Three-dimensional Structure of G-banded Human Metaphase Chromosomes Observed by Atomic Force Microscopy*

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Summary. The structure of G-bands in human metaphase chromosomes was analyzed by comparison between light microscopic and atomic force microscopic (AFM) images of the same chromosomes. G-bands of the chromosomes were made by trypsin treatment followed by staining with a Giemsa solution. The banded chromosomes examined by light microscopy were dried either in air or in a critical point-drier, and observed by non-contact mode AFM. Air-dried chromosomes after G-band staining showed alternating ridges and grooves on their surface, which corresponded to light-microscopically determined G-positive and G-negative bands, respectively. At high magnification, the G-positive ridges were composed of densely packed chromatin fibers, while the fibers were loose in the G-negative grooves. Fibers bridging the gap between sister chromatids of a mitotic pair were often found, especially in the G-positive portions. These findings suggest that the G-banding pattern reflects the high-order structure of human metaphase chromosomes.

Chromosomes treated with suitable preparation methods show distinctive and reproducible patterns of transverse stained bands (for review, see BABU and VERMA, 1987; BICKMORE and CRAIG, 1997). These chromosome bands are important both for the identification of karyotypes as well as their key role in gene regulation. While a number of banding techniques have been reported, G-banding is widely used for the study and recognition of human chromosomes. G-bands are obtained when the chromosomes are pretreated with a proteolytic enzyme, such as trypsin, before staining with a Giemsa solution (SEABRIGHT, 1971; SUMNER et al. 1971). This tech-

nique allows some 300 bands to be readily distinguishable in human chromosomes (Paris Conference, 1971 [1972]). Biochemically, it has been postulated that G-positive bands are based on differences in their content of AT-rich and CG-rich DNA (HOLMQUIST et al., 1982). On the other hand, previous investigators have attempted to clarify the correlation between the chromosome structure and the production of G-banding patterns by microscopy, especially by transmission electron microscopy (TEM) of replicas (SCHWARZACHER et al., 1975; BATH, 1976) and scanning electron microscopy (SEM) (HARRISON et al., 1981, 1983). These microscopic studies indicated that the chromosome bands are produced in relation to the high-order structure of chromosomes, though the present study represents the first definite conclusion of this as it was performed to clarify the mechanism of G-banding in relation to the structure of chromosomes.

For this purpose, we analyzed human metaphase chromosomes with an atomic force microscope (AFM). The AFM use a sharp probing tip to scan the sample surface while monitoring the interaction force between the tip and sample. This microscope has advantages in obtaining images of the surface topography of non-conductive samples without conductive treatment at high magnification. Thus, recent researchers have applied the AFM to observations of various biological samples such as macromolecules (e. g., ENGEL et al., 1999; YAMAMOTO et al., 2000), collagen fibrils (YAMAMOTO et al., 1997), chromosomes (PUTMAN et al., 1992; TAMAYO and MILES, 2000) and living cells (USHIKI et al., 1999, 2000) (see also review by USHIKI et al., 1996; CZAJKOWSKY et al., 2000). The present study examined the surface topography of

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G-banded chromosomes of the human metaphase at high resolution by AFM and discusses the correlation between the chromosome structure and the production of G-banding patterns in relation to the high-order structure of chromosomes.

MATERIALS AND METHODS

Chromosome preparation

Heparinized peripheral blood was cultured at 37°C for 72 h in Karyotyping medium (GIBCO BRL), containing 1.5% phytohaemagglutinin, 15% fetal calf serum, and antibiotics. After the lymphocytes were arrested with 0.05 µg/ml colcemide for 60 min, the cell suspension was exposed to 75 mM KCl and fixed with a mixture of methanol and acetic acid (3:1). Spreads of chromosomes were made by dropping the cell suspension onto glass slides, followed by air drying. For G-banding, these chromosomes were treated with 0.025% trypsin at 4°C for 5–15 sec and stained with a Giemsa solution. Some of these specimens were simply dried in air, while others were fixed in glutaraldehyde, treated with a 1% tannic acid solution and 1% OsO₄, and critical point dried in liquid CO₂. They were dried in air and observed with a light microscope (LM) to check the G-banding pattern. The chromosomes observed by LM were then examined by dynamic force mode AFM in air. As control, chromosomes without trypsin treatment were also prepared, observed with a LM and examined by AFM.

Atomic force microscopy

AFM studies were carried out using a SPA-300 or SPA-400 scanning probe microscope controlled by an SPI 3700 or SPI 3800 probe station (Seiko Instruments Inc., Chiba, Japan). This microscope was equipped with a piezo translator with a maximum xy scan range of 20 µm width and a z range of about 1.2 µm. Cantilevers used were rectangular, with a force constant of 35 N/m and a resonance frequency of 320 kHz (SI-DF40, Seiko Instruments Inc., Chiba, Japan). All images were collected simultaneously as the constant force images (i. e., height mode images) and the variable deflection images in a dynamic force mode in air at room temperature. The resonance frequency shift was measured using the slope detection method at a slightly lower frequency of the cantilever than its resonance frequency. The constant force images were usually displayed by a computer in the “gradation mode” that represents the height of specimens as color gradations.

RESULTS

Chromosomes without trypsin treatment

Air-dried chromosomes without trypsin treatment were composed of the highly condensed chromatids of a mitotic pair (Fig. 1). The width of the chromatids was about 1 µm and the average height of the chromatids was about 200 nm. At high magnification, the chromosomes showed granular appearance in surface, though the details were not clearly revealed in these specimens.

Chromosomes dried in a critical point dryer were estimated 280 nm in height. The surface morphology of the chromosomes was basically the same as that of the air-dried chromosomes (micrograph not demonstrated).

G-banded chromosomes

In air-dried chromosomes after G-band staining, alternating ridges and grooves were found on their surface, which corresponded light-microscopically to dark (G-positive) and pale (G-negative) bands, respectively (Figs. 2–4). At high magnification, G-positive ridges were produced by an aggregation of fibrous structures about 50–100 nm in diameter (Fig. 5). These fibrous structures were loose in G-negative grooves. Fibers spanning the gap between sister chromatids of a mitotic pair were found predominantly in the G-positive portions.

In critical point-dried chromosomes, grooves were very shallow or sometimes not found in the G-negative portions (Fig. 6). At high magnification, the paired chromatids were composed of coiled or twisted chromatin fibers, 30–50 nm in diameter (Fig. 7). These chromatin fibers tended to be looser in the G-negative portions than in G-positive portions; in the latter portions, chromatin fibers took a strongly winding course as compared with fibers in the former portions. Interchromosomal fibers were evident in the G-positive portions as shown in air-dried chromosomes.

DISCUSSION

The present study has documented the three-dimensional structure of G-banded chromosomes by AFM as well as by light microscopy. Though several investigators actually applied AFM to observations of G-banded chromosomes, no clear image was obtained due to the lower resolution of their AFM

Fig. 1. AFM images of human metaphase chromosomes, fixed in methanol-acetic acid, spread and air-dried on a glass slide, stained with a Giemsa solution without tryprin treatment, and air-dried again. **a.** Chromosome 1, observed in the center of this micrograph, is characterized by the presence of the long chromosome arms separated by the centromere. **Inset:** A light microscopic picture of the corresponding portion. **b.** A closer view of the chromosome. The surface has granular appearance, though the details are not clearly revealed in this specimen.

images (MUSIO et al., 1997; SAHIN et al., 2000; THALHAMMER et al., 2000). The present study has succeeded in analyzing the relationship between the chromosome structure and banding patterns by AFM more precisely than before. Comparison of the light microscopic and AFM images in the same portions is also very effective to perform an accurate analysis of G-banded chromosome structures.

Previously, BATH (1976) examined platinum-carbon replicas of G-banded chromosomes by TEM and reported that the ridges observed in replicas correspond to G-positive bands observed by light microscopy. He also mentioned that the G-positive bands appeared as an area of tightly compacted fibrils on the surface of the chromosomes. In contrast, HARRISON et al. (1981) investigated the topography of the G-banded human metaphase chromosomes by SEM and stated the presence of circumferential grooves which correspond to the G-banding pattern. Their findings were quite different from the findings reported by BATH (1976). The present study has clarified that the surface topography of G-banded chromosomes varies, according to the drying methods for sample preparation.

One noteworthy finding is the presence of grooves

and ridges in air-dried G-banded chromosomes. Our observations have clearly shown that these grooves and ridges correspond to the pale and dark staining bands of G-banded chromosomes. As already noticed by BATH (1976), G-positive ridges were composed of an aggregation of fibrous structures about 50–100 nm in width, while these fibrous structures were sparse and loose in G-negative grooves. Our findings merit consideration in comparison with previous studies on the stretching of G-banded chromosomes (HLISCS et al., 1997). Mechanically stretching G-banded chromosomes with very fine tweezers allows G-positive dark bands to be stretched more easily than G-negative light bands and split into dark and light subbands. Previous biochemical studies, on the other hand, showed that the DNA of G-positive bands is AT rich compared with the GC-rich content of G-negative bands (HOLMQUIST et al., 1982). Thus, it is probable that AT-rich DNA is packed as relatively highly condensed heterochromatin, while CG-rich DNA with relatively high concentrations of genes is arranged as a relatively loose structure.

It is interesting that the grooves were very shallow, or sometimes absent from the G-negative portions of critical point-dried chromosomes. This implies that

Fig. 2. Light microscopic (a) and AFM (b) images of G-banded chromosomes, which were air-dried after G-banding treatment. G bands of variable staining intensity are observed on individual chromosomes by light microscopy. By AFM, alternating ridges and grooves appear on the surface of chromosomes, which correspond to light-microscopically dark (G-positive) and pale (G-negative) bands, respectively.

Fig. 3. Two pairs of light microscopic (a, c) and AFM (b, d) images of G-banded chromosomes 1, which were air-dried after G-banding treatment. Light-microscopically dark (G-positive) and pale (G-negative) bands correspond to ridges (*arrows*) and grooves (*arrowheads*), respectively, on the surface of chromosomes in these AFM images. **e.** The ideogram of the chromosome 1.

Fig. 4. Three-dimensional representation of the chromosome 1, shown in Figure 3b.

Fig. 5. Closer view of a part of Figure 3b. G-positive ridges (*p*) are observed as an aggregation of chromatin fibers, while these fibers are loose and sparse in G-negative grooves (*n*). Note fibers (*arrowheads*) spanning the cleft between the sister chromatids in the G-positive portions.

Fig. 6. Light microscopic and AFM images of G-banded chromosome 1, which was critical point-dried after G-banding treatment. Dark (G-positive) and pale (G-negative) bands are observed light microscopically (**a**), while the corresponding ridges and grooves in the AFM image (**b**) are not clear because the grooves are very shallow in this specimen. Arrows indicate G-positive bands and ridges.

Fig. 7. Closer views of parts of Figure 6. **a.** Paired chromatids composed of coiled or twisted chromatin fibers, 30–50 nm thick. These fibers are looser in the G-negative portions (*n*) than in G positive portions (*p*). **b.** Higher magnification of the upper right of **a**. Note also fibers (arrowheads) spanning the cleft between the sister chromatids in the G-positive portions.

G-negative grooves of air-dried chromosomes were probably produced by a squashing of the chromosomes during air-drying, probably due to the loose arrangement of chromosome fibers in the G-negative portions. This finding also supports the idea that certain proteins were extracted especially from G-negative portions by trypsin treatment, thus forming loose and fragile structures of chromatin fibers.

Several packing orders of chromatin fibers have been reported in metaphase chromosomes (COOK, 1995). The basic chromatin fibers are thought to be 20–30 nm in diameter, while the folding and/or coiling pattern of these fibers in metaphase chromosomes is still controversial. There are several models, such as a multi-strand model (KAUFMANN, 1956; RIS, 1956), folded fiber model (DUPRAW, 1965, 1966) and coiled-coil model (SEDAT and MANUELIDIS, 1978). On the other hand, Ohnuki (1968) demonstrated that chromosome arms are elongated and show a characteristic spiral pattern of chromatid fibers when chromosomes are treated with a specialized hypotonic solution. Further studies on G-banding are necessary in relation to the helical substructure of chromosomes.

The presence of interchromosomal fibers in G-positive portions was also reported in TEM images of chromosome replicas by BATH (1976). Although the nature of the interchromosomal fibers is still unknown, several possibilities are considered. One is that the fibers were artificially produced by trypsin treatment. Another possibility is that the fibers were actually present in the metaphase chromosomes. It is probable that chromatid separation is completed more slowly in the G-positive portions than in the G-negative portions. Recently, several investigators introduced the presence of certain molecules for sister chromatid cohesion in metaphase chromosomes (SONODA, 2001). The interchromosomal fibers may also affect the existence and distribution of such cohesion molecules as cohesin.

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