

# APPEARANCE OF "HOLES" IN SUB-TELOMERIC REGIONS OF HUMAN AND CHINESE HAMSTER OVARY CELL CHROMOSOMES DUE TO PROLONGED INCUBATION IN T-BANDING BUFFER, FOLLOWED BY GIEMSA STAINING\*

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## ABSTRACT

Prolonged incubation of human and Chinese hamster ovary cell (CHO) chromosomes in hot T-banding buffer induces the appearance of minute holes in the sub-telomeric area and in the paracentric regions of sister chromatids. Holes induced in the chromosomes were also observed in polyploid and endoreduplicated CHO cells. Reflection microscopy showed chromosome structures as dark empty and very sharp holes. These holes may be an indication that portions of chromosome segments are specifically removed by this method, which may be due to a segmentary molecular composition.

## INTRODUCTION

Dutrillaux (1973) reported a method for the specific staining of terminal segments of human chromosomes. This technique is a modification of the procedure for

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R-banding (Dutrillaux and Lejeune, 1971). The method consists mainly of incubation of chromosome preparations in a buffered salt solution at a high temperature, followed by Giemsa staining. T-banded regions are darkly stained segments with the rest of the chromosome being lightly stained.

T-bands are considered to be a subset of GC-rich R-bands and are highly resistant to heat denaturation and to digestion with the restriction endonuclease *Mse* I (Ludeña *et al.*, 1991). With the scanning electron microscope, T-bands in human chromosomes appear as intricate fibrous structures and the remaining areas have an amorphous flattened aspect (Allen *et al.*, 1988).

We describe the induction of minute chromosome holes in the sub-telomeric and paracentric segments of human and Chinese hamster ovary cell (CHO) chromosomes by a combined treatment of prolonged incubation with the T-procedure, followed by Giemsa staining.

## MATERIALS AND METHODS

### *Cell culture procedures*

CHO cells were grown as monolayers at 37°C in flasks or in Petri dishes in McCoy's 5A medium, supplemented with 10% fetal calf serum, 200 mM glutamine and antibiotics (penicillin 100 units/ml and streptomycin 0.1 mg/ml) in an atmosphere of 5% CO<sub>2</sub> in air. Cells were prepared after 2 h exposure to Colcemid (0.08 µg/ml), using routine methods. Human peripheral lymphocytes (HPL) were cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum, phytohemagglutinin and antibiotics. The cells were prepared after 72 h, including a 4 h treatment with Colcemid, using routine methods.

### *T-banding technique*

A method for visualizing T-bands with Giemsa stain (Dutrillaux, 1973) was used, with the following modifications: (1) slides with air dried metaphases were immediately frozen and stored at -20°C; (2) slides were de-frozen and transferred to an incubator at 60°C for 4 h before T-banding; (3) preparations were incubated in hot (87 ± 0.1°C) 0.1 M sodium phosphate-citric acid buffer (pH 5.1) for 8-10 min (CHO cells) or 12-14 min (HPL) for usual T-banding; prolonged incubation in hot buffer results in over-denaturation of chromosomes (the exposure time must be carefully adjusted to obtain consistent results); (4) slides were washed in buffer solution at room temperature for 1 min and in bidistilled water for 5 min; (5) metaphases were stained in Giemsa stain

prepared by dissolving 0.75 ml (CHO) or 1.5 ml (HPL) of Giemsa stock solution (Merck) in 50 ml bidistilled water, containing 1.5 ml methanol and 1.5 ml sodium phosphate buffer at pH 6.8, resulting in 1.5% or 3% Giemsa solution, respectively.

### *Reflection light microscopic observations*

The reflected light microscopic observations were performed according to reported methods (Lejeune *et al.*, 1986; Folle, 1987). A Zeiss Photomicroscope II with a Vertical Illuminator III C/45 mm system, a reflector H-PI-Pol, a reflection light aperture stop insert and a Phanapochromat 1.3 immersion oil objective with adapter ring were used.

## RESULTS AND DISCUSSION

Figures 1 and 2 show that prolonged incubation of chromosome preparations in hot buffer leads to the appearance of holes in the T-banded areas of human and CHO chromosomes. This feature was observed in about 350 CHO and 50 human well spread metaphases. Holes were observed in one chromatid or in both sister chromatids. With reflected light microscopy the holes can be seen as black areas and their locations and size much more precisely determined (Figure 1b and Figure 2b). Shorter periods of incubation produced T-bands and holes in chromosomes of the same metaphase.

Holes were also induced in chromosomes of polyploid CHO metaphases (Figure 1c). In 18 analyzed endoreduplicated CHO metaphases holes were preferentially seen in the outer chromatids indicating differences in the reaction of the four chromatids to the staining procedure (Figure 1d). In 26 out of 1050 CHO metaphase chromosomes examined, holes were detected in paracentric segments (Figure 1a and 1c, arrows). This finding was also observed in human metaphases (Figure 1a, arrow).

Terminal chromosome segments reportedly contained a variable number of DNA repeats in mammals and higher plants. Long tandem arrays of repeated telomere-associated units were found in *Chironomus* and in *Secale*, appearing as large blocks of C-banded heterochromatin (Bedbrook *et al.*, 1980; Saiga and Edström, 1985). Middle repetitive elements or telomeric-associated sequences have also been found in subtelomeric regions (Blackburn and Szostak, 1984; Zakian, 1989). Families of repeated sequences were also detected in other chromosome regions. A 12 kb fragment of *Drosophila melanogaster* DNA was found to contain sequences homologous to DNA found at the ends of the polytene chromosomes and to the pericentric sequences present in the beta heterochromatin by *in situ* hybridization (Young *et al.*, 1983). The repeated

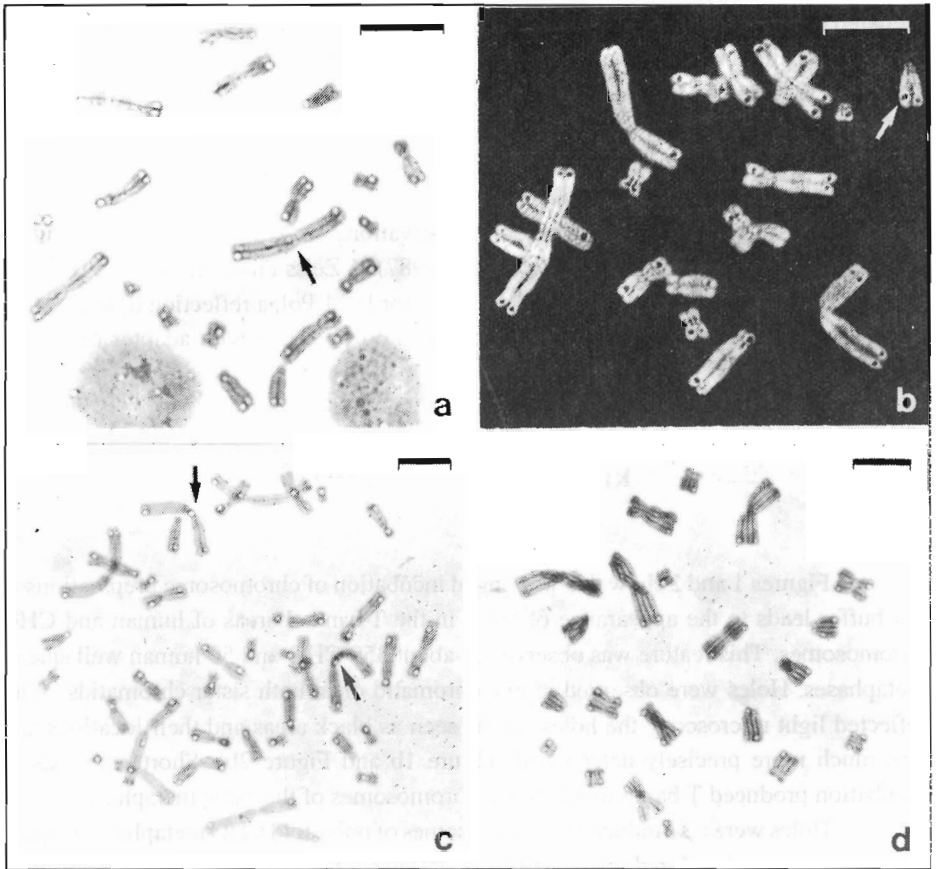


Figure 1 - Over-denatured CHO chromosomes. (a) Metaphase with holes at subtelomeric areas. (b) Holes observed in CHO metaphase chromosomes with reflection microscopy, appearing as empty dark structures. A telocentric chromosome showing holes located at different distances from the ends of the sister chromatids is indicated by an arrow. (c) Holes in all chromosomes of a polyploid cell. (d) Holes in an endoreduplicated cell. Bar = 10  $\mu$ m.

sequence detected in human telomeres has also been found in the pericentric heterochromatic region and in non-telomeric sites in chromosomes of other mammals (Meyne *et al.*, 1990).

The induction of tiny holes in the sub-telomeric area and in the paracentric regions of CHO and human chromosomes may suggest that portions of this material are specifically removed by over-denaturation with the T-method, which could be due to the organization of the repeated DNA sequences present in these chromosome regions.

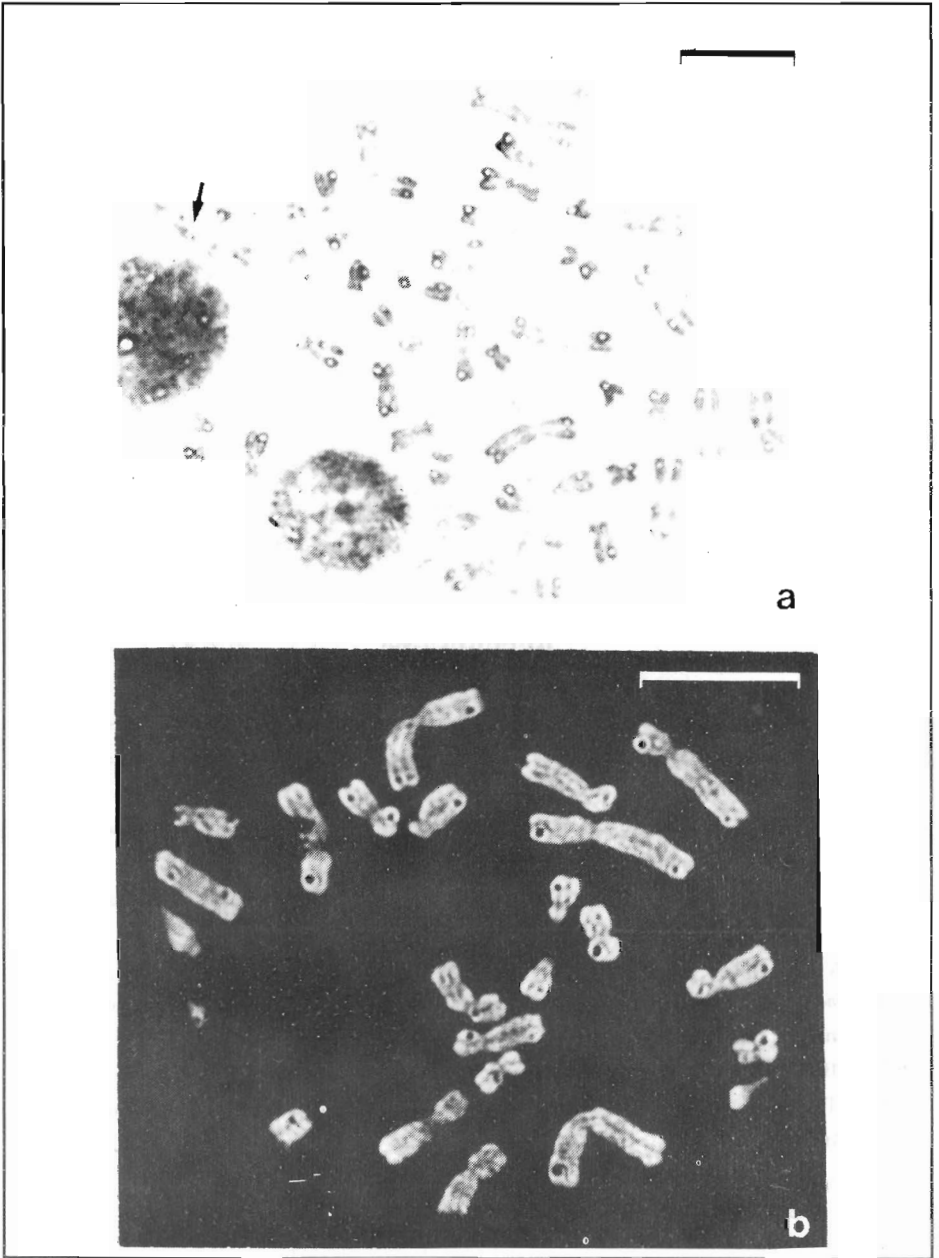


Figure 2 - Chromosome holes in human chromosomes. (a) Metaphase showing holes at subteleric and paracentric segments (arrow). (b) Partial view of a human lymphocyte metaphase, showing chromosome holes as seen with reflection microscopy. Bar = 10  $\mu$ m.

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## RESUMO

A incubação prolongada de cromossomos humanos e de células de ovário de hamster Chinês (CHO) em tampão bandeamento T quente, induz o aparecimento de pequenos orifícios na área subtelomérica e nas regiões paracêntricas de cromátides irmãs. Orifícios induzidos nos cromossomos também foram observados em células CHO poliplóides e endoreduplicadas. Microscopia de reflexão mostrou estruturas cromossômicas escuras, vazias e bem definidas. Esses orifícios podem ser indicação de que porções de segmentos cromossômicos são removidos especificamente através deste método, o qual se deve a uma composição molecular segmentária.

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