High order structure of *Chironomus* polytene chromosomes by scanning electron microscopy

BY Toshihiko TANIGUCHI and Susumu TAKAYAMA

*Biological Laboratory, School of Science, Kwansei Gakuin University,
Nishinomiya 662*

(Received August 24, 1987)

ABSTRACT

Polytene chromosomes isolated from *Chironomus* salivary gland cells were processed with the OsO₄/TCH technique and examined by scanning electron microscopy (SEM) mainly using stereo techniques. When chromosomes were somewhat extended for preparation, band and interband regions became clearly visible. The bands appear to be dense masses of kinky fibers while the interband regions consist of the 30 nm chromatin fibers. On the other hand, in less extended chromosomes where band and interband regions were less distinctive, most of the component fibers appear to have coiled configurations and their diameters range 150 to more than 200 nm. We conclude, therefore, that the natural chromonemata of the polytene chromosomes are about 200 nm in diameter rather than 30 nm even in the interband regions. The polytene chromosomes seem to have a cable-like structure.

1. INTRODUCTION

Despite a large number of studies on cytogenetics as well as on the organization of the genome at the DNA level, our understanding of the chromatin fiber structure in eukaryotic chromosomes is still controversial. A number of models for the higher-order structure of the metaphase chromosomes have been presented (Dupraw 1965; Stubblefield and Wray 1971; Paulson and Laemmli 1977; Coming 1978; Okada and Comings 1979; Zatsepin *et al.* 1983). In all of these models, however, the only agreement we have at present is the existence of 30 nm chromatin fibers, and the higher-order structure of the chromatin fibers in metaphase chromosomes still remains uncertain.

Recently Rattner and Lin (1985) have suggested that the metaphase packing is achieved by compaction through helical coiling of a 200-300 nm fiber which is further composed of looped 30 nm fibers. The present authors (Taniguchi and Takayama 1986) have also reported that metaphase chromosome preparations specially processed for scanning electron microscopy (SEM) show fairly homogeneous fibrous structures measuring about 200 nm in diameter which in turn appear to be coiled 30 nm fibers.

Polytene chromosomes seem to provide useful information for the funda-

mental understanding of the organization of chromatin fibers in metaphase chromosomes because of their structural characteristics, i. e., the occurrence of the reproducible patterns of bands and interbands. There have been number of studies on polytene chromosomes by transmission electron microscopy (TEM), but information on their whole structure available from these studies are rather limited. On the other hand, observations by SEM seem to make it possible to reveal the characteristic architecture of polytene chromosomes in a three dimensional figure. However, SEM studies on this type of chromosomes so far published are rather incomplete (Brady *et al.* 1977; Sedat and Manuelidis 1978; Iino and Naguro 1980; Mortin and Sedat 1982).

In this study, we applied our SEM technique (Taniguchi and Takayama 1986), which enabled us to show the presence of 200 nm fibrous structures in the mammalian metaphase chromosomes, to the *Chironomus* polytene chromosomes, and obtained some interesting findings on their chromatin fiber structure.

2. MATERIALS AND METHODS

Salivary gland of *Chironomus plumosus* larvae were isolated on glass slides and treated with 70% acetic acid for 15 min. Under cover glasses, they were squashed carefully to isolate polytene chromosomes. After a brief wash in a 1/15M phosphate buffer solution (pH 6.8), chromosomes were processed for SEM by the application of OsO₄/thiocarbohydrazide (TCH) technique (Ip and Fischman 1979; Harrison *et al.* 1982). Chromosomes were fixed with 3% glutaraldehyde in the phosphate buffer solution for 30 min, rinsed in the buffer three times, and further treated as follows: (i) fixation in 1% OsO₄ in the buffer for 30 min followed by three time rinses in distilled water; (ii) 20 min incubation in a freshly prepared and filtered saturated solution of TCH in distilled water followed by three time rinses in distilled water. These two steps were repeated more than three times. The specimens were then dehydrated through a graded ethanol series (30% to 100%) and three changes of amyl acetate. Finally they were critical-point dried from liquid carbon dioxide with amyl acetate as the transitional fluid. After sputter coating with 10 nm of gold, chromosomes were viewed with the JEOL JSM-T200 SEM operated at 25 KV. Photographs were taken using stereo techniques.

3. RESULTS

The chromosome shown in pairs of stereo photographs in Figure 1 seems to have been somewhat extended as a result of preparative artifacts. There are two prominent cross bands and several much less conspicuous bands with interband regions between them. Such a topography is in a good agreement with the views so far reported by TEM. In interband regions, separated thin

Fig. 1. Stereo pair scanning electron micrographs of part of a polytene chromosome from the *Chironomus* salivary gland cell. a: The chromosome appears somewhat extended, showing distinct band and interband regions. b: Part of the same chromosome as shown in a, showing highly prominent bands which consist of thicker fibers compared those in the adjacent interbands. Bars indicate $1\text{ }\mu\text{m}$.

fibers which might correspond to 30 nm chromatin fibers are seen to run parallel to the long axis. Such fibers, however, frequently appear somewhat thicker than 30 nm, since they tend to have some nodular or kinky appearances.

Fig. 2. Part of a relatively extended chromosome; there are several minute bands and interbands gathering close to each other. Bar indicates $1\ \mu\text{m}$.

On the other hand, banded regions appear to consist of densely packed nodules or highly kinked fibers. The larger the bands are the more kinky they appear. Bands, therefore, can vary in a stereo viewer. This situation is clear in the two prominent band regions shown in Figure 1 b. These band regions appear to consist of highly kinked and of raised fiber components which are fairly homogeneous in diameter measuring about $120\ \text{nm}$. The difference in size is clearly revealed in the portion near the bottom of the Figure 1 b, where the boundary between band and interband is visible.

Figure 2 is a portion of another chromosome. Considerable numbers of minute bands are distinguishable as small masses of kinky fibers which are located close to each other in a relatively small region.

The chromosome shown in Figure 3 seems somewhat less extended compared to the one in Figure 1 while the bundles of component fibers, chromonemata, appear twisted and disturbed to some extent. In this chromosome, most chromatin fibers appear clearly coiled even in the interband regions (Fig. 3 a). The diameters of such coiled fibers are roughly $130\ \text{nm}$. Figure 3 b reveals a portion of another chromosome likewise less extended where all the chromatin fibers are seen to have distinctly coiled appearances.

Figure 4 shows a chromosome which seems little extended, probably revealing an appearance close to its native state. Although the topography appears to be reflecting the band-interband configuration, typical interband regions are no longer discernible in this chromosome. It appears to consist of nodular or kinky fibers which are considerably thicker than the fibers ob-

Fig. 3. Stereo pair micrographs of part of a relatively less extended chromosomes. a: Most chromonemata appear clearly coiled. b: The similar situation is seen in another chromosome. Bars indicate $1\text{ }\mu\text{m}$.

served in Figures 1-3 ranging from 150 to more than 200 nm in diameter (Fig. 4b).

Figure 5 shows a chromosome with a broken end which probably happened to occur during the preparation. The appearance of this broken end seems to

Fig. 4. Part of a chromosome little extended. a: Stereo pair micrographs. The band-interband configuration appears obscure. b: Part of the same chromosome as shown in a. The thickness of the chromonemata ranges from 150 to more than 200 nm in diameter. Bars indicate 1 μ m.

suggest that the polytene chromosome is made of fairly packed chromatin fibers without any hollow. Observations on some other chromosomes encountered in this study were also suggestive of the presence of such a cable-like architecture in them.

4. DISCUSSION

Our SEM method with stereo techniques have allowed to reveal the chromonema arrangement in both band and interband regions. The demonstration

Fig. 5. Stereo pair micrographs of the broken end of a chromosome, being suggestive of the cable-like architecture. Bar indicates $1\ \mu\text{m}$.

of 200 nm fibers in the polytene chromosomes seems quite important since thick chromatin fibers of this size have been reported in the mammalian metaphase chromosomes by the present authors (Taniguchi and Takayama 1986). This has led us to assume that such 200 nm fibers may be a common high-order structure of chromatin fibers not only in the mammalian metaphase chromosomes but also in polytene chromosomes which are in general considered to be prophasic but not metaphasic. Since the coiled fibers shown in the present study were observed mainly in less or little extended chromosomes but not in extended ones, such a configuration might have been preserved only in the chromosomes prepared in the intention of keeping chromosomes as less extended as possible. TEM images of polytene chromosomes so far published are based on the highly extended chromosomes unless using sectioned materials. In such preparations, chromonemata in the interband regions appear to be 30 nm chromatin fibers (Ananiev and Barsky 1985). In the present study, as reported in other SEM studies (Brady *et al.* 1977; Iino *et al.* 1980) we also found fibers roughly corresponding to the 30 nm fibers in the interband regions of extended chromosomes. On the other hand, the prominent band regions of less extended chromosomes appeared to consist of coiled 200 nm fibers.

On the basis of these findings, we tried to make a model for the polytene chromosome organization (Figure 6). In this model, the polytene chromosome

Fig. 6. Schematic representation of coiled coil model of the polytene chromosome. The 30 nm chromatin fibers coil to form 200 nm fibers and bands (B) occur as the result of further coiling of the 200 nm fibers while interbands (IB) contain minute bands (MB) and minute interbands (MIB) which result from local loosening of the 200 nm fibers.

is shown to be a cable-like bundle of chromonemata. The chromonemata basically assume 200 nm fibers which in turn result from coiling of 30 nm ones. Even the interband regions, therefore, consist of such coiled fibers whereas the coiling of 30 nm fibers may be loosened to various degrees in some portions. In other words, there is a possibility that a so-called interband between two bands (prominent bands) can contain minute bands and minute interbands as well. The clustered minute bands with the interbands seen in Figure 2 may represent some of the possible configurations resulting from such loosening.

The presence of band and interband configurations in polytene chromosomes is one of the most conspicuous characteristics under light microscopy. It is naturally impossible, however, to distinguish the presence of extremely minute bands and interbands in the conventionally stained preparations by light microscopy. In addition, it is true that the polytene chromosomes prepared for light microscopy are highly extended. Such artificial extension of the chromosomes seems to occur more severely in the whole-mounted preparations for TEM and thus minute bands would disappear in such preparations. It is, therefore, impossible to exactly correlate band and interband configurations in the stained preparations with those from TEM images. The necessity of TEM for the detection of "real" interbands in the sectioned materials has been claimed by Vlassova *et al.* (1985).

On the other hand, we interpret the prominent bands as the clusters of gyres resulting from coiling of 200 nm fibers. If this is true, the configuration of the prominent bands would correspond to that of the metaphase chromatid as the highest order of coiling in the hierarchies of chromatin condensation,

and thus the chromonema organization of the polytene chromosome would be comparable to that of the mammalian metaphase chromosome (Taniguchi and Takayama 1986). However, if such gyres as the constituents of the prominent bands are too fragile to endure the preparative forces, they would become somewhat loosened and unwound. This may account for the fact that in the present study the prominent bands showed highly raised but complicated appearances in the fiber arrangement.

There have been considerable numbers of studies on the functional significance of interbands and some results are contradicting to each others. Some recent studies have claimed the transcriptional activity of interbands (Jamrich *et al.* 1977, Semenshin *et al.* 1979, 1986, Vlassova *et al.* 1985). This seems quite probable since decondensation of chromatin fibers should be at least related to transcriptional activity. However, it seems possible that some bands and interbands are convertible from one to the other and the minute bands and interbands mentioned above might belong to this type of structures. In this context, it is also possible that at least some, if not all, prominent bands are essentially different from minute bands in the chromatin constituents as well as in the function. In our model, these two types of bands are illustrated to be different at the level of the chromatin condensation.

REFERENCES

- ANANIEV, F. V. and BARSKY, V. E. (1985) Elementary structure in polytene chromosomes of *Drosophila melanogaster*. *Chromosoma* **93**, 104-112.
- BRADY, T., BAILEY, J. E. and PAYNE, M. B. (1977) Scanning electron microscopy of isolated *Chironomus* polytene chromosomes. *Chromosoma* **60**, 179-186.
- COMINGS, D. E. (1978) Mechanisms of chromosome banding and implication for chromosome structure. *Annu Rev Genet* **12**, 25-46.
- DUPRAW, E. J. (1965) Macromolecular organization of nuclei and chromosomes: a folded fibre model based on wholemount electron microscopy. *Nature* **206**, 338-343.
- HARRISON, C. J., ALLEN, T. D., BRITCH, M. and HARRIS, R. (1982) High-resolution scanning microscopy of human metaphase chromosomes. *J Cell Sci* **56**, 400-422.
- IINO, A. and NAGURO, T. (1980) Polytene chromosomes observed by scanning electron microscope. *Cytobios* **27**, 157-165.
- IP, W. and FISCHMAN, D. A. (1979) High resolution scanning electron microscopy of isolated *in situ* cytoskeletal elements. *J Cell Biol* **83**, 249-253.
- JAMRICH, M., GREENLEAF, A. L., BAUTZ, F. A. and BAUTZ, E. K. (1977) Functional organization of polytene chromosomes. *Cold Spring Harbor Quant Biol* **42**, 389-396.
- MORTIN, L. I. and SEDAT, J. W. (1982) Structure of *Drosophila* polytene chromosomes. Evidence for a toroidal organization of the bands. *J Cell Sci* **57**, 73-113.
- OKADA, T. A. and COMINGS, D. E. (1979) Higher order structure of chromosomes. *Chromosoma* **72**, 1-14.
- PAULSON, J. R. and LAEMMLI, U. K. (1977) The structure of histone-depleted chromosomes. *Cell* **12**, 817-828.
- RATTNER, J. B. and LIN, C. C. (1985) Radial loops and helical coils coexist in metaphase chromosomes. *Cell* **42**, 291-296.
- SEDAT, J. W. and MANUELIDIS, L. (1978) A direct approach to the structure of eukaryotic chro-

- mosomes. *Cold Spring Harbor Symp Biol* **42**, 331-350.
- SEMENSHIN, V. F., ZHIMULEV, I. F. and BELYAEVA, E. S. (1979) Electron microscope autoradiographic study on transcriptional activity of *Drosophila melanogaster* polytene chromosomes. *Chromosoma* **73**, 163-177.
- SEMENSHIN, V. F., BELYAEVA, E. S., SHIMULEV, I. F., LIS, J. T., RIDHARDS, G. and BOUROUIS, M. (1986) Electron microscopical analysis of *Drosophila* polytene chromosomes. IV. Mapping of morphological structures appearing as a result of morphological structures appearing as a result of transformation of DNA sequences into chromosomes. *Chromosoma* **93**, 461-368.
- STUBBLEFIELD, E. and WRAY, W. (1971) Architecture of the chinese hamster metaphase chromosome. *Chromosoma* **32**, 262-294.
- TANIGUCHI, T. and TAKAYAMA, S. (1986) High-order structure of metaphase chromosomes: Evidence for a multiple coiling model. *Chromosoma* **93**, 511-514.
- VLASOVA, I. E., UMBETOVA, G. H., ZIMMERMANN, V. H., ALONSO, C., BELYAEVA, E. S. and ZHIMULEV, I. F. (1985) Immunofluorescence localization of DNA: RNA hybrids in *Drosophila melanogaster* chromosomes. *Chromosoma* **91**, 251-258.
- ZATSEPIONA, O. V., POLYAKOV, V. YU. and CHENTSOV, YU. S. (1983) Chromonema and chromomere. Structural units of mitotic and interphase chromosomes. *Chromosoma* **88**, 91-97.