Brazilian Journal of Genetics, 19, 4, 577-582 (1996)
(Revista Brasileira de Genética)

Microphotometric scanning of chromatid gaps and breaks induced by *Alu*I and *Bam*HI in Chinese hamster ovary cells

Wilner Martínez-López[1,2], *Rossana Bonomi*[1], *Gustavo A. Folle*[1] and *Máximo E. Drets*[1]

ABSTRACT

Chromatid gaps and breaks induced by the restriction endonucleases *Alu*I and *Bam*HI in the long arm of chromosome 1 of Chinese hamster ovary cells were microphotometrically scanned and mapped to a quantitative G-band map. More than 50% of chromatid breaks appeared as chromatin losses of greater than 5% of the total arm length. The majority of chromatid gaps and breaks as well as chromatin losses induced by both restriction endonucleases were non-randomly located in a region from 0.35 to 0.65 relative length units of the long arm of chromosome 1. We suggest that the access of these endonucleases to chromosomal DNA depends on the local organization of the chromatin.

INTRODUCTION

Different chromosome banding patterns reflect the underlying organization of the genetic material (Goldman *et al.*, 1984; Korenberg and Rykowski, 1988). The use of fluorescent staining methods has allowed the classification of human chromosome bands in five groups according to their base composition (Holmquist, 1990). A close relationship to some groups of bands has been found using data on the location of several oncogenes and exchange chromosome breakpoints in cancer cells (Trent *et al.*, 1989; Holmquist, 1992).

Although human and some primate chromosome banding patterns have been standardized (ISCN, 1985), no quantitative data on band localization, band size and band-interband junctions have been reported for any species. Therefore, microphotometric analysis of banded chromosomes could contribute to a more precise localization of induced breaks (Drets *et al.*, 1989).

Chinese hamster DNA contains *Alu*-like (type 1) short interspersed elements (SINEs) as well as *Bam*HI (MIF-1) long interspersed elements (LINEs), which bear recognition sites for the restriction enzymes *Alu*I and *Bam*HI, respectively. *Alu*-like SINEs dominate in early replicating Giemsa light (G-light) bands, *Bam*HI (MIF-1) LINEs are selectively partitioned in late replicating Giemsa dark (G-dark) bands (Holmquist and Caston, 1986).

Studies concerning the mechanism of the origin of chromosome aberrations have shown that restriction endonucleases (REs) are intense clastogenic agents on cultured mammalian cells (Obe *et al.*, 1985; Martínez-Lopez *et al.*, 1995), producing double-strand breaks in the DNA molecule as a unique DNA lesion (Obe *et al.*, 1992). The correlation of REs-induced lesions at the

[1] Division of Human Cytogenetics and Quantitative Microscopy, Instituto de Investigaciones Biológicas Clemente Estable, Avda. Italia 3318, 11600 Montevideo, Uruguay. Fax: (598 2) 47 55 48. E-mail: wlopez@iibce.edu.uy. Send correspondence to W.M.-L.

[2] Instituto de Biología, Facultad de Ciencias, Tristán Narvaja 1674, 11200 Montevideo, Uruguay.

molecular level with chromosome breakpoint localization is thus of interest since it may give additional information on the mitotic chromosome organization.

A method for the localization of breakpoints induced by *Alu*I in the long arms of chromosome number one of CHO cells by means of microphotometrical scanning has been reported (Drets *et al.*, 1994). In this study, a quantitative G-band map previously drawn for breakpoint localization was used.

MATERIAL AND METHODS

Cell culture and preparations

CHO is a transformed cell line with a modal number of 21 chromosomes (Siciliano *et al.*, 1985). This cell line contains nine normal chromosomes (one chromosome each of 1, 2, 5, 7, 9, 10, and X, and two chromosomes 8) and 12 rearranged chromosomes named Z (Z1 to Z10, Z12 and Z13). Chromosomes Z1 and Z6 originate from a small reciprocal translocation t(1;5) involving the tip of 1p and the distal region of 5q. Since the long arms of chromosomes 1 and Z1 are structurally identical (Drets *et al.*, 1994), they were used indistinctly in this study.

CHO cells were cultured as monolayers in plastic Petri dishes (94 x 16 mm) in McCoy's 5A medium (Gibco) supplemented with 10% fetal calf serum, 200 mM glutamine (Sigma) and antibiotics (100 units/ml penicillin and 125 μg/ml dihydrostreptomycin sulfate, Sigma). Mitotic cells were harvested, hypotonically treated with 1% sodium citrate at 37°C for 12 min, fixed twice in methanol-acetic acid (3:1) and spread on ice-cold slides. Chromosome preparations were stained with 3% phosphate buffered Giemsa stain (pH 6.8) for 5 min.

*Alu*I treatment

CHO cells (0.5 x 10^6) were cultured in 35-mm Petri dishes for 12 h at 37°C in a 5% CO_2 incubator. The cells were washed once with 2.5 ml McCoy's 5A medium and subsequently treated with 100 μl of a mixture containing 5 units of *Alu*I, 1.1 M glycerol (Merck; final concentration) and McCoy's 5A medium for 30 min at 37°C. After treatment, the cells were washed twice with prewarmed medium and incubated with complete medium for 8 h, including a 2-h treatment with Colcemid (0.08 μg/ml) (Ciba). Controls were carried out by treating CHO cells with *Alu*I storage buffer.

*Bam*HI treatment

Log phase CHO cell cultures growing in 96-mm Petri dishes were tripsinized, resuspended in 10 ml cold phosphate buffered sucrose (272 mM sucrose, 7 mM KH_2PO_4, pH 7.4 and 1 mM $MgCl_2$) and counted. Sterile electroporation cuvettes (0.4-cm electrode gap) were filled with 800 μl sucrose buffer containing 2 x 10^6 cells and 96 units (8 μl) *Bam*HI (Promega) and kept on ice for 5 min before electroporation. Cells were electroporated with a Bio-Rad Gene Pulser adjusted to 1000 volts/cm and 25 μF capacitance, kept on ice for 10 min, washed once with fresh McCoy's 5A medium and recovered in 35-mm Petri dishes containing complete medium for 8 h, including a 2-h treatment of Colcemid (final concentration 0.08 μg/ml). Controls were carried out by adding 8 μl of *Bam*HI storage buffer.

Scoring of chromosome damage

The following chromosomal aberrations were scored in buffer controls and REs-treated metaphases showing 18-22 centromeres (100 cells per experiment): polycentric chromosomes, ring chromosomes (Rings), double minutes (min), isochromatid/chromosome breaks (B"), chromatid interchanges (RB'), complex interchanges (RB'-complex), triradials (RB'B"), chromatid intrachanges (CI), chromatid breaks (B') and achromatic lesions (Gaps). To estimate the frequency of aberrations in the long arms of CHO chromosomes 1 and Z1, 1000 metaphases were scored both in controls and treated cells.

Localization of chromatid gaps and breaks

Chromatid gaps and breaks were photographed using a Zeiss Photomicroscope II with a Neofluar oil immersion lens (100 x) and phase contrast. Technical Pan film (Kodak) was exposed at DIN 12 and developed with Microdol (Kodak) at 20°C for 9 min. Negatives were enlarged with a Durst DA900 enlarger on Fine Grain Positive film (Kodak) and developed with Dektol (Kodak) for 2 min at 20°C. Film enlargements thus obtained were photometrically scanned for breakpoint localization as described previously (Drets *et al.*, 1994). The proximal chromosome segment of the broken chromatid was measured from the centromere to the first broken end (Figure 1, A), and the distal fragment from the second broken end (Figure 1, B) to the telomere. The length of the intact chromatid was measured and the value obtained considered as 1.00. The extent of chromatin loss between both broken ends (A and B) was estimated by comparing the length of the

intact and broken sister chromatids (Figure 1, S). A chromatin loss greater than 0.05 relative units (5% of the total arm length), which corresponds to an average CHO chromosome band, was defined as a loss of chromatin in terms of quantitative data.

A Zeiss microscope photometer MPM including a 0.25-μm step scanning stage, a Luminar objective (25 mm 1:3.5/A 0.15) and a LD planachromatic lens (40/0.60 - 160/1.1-1.5) as the microscope condenser were used for chromosome scanning. The system included the MSP 65 microprocessor (Zeiss) associated "on line" to a Digital Micro-Vax 3300 computer and a Tektronix Graphics Color Terminal 4211. Chromosome linear scanning was carried out using a rectangular luminous diaphragm (6 x 0.1 mm) and a photometric field diaphragm (1.5 x 0.1 mm) which cover at least 75% of the chromatid width.

RESULTS

The exposure of CHO cells to *Bam*HI mainly induced chromatid type aberrations after 8 h of recovery time. *Alu*I treatment produced the same type of chromosome lesions (Table I). The frequency of chromosomal aberrations induced by *Bam*HI was lower than that obtained with *Alu*I.

A total of 100 chromatid gaps and breaks induced by *Bam*HI (96 units) were localized on a quantitative G-band map of the long arm of CHO chromosome 1 and compared with the localization of 100 chromatid gaps and breaks induced by *Alu*I (5 units) (Drets *et al.*, 1994) (Figure 1).

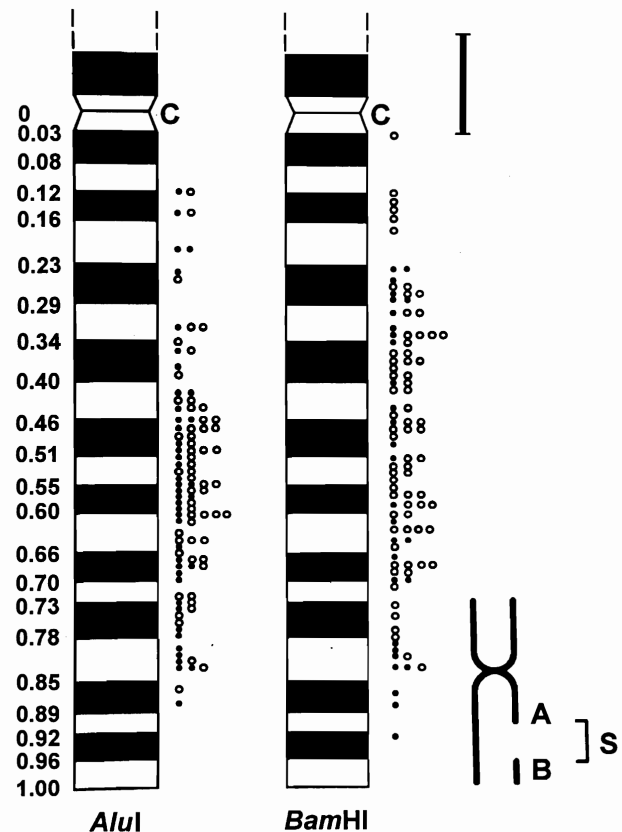

Figure 1 - Comparative diagram showing the relative position of 100 chromatid gaps and breaks induced by *Alu*I and *Bam*HI. Chromosome lesions were localized according to the relative position of G-bands in the long arm of CHO chromosome 1. Solid circles denote chromatid gaps and open circles indicate chromatid breaks. Inset shows the chromatid segment used for breakpoint quantitative estimation (see text). C = Centromere. Bar = 1 μm.

Table I - Chromosomal aberrations induced in CHO cells following treatment with either 5 units *Alu*I or 96 untis of *Bam*HI (recovery time: 8 h). Pooled data from three experiments for *Alu*I and four different experiments for *Bam*HI.

Treatment	Number of metaphases	Percent of aberrant metaphases	Aberrations per 100 metaphases								Total aberrations in 1q-Z1q per 1000 metaphases
			Polycentrics (No. of chromosomes)	Rings + min	B"	RB′ (RB′-complex)	RB′B"	B′ + Gaps	CI	Total	
*Alu*I Buffer control	300	10.66	0.33 (2)	0.0	1.33	3.66 (0.0)	0.0	9.0	0.66	15.00	21
*Alu*I 5 units	300	49.33	3.33 (2)	2.66	20.33	31.00 (1.0)	5.33	30.33	12.33	106.33	236
*Bam*HI Buffer control	400	5.25	0.25 (2)	0.5	1.00	1.50 (0.0)	0.0	5.50	0.75	9.50	17
*Bam*HI 96 units	400	29.00	1.75 (2)	1.25	5.75	9.25 (2.0)	2.00	17.50	10.50	48.00	129

For abbreviations see Material and Methods.

*Alu*I-induced lesions included 43 chromatid gaps and 57 chromatid breaks, while 30 chromatid gaps and 70 chromatid breaks induced by *Bam*HI were analyzed. No differences between the distribution of chromatid gaps and chromatid breaks induced by both REs were found. The breakpoints induced by *Alu*I were highly concentrated from 0.45 to 0.65 relative units and *Bam*HI-induced breakpoints were mainly located from 0.35 to 0.65 relative units (Figure 1), involving 2 G-light and 2 G-dark bands for *Alu*I and 3 G-light and 3 G-dark bands for *Bam*HI.

More than 50% of chromatid breaks (34/57 for *Alu*I and 47/70 for *Bam*HI) showed a chromatin loss greater than 5% of the total arm length. A schematic representation of the chromatin losses greater than 0.05 relative units (Figure 1, segment S) is presented in Figure 2.

Figure 3 shows the cumulative frequencies of chromatin losses (Figure 1, segment S) from chromatid breaks induced by *Alu*I or *Bam*HI. Chromatin losses were non-randomly located along the long arm of CHO chromosome 1 showing, at least two preferential sites for the action of both REs (Figure 3, see arrows).

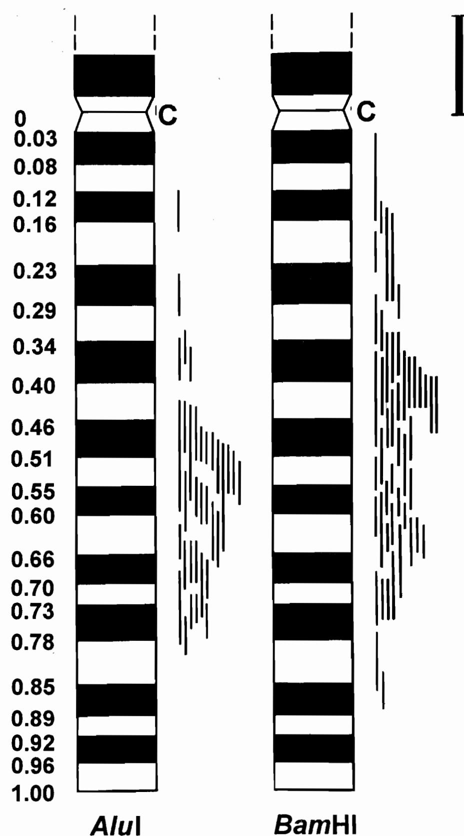

Figure 2 - Comparative diagram showing the localization of chromatin losses ("S" segments) greater than 0.05 relative units produced by *Alu*I and *Bam*HI according to the relative position of G-bands in the long arm of CHO chromosome 1. C = Centromere. Bar = 1 μm.

DISCUSSION

The recognition sequence for *Alu*I is AG ↓ CT, which is distributed preferentially in G-light bands, while the recognition sequence for *Bam*HI is G ↓ GATCC and it is located preferentially in G-dark bands in mammalian chromosomes (Holmquist and Caston, 1986). The fact that *Alu*I is more effective as a chromosome breaking agent than *Bam*HI has been studied by Obe and Johannes (1987).

Several investigations concerning the localization of breakpoints induced by X-rays in human and rodent chromosomes (Barrios *et al.*, 1989; Slijepcevic and Natarajan, 1994) have shown that more breakpoints are mapped to G-light than to G-dark bands. Folle and Obe (1995) found that the breakpoints induced by *Alu*I

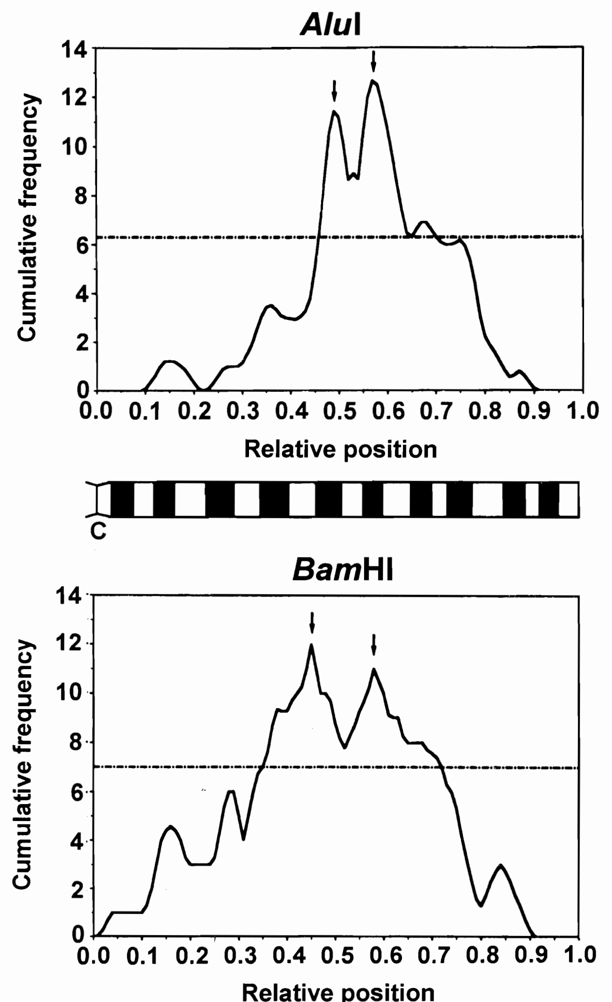

Figure 3 - Cumulative frequency curves showing the distribution of the extent of chromatin losses of all chromatid breaks induced by *Alu*I and *Bam*HI, respectively. Dashed lines represent a random distribution of "S" segments (averaged) estimated from the total amount of chromatid breaks. A G-band diagram of the long arm of CHO chromosome 1 with the same scale magnification as the relative position axis appears in the center of the figure (C = centromere).

and *Bam*HI in the G1 phase of CHO cells localize preferentially in G-light bands.

Savage (1977) pointed out that mapping chromosome breakpoints could give an erroneous result on band pattern recognition since the human eye tends to assign this kind of event to light bands. In this respect, Buckton (1976) and Dubos *et al.* (1978) observed that when the same chromosomes are sequentially R- and G-banded, breakpoints appear to be mostly located at G-/R-band junctions.

We have shown that it is possible to draw quantitative G-band maps to precisely localize chromosome breaks using a computerized microphotometric system (Drets *et al.*, 1994). With this system, we were able to detect that more than 50% of the chromatid breaks presented chromatin losses equal or greater than 5% of the total arm length, which could have resulted from the production of two breakpoints, probably followed by deletion of the intercalary segment.

According to our observations, breakpoints induced by the REs *Alu*I and *Bam*HI in the long arm of CHO chromosome 1 are non-randomly distributed. The overlapping of the chromatin losses from chromatid breaks induced by both endonucleases showed peaks located at 0.47 and 0.58 relative units for *Alu*I and 0.43 and 0.58 relative units for *Bam*HI.

Our results suggest that the localization of chromatid lesions induced by *Alu*I and *Bam*HI is not only dependent on the presence of their recognition sequences, but also on the accessibility of these REs to the chromosomal DNA, which in turn could be determined by the regional organization of the chromatin.

ACKNOWLEDGMENTS

We are grateful to P.J. Queirolo for computer program development and T. Abadie for statistical advice. Research partially supported by the Commission of European Communities (Contract CI*.0433.UY) and Programme for Development of Basic Sciences (PEDECIBA).

RESUMO

Falhas e quebras de cromátides induzidas pelas endonucleases de restrição *Alu*I e *Bam*HI no braço longo do cromossomo 1 de células do ovário de hamster chinês foram exploradas microfotometricamente e mapeadas em um mapa quantitativo de bandagem G. Mais de 50% das quebras de cromátides apareceram como perdas de cromatina maiores que 5% do comprimento total do braço. A maioria das falhas e quebras de cromátides assim como as perdas de cromatina induzidas por ambas as endonucleases de restrição localizaram-se não aleatoriamente em uma região que se estendia de 0,35 a 0,65 unidades de comprimento relativo do braço longo do cromossomo 1. Sugerimos que o acesso dessas endonucleases ao DNA cromossômico depende da organização local da cromatina.

REFERENCES

Barrios, L., Miro, R., Caballin, M.R., Fuster, C., Guedea, F., Subias, A. and **Egozcue, J.** (1989). Cytogenetic effects of radiotherapy breakpoint distribution in induced chromosome aberrations. *Cancer Genet. Cytogenet. 41*: 61-70.

Buckton, K.E. (1976). Identification with G and R banding of the position of breakage points induced in human chromosomes by *in vitro* X-irradiation. *Int. J. Radiat. Biol. 29*: 475-488.

Drets, M.E., Folle, G.A. and **Monteverde, F.J.** (1989). Quantitative detection of chromosome structures by computerized microphotometric scanning. In: *Chromosomal Aberrations. Basic and Applied Aspects* (Obe, G. and Natarajan, A.T., eds.). Springer-Verlag, Berlin-Heidelberg, New York, pp. 1-12.

Drets, M.E., Folle, G.A., Martínez, W., Bonomi, R., Duarte, J., Mechoso, B.H. and **Larrañaga, J.** (1994). Quantitative localization of chromatid breaks induced by *Alu*I in the long arm of chromosomes number 1 and Z1 of Chinese hamster ovary (CHO) cells by microphotometric scanning. In: *Chromosomal Alterations. Origin and Significance* (Obe, G. and Natarajan, A.T., eds.). Springer-Verlag, Berlin-Heidelberg, New York, pp. 169-183.

Dubos, C., Pequignot, E.V. and **Dutrillaux, B.** (1978). Localization of gamma-ray induced chromatid breaks using a three consecutive staining techniques. *Mutat. Res. 49*: 127-131.

Folle, G.A. and **Obe, G.** (1995). Localization of chromosome breakpoints induced by *Alu*I and *Bam*HI in Chinese hamster ovary (CHO) cells treated in the G1 phase of the cell cycle. *Int. J. Radiat. Biol. 68*: 437-445.

Goldman, M.A., Holmquist, G.P., Gray, M.C., Caston, L.A. and **Nag, A.** (1984). Replication timing of genes and middle repetitive sequences. *Science 224*: 686-692.

Holmquist, G.P. (1990). Mutational bias, molecular ecology, and chromosome evolution. In: *Advances in Mutagenesis Research* (Obe, G., ed.). Springer-Verlag, Berlin-Heidelberg, New York, pp. 95-126.

Holmquist, G.P. (1992). Review article: Chromosome bands, their chromatin flavor, and their functional features. *Am. J. Hum. Genet. 51*: 17-37.

Holmquist, G.P. and **Caston, L.A.** (1986). Replication time of interspersed repetitive DNA sequences in hamsters. *Biochim. Biophys. Acta 868*: 164-177.

ISCN (1985). An international system for human cytogenetic nomenclature. In: *Report of the Standing Committee on Human Cytogenetic Nomenclature* (Hardnden, D.G. and Klinger, H.P., eds.). Karger, Basel, pp. 118.

Korenberg, J.R. and **Rykowski, M.C.** (1988). Human genome organization: Alu, Lines and the molecular structure of metaphase chromosome bands. *Cell 53*: 391-400.

Martínez-López, W., Pieper, R. and **Obe, G.** (1995). Chromosomal aberrations induced in human whole blood cultures by pipetting cell pellets in the presence of *Alu*I. *Mutat. Res. 327*: 25-31.

Obe, G. and **Johannes, C.** (1987). Chromosomal aberrations induced by the restriction endonucleases *Alu*I and *Bam*HI: comparison with X-rays. *Biol. Zent. bl. 106*: 175-190.

Obe, G., Palitti, F., Tanzarella, C., Degrassi, F. and **De Salvia, R.** (1985). Chromosomal aberrations induced by restriction endonucleases. *Mutat. Res. 150*: 359-368.

Obe, G., Johannes, C. and **Schulte-Frohlinde, D.** (1992). DNA double-strand breaks induced by sparsely ionizing radiation and endonucleases as critical lesions for cell death, chromosomal aberrations, mutations and oncogenic transformation. *Mutagenesis 7*: 3-12.

Savage, J.R.K. (1977). Assignment of aberration breakpoints in banded chromosomes. *Nature 270*: 513-514.

Siciliano, M.J., Stallings, R.L. and **Adair, G.M.** (1985). The genetic map of the Chinese hamster and the genetic consequences of chromosomal rearrangements in CHO cells. In: *Molecular Cell Genetics* (Gottesman, M.M., ed.). John Wiley & Sons, New York, pp. 95-135.

Slijepcevic, P. and **Natarajan, A.T.** (1994). Distribution of radiation-induced G1 exchange and terminal deletion breakpoints in Chinese hamster chromosomes as detected by G-banding. *Int. J. Radiat. Biol. 66*: 747-755.

Trent, J.M., Kaneko, Y. and **Mitelman, F.** (1989). Report of the Committee on Structural Chromosome Changes in Neoplasia. *Cytogenet. Cell Genet. 51*: 533-562.

(Received May 8, 1996)