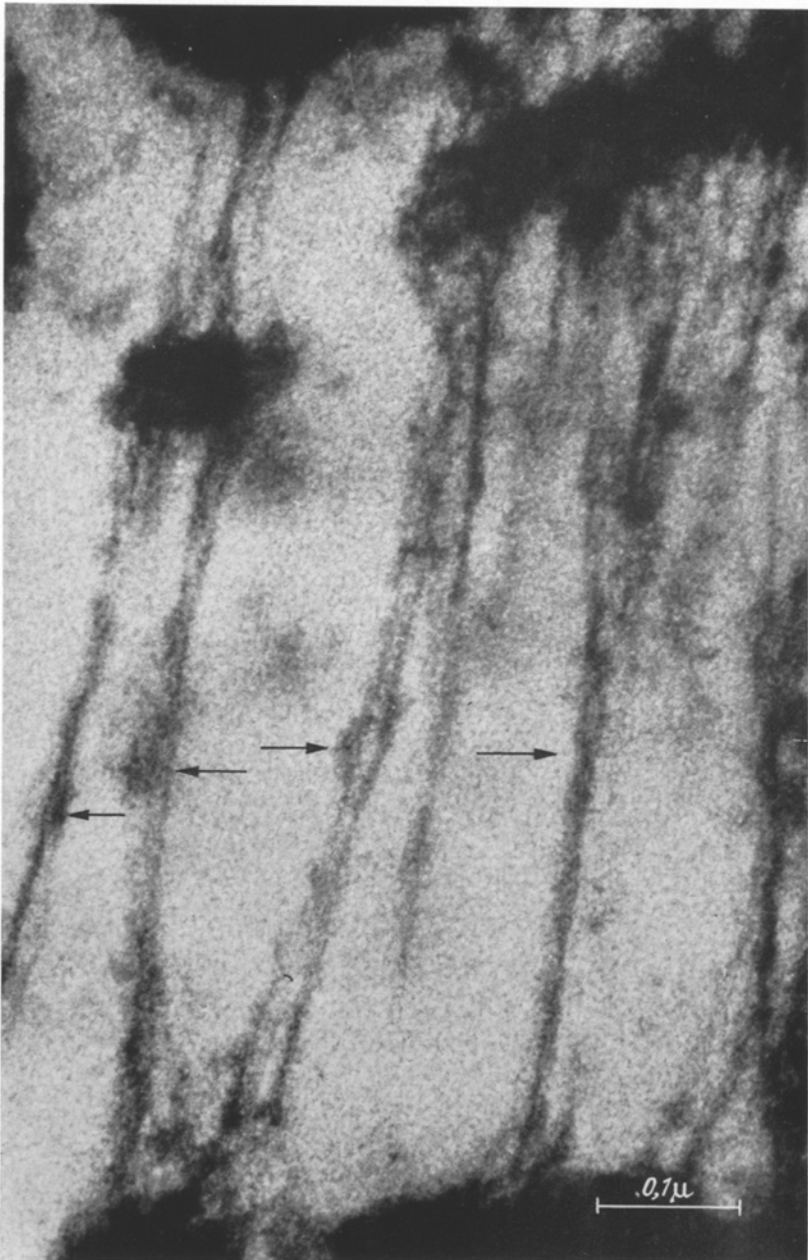


Fig. 3. Electron micrograph of the interband area in a salivary chromosome showing doubleness of the interband fibrils. Arrows point to the sites in the middle of the fibril, where a kinked thickening, presumed to be the relic of an uncoiled side-loop, can be seen (cf. Fig. 5 for explanation). $\times 193{,}000$

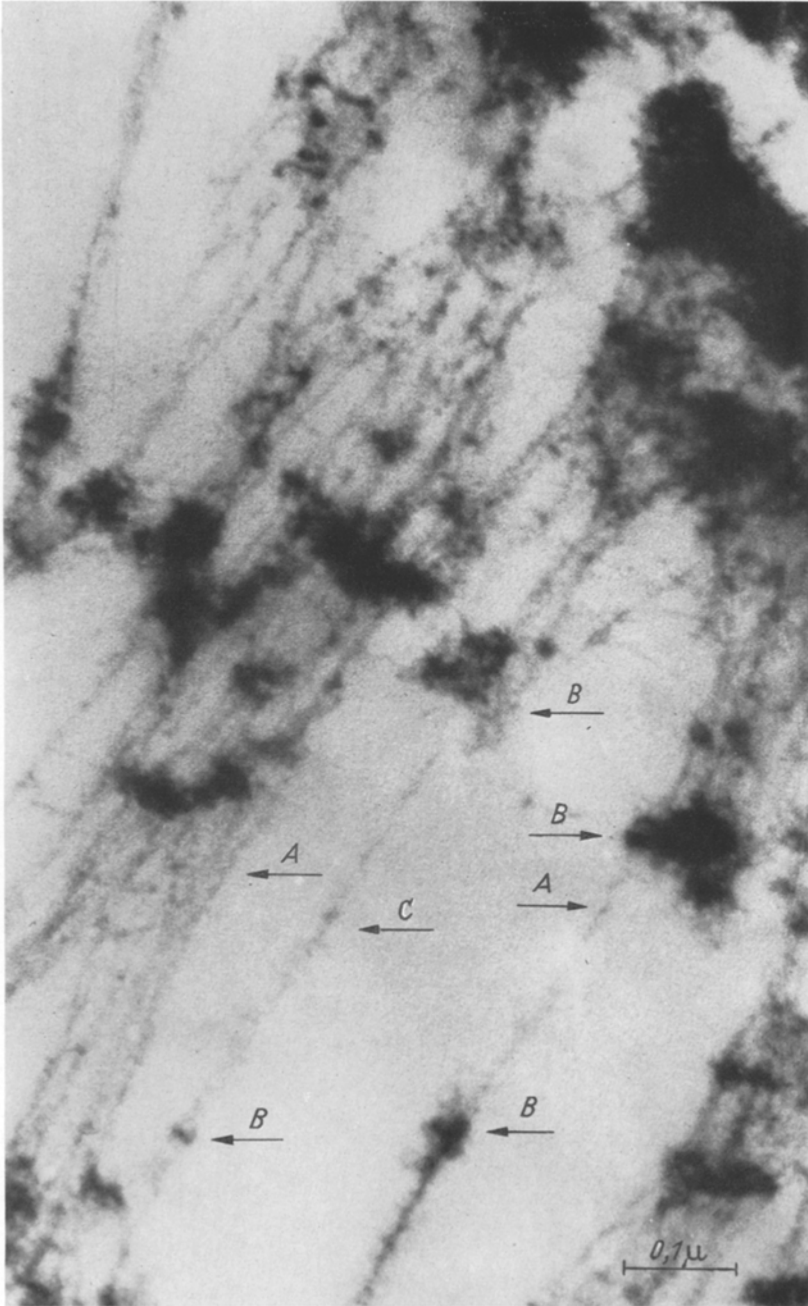


Fig. 4. Interband fibrils of a salivary chromosome. *A* Delicate coiling of the interband fibril. *B* Cylindrical particles, supposed to be linkers between the successive DNA-units. *C* Shortening of the interband fibril is supposed to begin in the middle part of each unit. (cf. Fig. 5 for explanation). ×150,000

The idea of the continuity of DNA in the chromosome axis has been supported by a great number of enzyme experiments in chromosomes of various organisms (Nebel and Coulon, 1962; Chorhazy, Bendich,

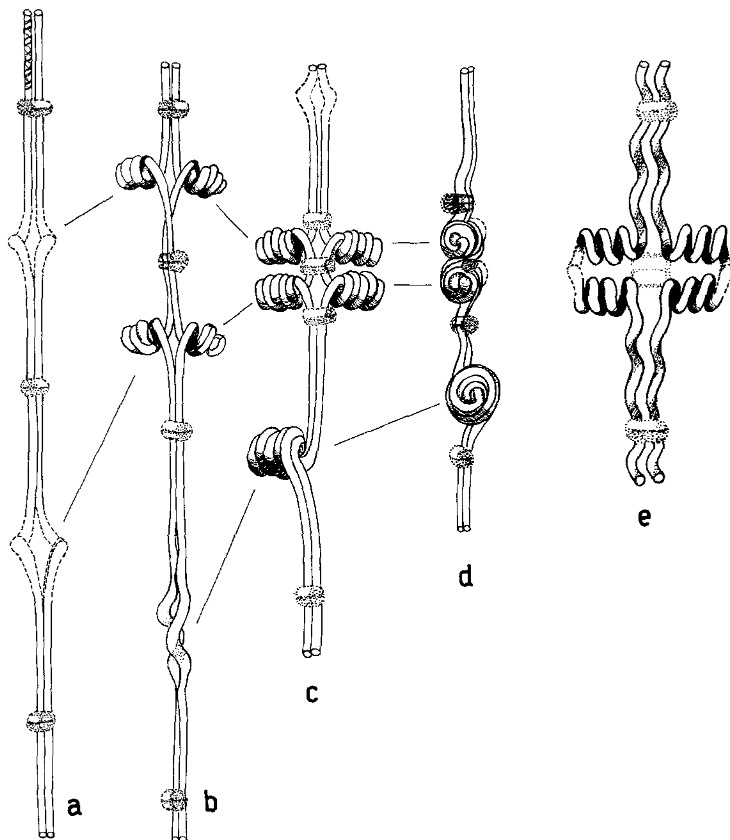


Fig. 5a—e. Tentative models representing the structure and coiling of an interband fibril of the polytene chromosome. a An interband fibril completely straightened. b Shortening of the fibril by transverse coiling. c Two hypothetical models for the coiling and formation of small chromomeres. d Side view of the same structure. e Another possible explanation for the double bar configuration of the interband chromomeres. The small cylindrical blocks are suggested to be linker particles combining the two parallel subfibrils and successive units together

Borenfreund and Hutchison, 1963; DuPraw, 1965; Trosko and Wolff, 1965). DNase has also been observed to destroy the interband areas of polytene chromosomes (Lezzi, 1965) as well as the interchromomeric strands and the lateral loops of the lampbrush chromosomes (Callan and McGregor, 1958). It thus seems legitimate to assume a general chromosome structure consisting of a linear orientation of DNA,

and most probably in successive basic units (FREESE, 1958; SCHWARZ, 1958, 1960; TAYLOR, 1962, 1963, 1966), which can even duplicate autonomously, as has been demonstrated for individual loci of several organisms (e. g. GABRUSEWYCZ-GARCIA, 1964; MILLER, 1964; KEYL, 1965, 1966; HESS, 1966).

We believe that our observations justify the following specific interpretation with respect to the structure of the polytene chromosome:

1. The interband fibrils are composed of two parallel subfibrils. These are assumed to be two nucleohistone fibers composing individual chromatids (cf. RIS, 1961). Such an interpretation is not in conflict with the results of earlier microphotometric analyses on *Sciara*, suggesting that "each individual unit of the polytene chromosome contains only one or two parallel double helices of DNA" (SWIFT, 1962).

2. The delicate coiling occasionally seen in interband fibrils (Fig. 4) is interpreted as suggesting a doubly coiled structure of the DNA-histone fibers.

3. The two parallel interband fibrils sometimes seem to be connected by a transverse cylindrical particle (Fig. 4). We believe it possible that identical particles also serve as linkers between successive DNA units along the chromatid (SORSA and SORSA, 1967).

4. Shortening of the interband fibrils is suggested to take place by formation of transversely coiled side-loops (Fig. 5). The chromomeres in the bands probably have a similar structure.

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