Interchromatidal central ridge and transversal symmetry in early metaphasic human chromosome one

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Interchromatidal central ridge and transversal symmetry in early metaphasic human chromosome one

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The topographic structure of GTG stained early metaphase human chromosomes adsorbed on glass was analysed by atomic force microscope using amplitude modulation mode [AM-AFM]. Longitudinal height measurements for the early metaphase human chromosomes showed a central ridge that was further characterized by transversal height measurements. The heterochromatic regions displayed a high level of transversal symmetry, while the euchromatic ones presented several peaks across the transversal height measurements, thus supporting evidence for interchromatidal interactions prior to disjunction. We suggest that this central ridge and symmetry patterns could point out a transitional arrangement of the early metaphase chromosome towards the typical disjunction of the sister chromatids.

Keywords: Chromosome structure; AFM, GTG banding.

I. INTRODUCTION

Chromosomes are vehicles of genetic information. Their structure and organization have been the focus of investigation before DNA was known to contain inheritance information. Nowadays nanometric resolution techniques like AFM, STM, SEM, and TEM are used to image the chromosome configuration; several captions of ultrastructures using these techniques have contributed to establish a less theoretical approach to the real conformation of the chromosomes [Harrison *et al.* 1982; Kenneth *et al.* 1986; Stark *et al.* 1998; García & Pérez, 2002]. According to these studies it is now accepted that the metaphasic chromosomes are super coiled strings of DNA and proteins, which have several hierarchical levels of organization showing the classical, light-microscopy chromosome shape [Cummings, 2003; Grigoryev, 2004].

Since AFM have been used to image human chromosomes, a more accurate documentation on the three dimensional structures of chromosomes have been developed. The height, width, volume, chromosomal aberrations, translocations, deletions and other features are already reported for some karyotypes and specific chromosomes [Rasch *et al.* 1993; Jiao & Schäffer, 2004; Zerrin *et al.* 2003] furthermore AFM images have been achieved either in native conditions, i.e. in liquid, without previous treatment [Hoshi *et al.* 2006], or with different staining methods, giving parameters to detect even the chromosome's damage caused by analysis techniques [Yangzhe *et al.* 2006; Kimura *et al.* 2004].

The classical chromosomal banding pattern is depicted with AFM as ridges and grooves, which can be correlated with the A-T and G-C rich portions respectively [Tamayo & Miles, 2000]. The centromeric

region is also a well defined chromosomal region in the literature [Fukushi & Ushiki, 2005].

Some polemical structures also have been reported. Most of them can be classified as "artifacts" from preparation methods or debris from the sample, in that regard "ghost strands" and satellite chromosomal ultrastructures can be distinguished [Hoshi & Ushiki, 2001]. However, other structures, like interchromosomal fibers, have been regarded as a normal part of chromosomes in some metaphasic stages, connecting the ridged regions in sister chromatids [Ushiki *et al.* 2002].

Fibers bridging the gap between sister chromatids have been reported since 1976 in TEM images from chromosomes replicas [Bath, 1976]. These can not be discarded as artificial products of trypsin or pre-TEM treatment, however there is not yet enough evidenced either against or in favor. The AFM images taken so far couldn't provide any strong evidence, basically because their interpretation at that scale needs a stronger biochemical background. Furthermore, the effects of contamination due to artifacts during the stain or fixation treatments could become significant at that level [Tamayo *et al.* 1999; Tamayo & Miles, 2000].

The existence of discrete interchromosomal structures such as interchromatidal fibers can represent an important step in the DNA organization from prophase heading towards metaphase, and thus, a hint to the full understanding of the process of chromosomal arrangement. However most of the AFM studies either focus on chromosomes with already split sister chromatids, or simply ignore the differences of the degree of disjunction of the sister chromatids. It could be even argued, that most of the observations of

interchromatid structures seem to be not intended as a research objective, but a casual observation while some other feature was being tested.

The aim of this study is to describe, using AFM, new early metaphasic chromosomal structures which could give hints on the processes undergoing the separation of sister chromatids.

II. MATERIAL AND METHODS

GTG banding

Whole heparinized human peripheral blood [6 ml] from healthy volunteers, around 20 years old, was cultivated in tubes containing 10 ml RPMI 1640 medium, supplemented with 20% fetal calf serum in 5% CO₂ atmosphere for 48 hours at 37°C. Lymphocytes were arrested in metaphase adding 1, 0 ml of 0.05 µg/mL colcemide. The cells were concentrated and washed by centrifugation during 8 minutes at 1200 rpm. Cell pellet was resuspended with 1, 5 ml KCl 0.0075 M following 18 minutes incubation at 37°C; then fixed using 3:1 methanol: acetic acid. GTG banding was performed incubating the glass slide in 0.1 % trypsin solution at 25°C for 20 seconds, and then 10 % Geimsa stain was applied.

Several preparations were examined in order to find metaphasic preparations showing the typical pattern of chromosomes in early metaphase, i.e. with the chromatids starting to split. One representative

Chromosome one was selected.

Longitudinal and transversal height measurements were taken. Three longitudinal height measurements were taken: two along each almost divided chromatid and one along the center of the whole chromosome. Transversal height measurements were taken on the euchromatic and heterochromatic regions.

Atomic force microscopy conditions

The AFM images were obtained with MFP-3D system [Asylum Research]. All images were collected in Amplitude modulation mode in air at room temperature, with 512 x 512 pixels of resolution. Scan speeds were approximately of 1Hz.

The Cantilever was rectangular, with a force constant of 2N/m and a resonance frequency of 70 kHz [AC240TS]. Under these conditions longitudinal and transversal height measurements from chromosome one were taken.

III. RESULTS

The imaged early metaphasic human chromosomes revealed the typical GTG pattern as seen using AFM [Cummings, 2003]. G-positive bands were clearly seen at micrometric scale [fig 1& 2]. The height and width for chromosome one were about 180 nm and 1.40 µm, respectively. These values are in agreement with other AFM measurements [Fritzsche *et al*;1997].

Figure 1. Topographic images of GTG stained metaphase human chromosome 1 a) Longitudinal height measures b) AFM image c) the eu- and heterochromatin according to genes density d) *Ensembl* ideogram used as a control for banding.

Figure 2. Topographic images of GTG stained metaphase human chromosomes. [Right] Each chromosome in early metaphase can be distinguished as having two recently built sister chromatids, however both of them are in tight contact previous to disjunction. [Left] Chromosomes in late metaphase, the centromere clearly binds the sister chromatids.

The banding pattern accomplished with the AFM images matched the light microscopically banding and the *Ensembl's* [Ensembl,2007] ideogram for chromosome one [Fig. 1].

Height graphics were chosen because they express the actual height and the compressibility of the sample being tested, providing information about the configuration and packing of the chromosome [Thalhammer *et al.* 2000; García & Pérez, 2002].

The longitudinal height graphics were quantitatively different depending on whether they were measured on the chromatids or in the middle of the chromosome. A pattern of ridges in the middle of the early chromosomes was observed. This height difference was greater in the G-positive bands than in the negative ones [fig. 1]. The transversal height graphics displayed a particular ridge always in the central region of the non-completely divided chromatids and showed that G-positive heterochromatic regions were thicker and more symmetric than the G-negative euchromatic regions [fig.3].

IV. DISCUSSION

The longitudinal height measurements for the chromosomes displayed compact and loose regions. The white ridges on the image can be distinguished as peaks in the height graphic, while relaxed regions are showed in dark colors [fig. 2]. A comparison of the distribution of ridges and the grooves on the Chromosome 1, either from the AFM image or from an *Ensembl* ideogram, has a coincident euchromatin and heterochromatin patterns [fig. 1] according to the distribution of known genes.

The chromosomes were examined for early metaphase, in which the sister chromatids are not completely disjointed [fig. 2] although they can be realized as having almost their own conformations [Tamayo & Miles, 2000; Sumner, 1991].

The longitudinal height measurements along each of the sister chromatids, and the middle of the chromosome,

showed that the higher points were predominantly located in their central longitudinal axis. This behaviour was previously realized while examining other samples for AFM in our laboratory. It was then confirmed using the chromosome 1 as example; several transversal height measurements displayed higher points towards the center of the chromosome. Therefore, at this stage within metaphase, the GTG treatment brought a symmetrical transversal pattern across the chromosome, which reaches its highest points at the center [fig. 3].

This symmetrical transversal pattern in early metaphasic chromosomes could not be expected, since the trypsin treatment eats away the chromosome from the outside and one feasible option would be that each sister chromatid has its own ridge at its own center. Another possibility would be that the chromosome doesn't have any transversal pattern when is explored along the same band, i.e. having a rather flat height level across the sister chromatids. Neither of the mentioned options seems to correspond with our observations.

The chromosome's height pattern could be influenced by external factors like the deposition of stain, irregularities on the slide surface, remaining biological layers and sample debris from the preparation process [Harrison *et al.* 1982; Stark *et al.* 1998; Tamayo *et al.* 1999; Tamayo & Miles, 2000]. To discard the above cited possibilities we checked for irregularities in the glass support that might have biased the height measurements, but it was fairly regular [Fig. 3]. We also considered a remaining protein and RNA layer over the chromosome, which is about is around 30 nm height [Tamayo *et al.* 1999; Harrison *et al.* 1982], but since we measured height differences even of 100 nm along the chromosome our observations can not be explained by artifact layers. Furthermore, a biological debris layer from the preparation process could also be deposited on the glass substrate along with some Giemsa stain and produce slopes in the chromosome's disposition on the glass substrate, however in our preparations this layer

had a typical value smaller than 20 nm. Therefore the differences in transversal and longitudinal height measurements, observed under our experimental conditions, were generated by intrinsic configurations of the chromosome after the GTG treatment.

The enzymatic trypsin digestion of the chromosomes has been argued to produce the GTG-bands due to the depletion of non histones proteins, or just the degradation of the proteins located at the most loose parts of the chromosome [Kenneth *et al.* 1986; Hoshi & Ushiki, 2001; Rasch *et al.* 1993]. In either case a differential arrangement of not only proteins, but also DNA is conceded. According to this, the transversal ridge and the symmetry pattern observed in our samples correspond to a specific arrangement of Proteins and DNA at a specific point of the metaphase.

Despite their common central ridge, the transversal height graphics displayed a more symmetrical pattern in heterochromatic zones, as for the bands p21.3 and q31.3 [fig. 3.b, 3.d]. On the other hand, the euchromatic bands, like p36.13 and q42.2, tended to appear as having several peaks across the chromatids [fig. 3.a, 3.e]. Thus the heterochromatic bands accounted for the highest and more symmetric regions.

The observation of these ridges in the middle of the Chromosome and the pattern of transversal symmetry, can show an interaction between the sister chromatids prior to their separation. It has been observed that “interchromatid interactions” take place in a noticeable way among heterochromatid bands [Grigoryev, 2004]. The presence of bridging fibers, for instance, has been often observed in the heterochromatic bands [Wu *et al.* 2006; Ushiki *et al.* 2002].

There is evidence for specific DNA-protein arrangements that holds the sister chromatids during metaphase avoiding improper orientation or disjunction;

one example of such arrangement is the phylogenetically conserved multiprotein complex called cohesin in humans and Rad21 or Rec8 in plants and mice respectively [Zhang *et al.* 2004]. There are also other proteic components that could be potentially associated with interchromatid interactions in heterochromatin and that could remain present after GTG-treatment, they might be proteins with similar functions to MeCP2 [methyl-CpG-binding protein 2], HP1 [heterochromatin protein 1], MENT [myeloid and erythroid nuclear termination stage-specific protein] among others; all of them interact strongly with DNA and/or Histones [and their respective methylated or acetylated variations] in order to organize spatial packing patterns in heterochromatin [Georgel *et al.* 2003; Nasmyth, 2003; Zhang *et al.* 2004; Kudo, 1998]. Thus, the relative great height and symmetry of the heterochromatic regions observed in this study could be thought as an indirect evidence for a differential arrangement of proteins and DNA, which can originate the posterior formation of structures like the bridging fibers observed in GTG stained chromosomes.

These results also support the role of heterochromatic regions in chromosome's stability during the process triggering the formation of sister chromatids from replicated chromatin.

Secondary peaks in both chromatids accompanied the central ridge in the Euchromatic bands [fig.3]. It can be theorized that these peaks display the way the chromatin is being distributed between the sister chromatids, and thus this direct observation indicates that the disjunction happens first in the euchromatic bands, which lose symmetry first. This confirms other results in the literature, which stress that disjunction is triggered first in euchromatic bands [Grigoryev, 2004].

Figure 3. Topographic image of GTG stained metaphase human chromosome 1. Transversal height measurements for euchromatic and heterochromatic bands.

According to our observations, a transitional chromosome arrangement leading towards the correct separation of the sister chromatids would be likely. This process would imply the primary condensation of chromatin, as seen in prophase; a secondary transitional state, mediated by a longitudinal central distribution of DNA and DNA associated proteins; then the symmetrical distribution of DNA in both chromatids and the final disjunction of the chromatids, delayed specially in the heterochromatic bands which contributes to the chromosome stability during this process. However more studies are to be carried out on this subject.

The normal preparations for chromosome structure studies come from "mature" metaphasic chromosomes with their sister chromatids already split. We would like to suggest with our work that other stages of the chromosomal assemblage, like early metaphasic conformations, should be structurally distinguished from mature chromosomes in AFM studies, since these stages could determine the final structure of the chromosome. After all, if we want to figure out the development and structure of chromosomes we need to understand a continuous process in time, not an quasi-static accumulation of molecules with very discrete transitions

V. CONCLUSION

In this paper, we described chromosomal features which suggest that a transitional state of chromosomes prior to the splitting of the sister chromatids was captured by AFM, which has the advantage of obtaining the quantitative height and compressibility information of adsorbed chromosomes.

We concluded that the AFM characterization of early metaphasic chromosomal features, such as the symmetry pattern seen in Human chromosome one, can give hints on the processes undergoing the separation of sister chromatids.

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