The Fourth Chromosome of *Chironomus tentans* Malpighian Tubules

An Ultrastructural Study

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**Abstract.** Morphology and banding pattern of the 4th chromosome in *Chironomus tentans* Malpighian tubules have been investigated by electron microscopy, using the squash and selection technique. The map we composed from our observations shows a remarkable increase (75%) in band numbers as compared to the map previously presented by Beermann for the 4th chromosome from salivary glands. Extrapolation of this increase to the entire genome would result in a total band number of about 3,500. The mean DNA content of bands can thus be calculated to be about 50 kb. Many bands show a complex structure, including the BR2 band. Some bands seem to result from fusion of smaller components. "Minibands" have also been observed. Some interbands contain RNP particles. In our material the interbands appeared to be made up of fibrils with a diameter of about 120 Å. On the basis of these results we estimate the DNA in the interbands as amounting to 2% of the entire genome. The results are discussed with respect to the organization of the polytene chromosomes and the functional significance of the banding pattern.

**Introduction**

In his light optical study of polytene chromosomes of *Chironomus tentans* Beermann (1952) not only mapped the bands on the chromosomes from the salivary gland cells, but he also observed that the banding pattern was largely identical in different larval tissues. Beermann also tried to relate some of these bands to the large puffs (Balbiani rings: BRs) which were observed in the salivary gland chromosomes. These puffs are known to be sites of intense gene activity (see for review: Beermann, 1972). Especially one Balbiani ring in *Chironomus tentans*, the BR2 in the 4th chromosome, has received much attention so far. According to Beermann, the sequence coding for the RNA product of the BR2 is located in a thin band, the size of which would probably not allow more than one coding sequence of the structural gene concerned (Beermann,
Similarly, the 37 kb coding sequence of the BR2 locus is probably unique (for review see: Daneholt et al., 1979).

However, recently a re-investigation of the BR2 region of chromosomes from both salivary glands and Malpighian tubules has shown that the site giving rise to the BR2 includes a broad band whose DNA content allows a repetitivity of the structural gene of 12 times (Derksen et al., 1980). It was also observed that many bands in the BR2 region, including the BR2 band, appeared sometimes as double or even triple bands in the light microscope (Derksen et al., 1980). Thick bands can artificially be made to look like double bands or doublets by varying the fixation procedures (Beermann, 1962; Berendes, 1970; Sorsa, 1979; Semeshin et al., 1979). The double and triple bands observed in the BR2 region however did not show the typical blurred or lens-like morphology of the so called “doublets”. Especially, since the BR2-band itself sometimes appeared to have a compound structure, we initiated an ultrastructural investigation of the BR2 region. For our electron microscopic investigation we have used a modification of the squash and selection technique (Derksen, 1978) which allows longitudinal sectioning of long stretches of chromosomes. We have used Malpighian tubule chromosomes, since salivary gland chromosomes exhibit less strict association of their constituent chromatids.

In this study we have established the banding structure of the BR2 region and we have shown that the BR2 band consists of 4 subunits, probably single bands.

Since the polytene chromosomes from C. tentans are reported to differ from those in D. melanogaster with respect to band number and DNA content per chromomere (see for review: Beermann, 1972), we have extended our observations to the entire 4th chromosome. Based on these observations we composed a map of the 4th chromosome, which shows considerable more bands than the map published before by Beermann (1952) for salivary gland chromosomes. Thus the number of bands in C. tentans would equal that in D. hydei (Grond et al., 1982a, b) and in D. melanogaster (Berendes, 1970).

Materials and Methods

Cultures of Chironomus tentans, strain Tübingen, were maintained as described by Beermann (1952), with some modifications: the larvae were abundantly aerated and supplied with undigested nettle powder and kept at a temperature of 21°C.

Malpighian tubules were isolated from prepupae, pupae and young adults, all females. The use of females appeared necessary since the cells in the Malpighian tubules of the males undergo one replication cycle less than the females and therefore are usually not suitable for further analysis (data not shown; see also Daneholt et al., 1979).

For the light microscopical analysis the tissue was fixed in ethanol-acetic acid (3:1), stained in 3% orcein in 70% acetic acid and squashed in 45% acetic acid. After removal of the coverslip the preparations were dehydrated in ethanol and mounted in euparal (for details see Derksen, 1978).

For electron microscopical analysis a modified squash and selection technique was used. Glands were fixed in 3% glutaraldehyde in 0,1 M Cacodylate buffer at pH 7,2, squashed in 45% acetic acid and after removal of the coverslip, transferred into a methanol-formalin mixture (9:1). After dehydration the preparations were stained in haematoxilin, dehydrated again, stained with uranyl-acetate and embedded in epon resin. Suitable chromosomes were selected and sectioned on a LKB Ultrotome III. For a full description of the procedure see Derksen (1978). Preparations were examined in a Philips EM 201 at 60 KV and photographed at various magnifications.