



Non-random distribution of high density chromatin detected at opposite ends of T-banded human metaphase chromosomes

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Abstract

Previous research using microdensitometric scanning and computer graphic image analysis showed that T-banded segments of human metaphase chromosomes usually exhibit an asymmetrical distribution of high density (HD) chromatin between sister chromatids. Here, we employed the same methods to analyze HD chromatin distribution at opposite ends of T-banded human lymphocyte chromosomes. This study revealed that in most chromosomes with an asymmetrical distribution of HD chromatin at both ends, the highest densities of each arm were located in opposite chromatids. The frequency of this configuration was 0.792 per chromosome, indicating that the highest chromatin densities of the terminal segments of T-banded human chromosomes were non-randomly distributed at opposite chromosome arms. The possible relationship of this observation to the mode of replication of the terminal chromosome region is briefly discussed.

Key words: human chromosomes, T-banding, chromatin structure, telomere, microdensitometry.

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It is well known that the usual banding procedures (C-, G-, R- and T-) reveal the underlying structure and composition of DNA and associated proteins in mitotic chromosomes (Therman and Susman, 1993). The specialized chromatin structure and the GC-richness of the telomeric/subtelomeric DNA (de Lange, 2005; Riethman *et al.*, 2004) confer high resistance to heat denaturation to the terminal regions of metaphase chromosomes which results in selective staining after T-banding (Dutrillaux, 1973). T-banding is a modification of R-banding procedure and is obtained through the incubation of chromosomes in a buffer at a high temperature followed by Giemsa staining. This procedure results in T-bands as darkly stained segments in lightly stained chromosomes. Scanning electron microscopy showed that T-bands are areas of highly aggregated chromatin fibres (Jack *et al.*, 1986; Allen *et al.*, 1988).

Microdensitometric scanning and computer graphic image analysis of high-resolution microphotographs of T-banded segments of human and Chinese Hamster Ovary (CHO) chromosomes allowed the detection of a differential distribution of HD sub-telomeric chromatin between sister chromatids. The different patterns observed were arbitrarily classified in three kinds of HD chromatin distribution, namely: *Type I*, HD segments were of similar size in

both sister chromatids; *Type II*, HD predominated in one of the chromatids; and *Type III*, HD was detected in only one chromatid (Drets *et al.*, 1992). Alternatively, the distribution of HD chromatin could be classified in a simplified way as *symmetrical* (pattern *type I*) or *asymmetrical* (patterns *type II* and *III*). These patterns were confirmed by scanning T-banded endoreduplicated CHO chromosomes in which the same interchromatid distribution of HD chromatin appeared in both sister chromosomes (Drets and Mendizábal, 1998). Nevertheless, quantitative computerized microdensitometrical analyses on the distribution of HD chromatin at opposite ends of T-banded chromosomes have not yet been reported. Therefore, we performed microdensitometric scanning and computer graphic image analysis to study HD chromatin distribution at T-banded human chromosomes that showed asymmetry at opposite ends.

Human chromosomes were obtained from peripheral lymphocytes using routine methods. Dutrillaux's T-banding method was used with minor modifications (Drets *et al.*, 1992). T-banded chromosomes were photographed under a Zeiss Photomicroscope II using a Neofluar oil immersion objective (100X), double immersion and phase contrast. Technical Pan film (Kodak) was exposed at 12 DIN and developed with Microdol-X (Kodak) at 20 °C for nine minutes. Negatives were enlarged using a DA900 Durst Professional enlarger with a Componon lens (NA

1:3.5, Schneider) on Fine Grain Positive Film (Kodak) and developed with Dektol (Kodak) for one minute at 20 °C. Twenty five metaphases with a large number of T-banded chromosomes were photographed. Well spread chromosomes with evident T-bands at both ends were selected for microdensitometric scanning. Chromosomes pairs 13, 14, 15, 19, 20, 21, 22 and the Y chromosome were excluded from the analyses because of their small short arm and length which prevent a detailed quantitative analysis of the terminal chromosome region. Our study involved the microdensitometric scanning of approximately 1000 chromatid ends corresponding to 250 T-banded chromosomes. A microscope photometer MSP65 system (Zeiss) equipped with a fast scanning stage (0.25 µm/step), a Zeiss Luminar lens (25 mm 1:3.5/A 0.15), and a LD Planachromatic Zeiss condenser objective (40/0.60-160/1.1-1.5) was used for scanning T-banded human chromosomes photomicrographs. The MSP65 microprocessor was connected with a DEC Micro-VAX 3300 computer (Digital) and a TEK Netstation 4211 (Tektronix) computer graphic color terminal which was used for generating quantitative images from the microdensitometric data. All chromosome scanings covered an object area of 100 x 100 stage steps, totaling 100 absorbance measurements per step that were averaged in order to reduce any eventual random noise produced by the electronic components. Averaged measurements were displayed into two dimensional image units (pixels) and sorted into twelve density intervals ranging from 0 to 100 absorbance units. Bicubic interpolation was used to increase image resolution allowing a more detailed visualization of the chromosome structure. Chromosome graphic images were photographed directly from the TEK Netstation terminal. The computer procedures used were reported elsewhere (Drets *et al.*, 1995).

Microdensitometric scanning and computer graphic image analysis produced quantitative images that closely corresponded to the chromosome morphology observed under the light microscope. However, the high magnification of the terminal chromosome region obtained with this method enabled the detection of subtle staining density differences, such as symmetric and asymmetric patterns of HD chromatin distribution, undetectable with light microscopy. We studied the distribution of the highest chromatin densities in chromosomes that exhibited asymmetric patterns in both ends. Thus, each T-banded human chromosome was first entirely scanned, which was followed by an individual scanning of both chromosome ends in order to get a more detailed quantitative image of the interchromatid distribution of HD chromatin. Counting of the number of pixels in each HD area through a graphic computational procedure provided an arbitrary quantitative criterion to discriminate between symmetrical and asymmetrical patterns. We considered as *symmetrical* those interchromatid differences lower than 25 percent and as *asymmetrical* differences higher than 25 percent between

sister chromatids. Chromosomes that exhibited T-banded segments with a *type I* density pattern or HD areas presenting minute sister chromatid exchanges were not considered in our analysis.

Two configurations were detected in chromosomes presenting asymmetry at both ends: the highest densities of each chromosome arm were either located in opposite chromatids (TRANS; Figure 1a) or in the same chromatid (CIS; Figure 1b). Microdensitometric analyses of opposite ends of T-banded human chromosomes revealed that of a total of 101 scanned chromosomes with an asymmetrical distribution of HD chromatin between sister chromatids in both arms, 80 had a TRANS configuration and 21 had a CIS configuration (Figure 1). The frequencies of each configuration per chromosome (TRANS: 0.792; CIS: 0.208) were considerably different than expected (Chi-square test, $p \ll 0.001$), indicating that the highest chromatin densities of the terminal segments of T-banded human chromosomes appeared non-randomly located at opposite chromosome arms. It is of interest to mention that we have recently reported similar observations in T-banded CHO chromosomes (Drets and Santiñaque, 2007).

T-banded segments of human and CHO chromosomes exhibit a complex distribution of HD chromatin between sister chromatids (Drets *et al.*, 1992). The detection of similar patterns in endoreduplicated T-banded CHO chromosomes through microdensitometric scanning and computer graphic image analysis indicated that the differential distribution of HD chromatin was not due to a methodological artifact since it replicated in the same manner in the terminal region of both sister chromosomes (Drets, 2000). Moreover, T-banded segments of human and CHO chromosomes sometimes exhibited HD chromatin areas distributed like minute sister chromatid exchanges (Drets *et al.*, 1992), what we recently called ter-SCE (Drets *et al.*, 2006). In this work we showed that HD chromatin was non-randomly distributed at opposite ends of T-banded human chromosomes, with the highest chromatin densities located in a TRANS configuration in most chromosomes. These observations suggest that a normal phenomenon occurring in the terminal chromosome region may be involved.

Significant differences in telomeric signal intensities between sister chromatids of human metaphase chromosomes were reported using fluorescent labelled telomeric peptidic nucleic acid (PNA) probes and three-dimensional confocal microscopy (Bekaert *et al.*, 2002). These findings conform with the differential distribution of HD chromatin observed in T-banded human chromosomes. This may indicate that the highly heat-resistant HD areas could interfere with DNA accessibility to telomere probes. In human metaphase chromosomes, HD chromatin T-banded areas co-localize with fluorescent signals of FITC-labeled telomere PNA probes (CCCTAA), which are non-strictly localized at the end of the chromatids (Drets, 2004; Moyzis *et al.*,

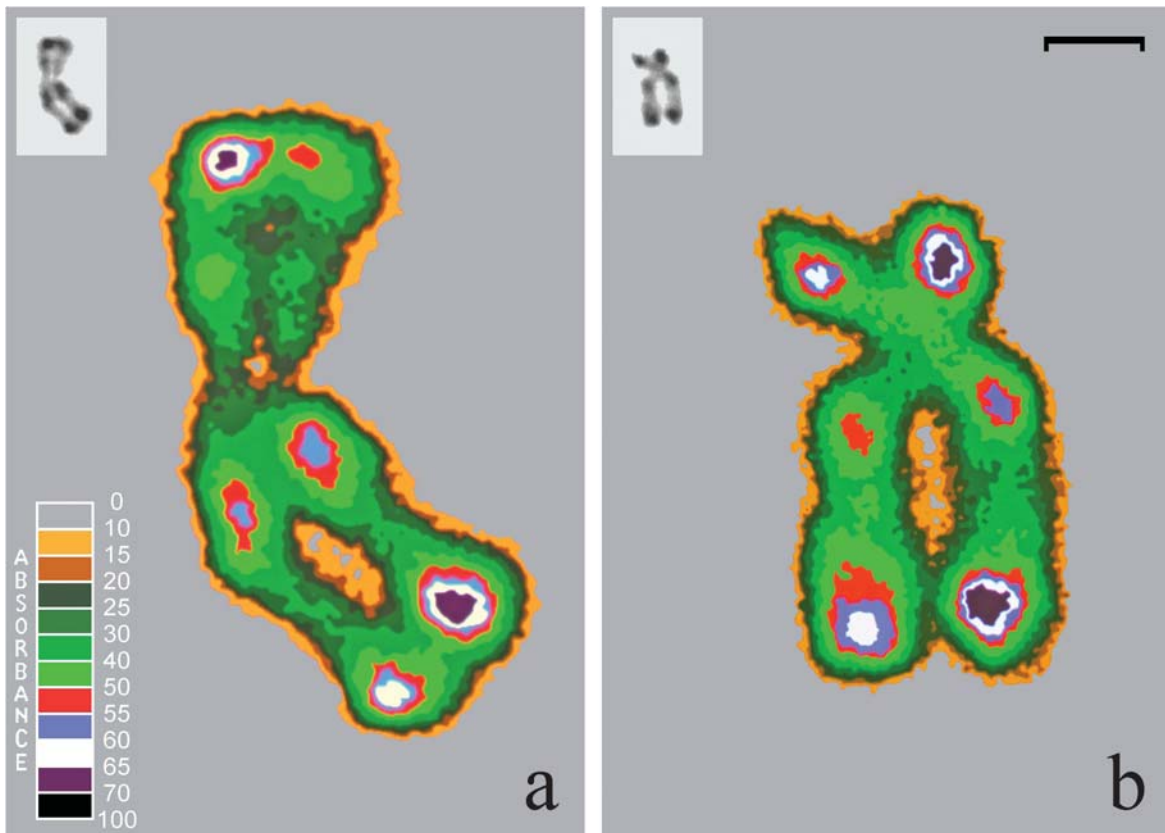


Figure 1 - Graphic images of T-banded human chromosomes showing TRANS (a) and CIS (b) configurations obtained through microdensitometric scanning and computer graphic image analysis. Remnants of R-bands are still observed since T-bands are a sub-set of them. Insets illustrate microscopic images of the scanned T-banded chromosomes. Bar = 1 μ m.

1988; Steinmüller *et al.*, 1993). Interestingly, the TRANS and CIS configurations frequencies found here are comparable to those obtained in normal human mitotic chromosomes using telomeric CO-FISH, a method that allows to differentiate between telomeres that replicated through lagging- or leading-strand synthesis (Cornforth and Eberle, 2001). These authors suggested that the CIS configuration could be explained in several ways: (a) by the occurrence of an odd number of sister chromatid exchanges along the chromosome, (b) by an overlap of sister chromatids near the centromeric region, and, (c) by spontaneous recombination in the subtelomere, that resulted in TRANS to CIS pattern conversions.

Although the molecular analysis of the terminal chromosome region is producing considerable data about its structure and function, we have no explanation for our observations. Since the phenomenon that we detected in normal T-banded chromosomes has a similar frequency to that obtained using telomeric CO-FISH and that HD chromatin areas colocalize with telomeric DNA, we suppose that they may be related to the specialized high-order chromatin structure nucleated by the telomeric TRF2, TRF1, POT1, TIN2, TPP1 and Rap1 proteins (de Lange, 2005). Accordingly, our observations could reflect a non-random segregation of some telomeric components of the HD chromatin

to only one sister telomere during its replication. The chromatin in this telomere would be more compact than in the other telomere which would later incorporate those components to nucleate the HD chromatin state. This agrees with recent findings showing that sister telomeres are unequally susceptible to the transient expression of dominant mutants of the protective protein TRF2, that leads to deletions or end-to-end chromatid-type fusions in a TRANS configuration involving only leading-strand telomeres (Bailey *et al.*, 2001; Bailey and Murnane, 2006; Wang *et al.*, 2004). Additional experimental data are extremely important to test this hypothesis since other possibilities cannot be excluded at present. Thus, a combined molecular and cytogenetic analysis would provide a better understanding of the high-order chromatin organization and replication of the terminal chromosome region.

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